Mémoire présenté pour l'obtention de l'Habilitation à Diriger des Recherches

Martin Daufresne

Soutenue le 06 juillet 2017

Impacts du réchauffement climatique sur les écosystèmes aquatiques

Irstea, UR RECOVER, 3275 Route de
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N° d'ordre Année 2017

Habilitation à diriger des recherches

présentée
devant l'université Lille 1, école doctorale SMRE (Sciences de la Matière, du Rayonnement de l'Environnement)

par

Martin DAUFRESNE

IMPACTS DU RÉCHAUFFEMENT CLIMATIQUE SUR LES ÉCOSYSTÈMES AQUATIQUES.

Soutenue le 6 juillet 2017

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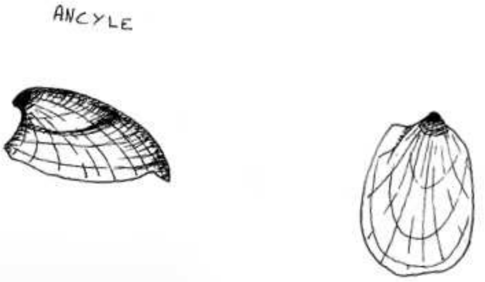
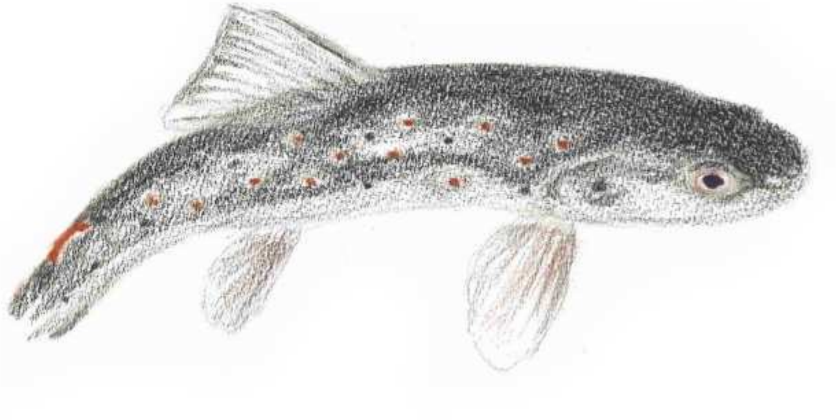
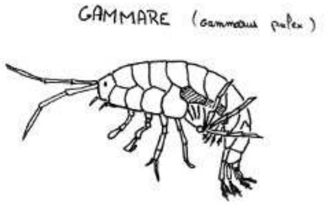
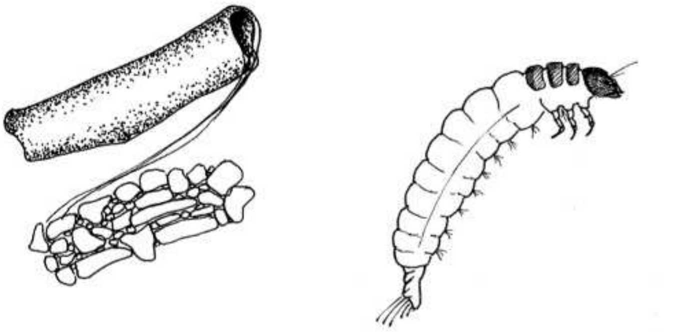








Merci aux anguilles de la Dive, aux truites de la Touques, aux truites de Berval.
Merci à Jacques-Yves Cousteau et à Théodor Monod.
Merci à ma famille, à ma femme, à mes enfants.

À ma soeur jumelle Benoîte qui, d'une manière ou d'une autre, m'a toujours accompagné.

Préambule

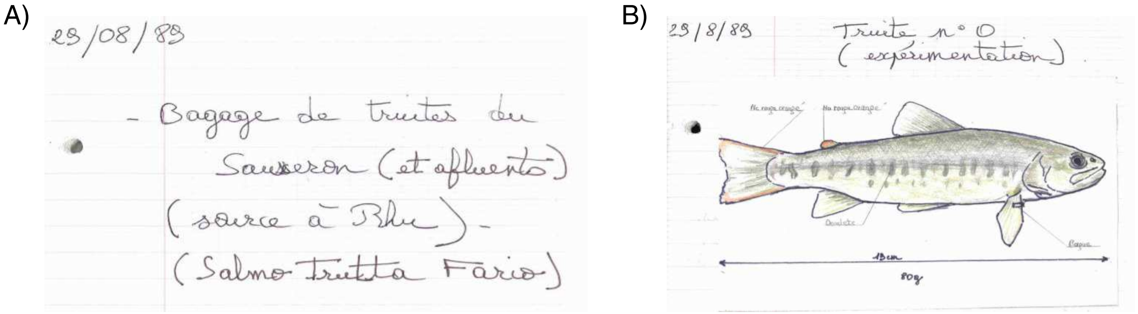
– Ecotone. Une ronce s'aventure à la frontière du milieu aquatique. Huveaune, Roquevaire, Février 2016. –

D'aussi loin que je me souvienne, le monde aquatique m'a toujours fasciné. C'est très certainement sa remarquable insoumission physique à l'espèce humaine qui m'a conduit jusqu'ici, bien plus que mon attachement à l'école ou au monde académique convenons-en. Vous trouverez dans ce document une présentation des mes activités de recherche passées, présentes et futures portant sur ces milieux si chers à mon coeur. Il est traditionnellement admis de débuter la rédaction d'une HDR par la présentation des activités menées en thèse, ou juste avant la thèse. Je ne peux me résoudre à faire cela tant cette démarche a commencé antérieurement à ma thèse de doctorat. Deux faits principaux me paraissent plus appropriés pour évoquer ce que je considère comme le début de mon parcours d'écologue et d'ichtyologue.

1 Daufresne & Daufresne (1989–1995). Étude de la population de truites du Ru de Theuville

.

Ma passion pour les milieux aquatiques m'a poussé à passer beaucoup de temps au bord des rivière, la plupart du temps avec mon frère aîné, Tanguy Daufresne. Si nos activées étaient d'abord principalement liées à la pêche à la ligne, dans la Dive, la Touques ou la Viosne, nous avons assez rapidement centré notre attention sur la capture, à la main, de truites du ru de Theuville, un affluent du Sausseron (Fig. 1).



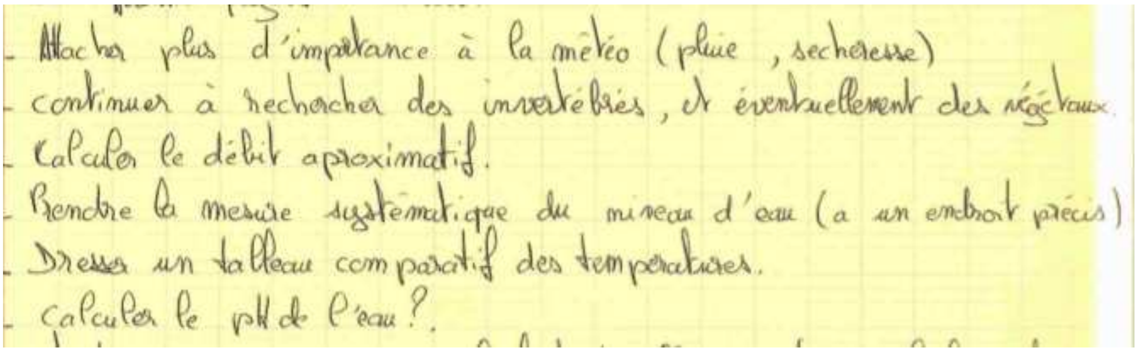
29/08/89

- Bagage de truites du Sausseron (et affluents) (source à Blu) - (Salmo Trutta Fario)

29/8/89 Truite n° 0 (expérimentation)

FIGURE 1 – Étude de la population de truites du ru. A) Page de garde du cahier de manipulation. B) Dessin de la première truite marquée.

Cette étude était purement descriptive mais l'étude de l'influence de variables environnementales biotiques (abondance et diversité des macroinvertébrés) ou abiotiques (hauteur d'eau, débit, ph) sur la morphologie, le comportement ou l'abondance de truites a rapidement été envisagée, avec déjà un attrait pour le paramètre thermique (Fig. 2).



- Attacher plus d'importance à la météo (pluie , secheresse)
- continuer à rechercher des invertébrés, et éventuellement des végétaux
- Calculer le débit aproximatif.
- Rendre la mesure systématique du niveau d'eau (a un endroit précis)
- Dresser un tableau comparatif des températures.
- Calculer le pH de l'eau ?.

FIGURE 2 – Extrait du cahier de manipulation (25 avril 1991).

Le site d'étude consistait en un cours d'eau principal et 3 de ses affluents principaux (Fig. 3). L'affluent numero 2 à été le plus étudié du fait de sa petite taille et de la facilité d'y prélever des truites.

Les truites étaient mesurées, pesées et éventuellement photographiées avant d'être relâchées. La pesée s'effectuait à l'aide d'un dynamomètre fourni par mon professeur de physique, Mr Garnier (je réalise qu'il l'avait gracieusement subtilisé à l'établissement pour la "bonne" cause.... Merci à lui). La prise de photo du flanc droit et gauche est devenue systématique à partir de 1994. Un descriptif de leur morphologie était consigné dans des fiches de terrain dédiées (Fig. 4) ayant légèrement évoluées au cours de l'étude.

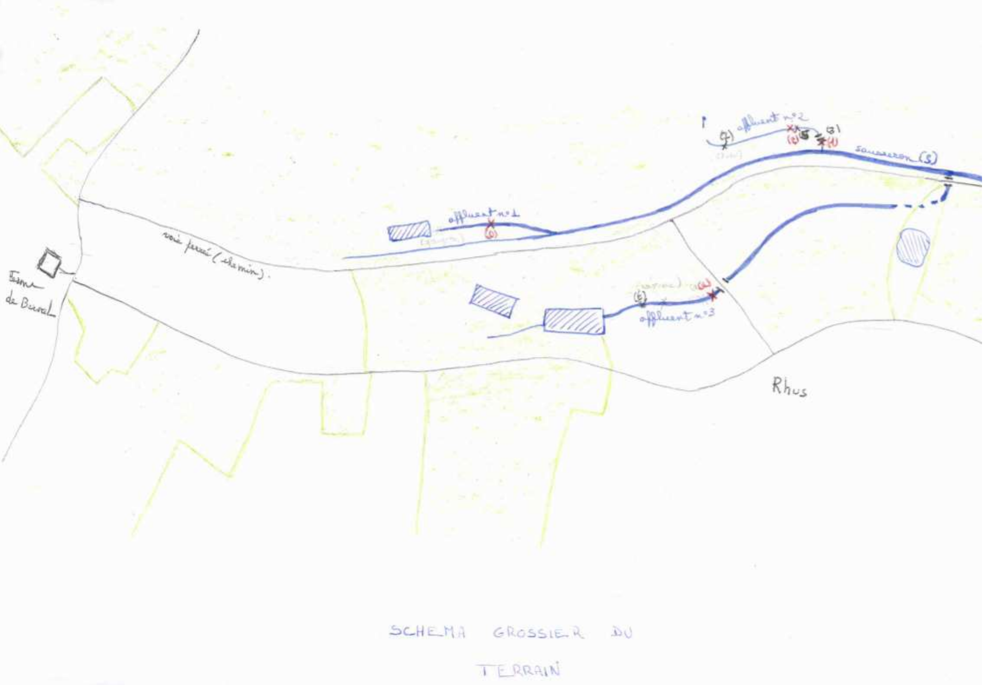


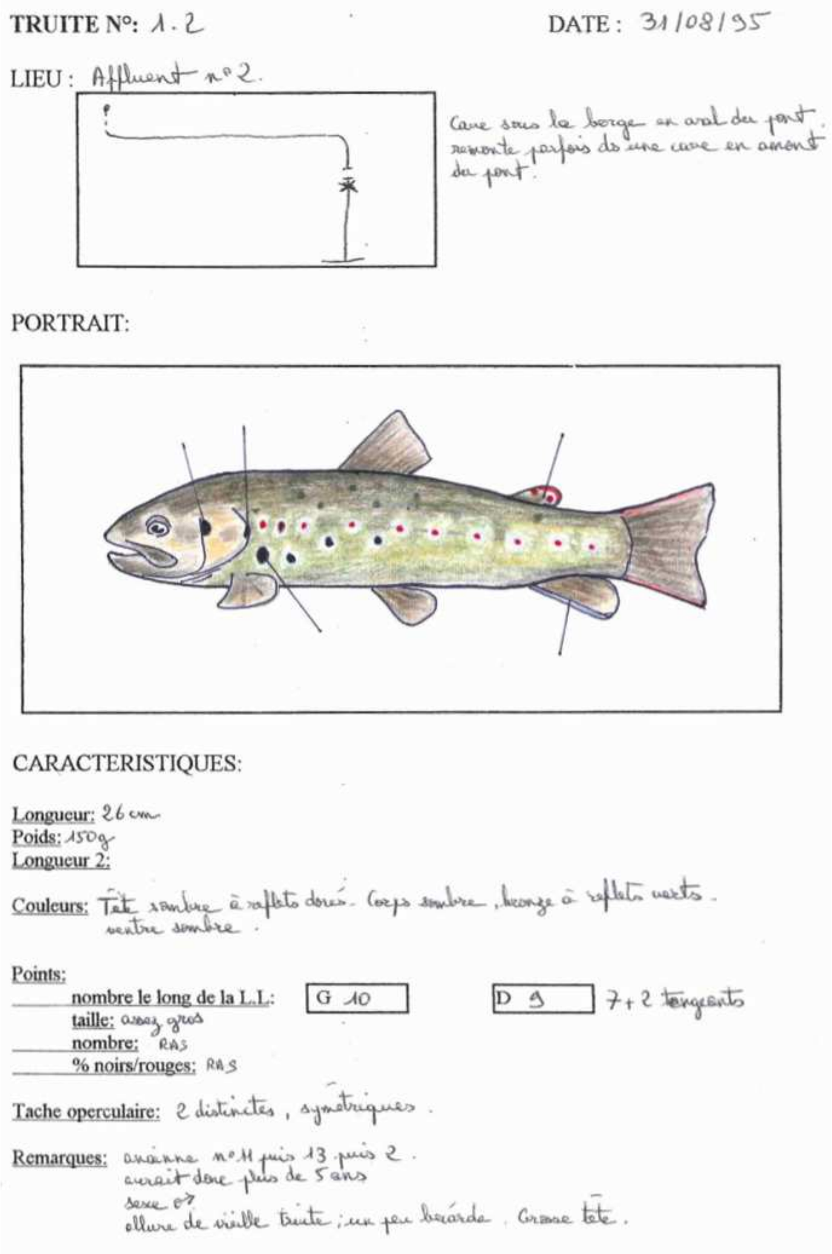
FIGURE 3 – Site d'étude. Les différents affluents du cours d'eau principal sont numérotés. La localisation des x premières truites (numéros 1 à x en noir) capturées est indiquée (novembre 1989).

Les truites étaient marquées à l'aide de bagues qui ont successivement consisté en des languettes métaliques, des plaques plastiques fixées sur des liens en cuivre et des agrafes (Fig. 5). Ce dernier système c'est montré relativement efficace en nous permettant en particulier de suivre pendant plusieurs années la truite “numero 2” (Fig. 4, Fig. 6).

Les données relatives à cette étude n'ont jamais été formellement analysées et ne le seront probablement jamais. Néanmoins la démarche adoptée, les campagnes de terrain et la consignation des resultats sont très proches du travail que j'ai pu effectuer par la suite dans diverses études empiriques ou expérimentales. Par curiosité, j'ai simplement ici regardé la relation taille-poids sur un échantillon non exhaustif des données (représentée en échelle log-log sur la Fig. 7) et constaté que l'exposant de la relation (2.6) était inférieure à la moyenne donnée dans Fishbase (3.03, Froese & Pauly 2014), soulignant le caractère longiligne des truites échantillonnées.

2 L'Ichtyologue de novembre 1989

Le deuxième fait ayant motivé de manière forte mon parcours a été la lecture du livre *Le Monde du Silence* (Cousteau & Dumas 1953) en novembre 1989. Le contenu m'a bien évidemment interpellé mais c'est surtout l'évocation du fait que Théodore Monod fut le premier scientifique à descendre dans le Bathyscaphe, suite à un tirage au sort pour designer la personne accompagnant le professeur Piccard qui marqua une étape importante de mon parcours. En effet, je découvris alors de proche en proche le terme d' ”ichtylogiste” et sa signification (Fig. 8) et le laboratoire d'ichtyologie du Museum national d'Histoire Naturelle de Paris, où Théodore Monod avait son bureau. Émerveillé par le fait que la recherche sur les poissons pouvait être considérée comme un travail, je décidais immédiatement de devenir ichtyologue. S'en suivront deux visites au muséum, l'une en novembre 1989 l'autre en 1999. Je dois l'avouer, ma première visite fut un relatif échec, le personnel du laboratoire, regroupé dans la salle café pour la pause de 10h, m'ayant réservé un accueil assez froid.... La surprise sans doute. Je pu rencontrer Théodore Monod lors de ma



TRUITE N°: 1.2 **DATE :** 31/08/95

LIEU : Affluent n°2.

Cave sous la berge en aval du pont. remonte parfois ds une cave en amont du pont.

PORTRAIT:

CARACTERISTIQUES:

Longueur: 26 cm
Poids: 150g
Longueur 2:

Couleurs: Tête sombre à reflets dorés. Corps sombre, bronze à reflets verts. ventre sombre.

Points:
nombre le long de la L.L: G 10 D 9 7+2 tangents
taille: assez gros
nombre: RAS
% noirs/rouges: RAS

Tache operculaire: 2 distinctes, symétriques.

Remarques: ancienne n°11 puis 13 puis 2.
aurait donc plus de 5 ans
sexe ♂
allure de vieille truite ; un peu bécarde. Grosse tête.

FIGURE 4 – Fiche individuelle (version 2). Le numéro et les caractéristiques morphologiques de chaque truite sont consignés.

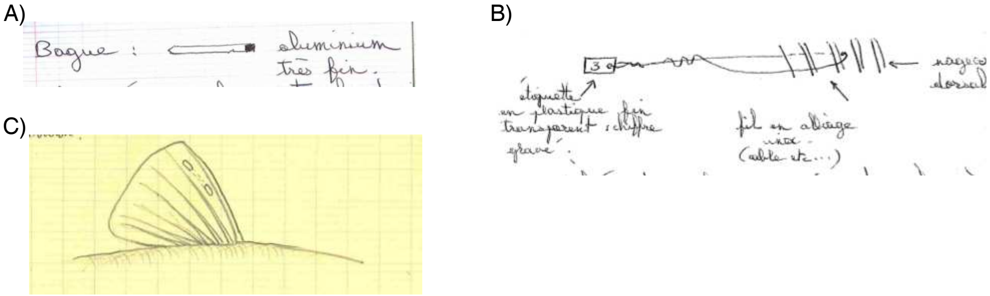


FIGURE 5 – Bagues utilisées pour le marquage des individus. A) Août 1989. B) Décembre 1989. C) Avril 1991, agrafes peintes.

Figure 6 – Truites du Ru de Theuville. A) Truite numéro 2. B) Truite numéro 2 dans son milieu. C) Truite conservée quelques jours en aquarium.

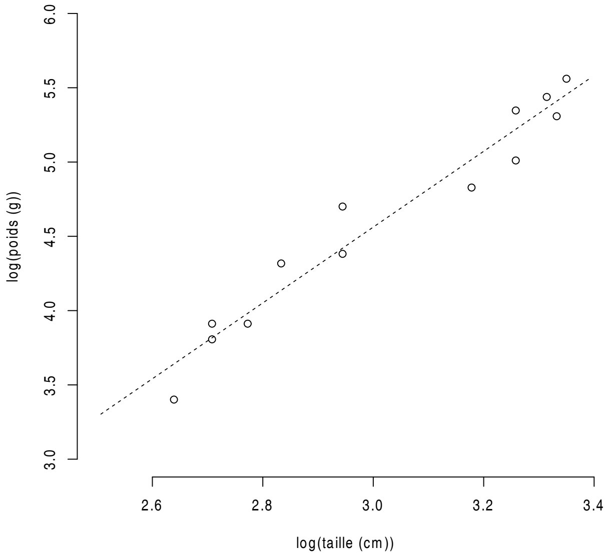
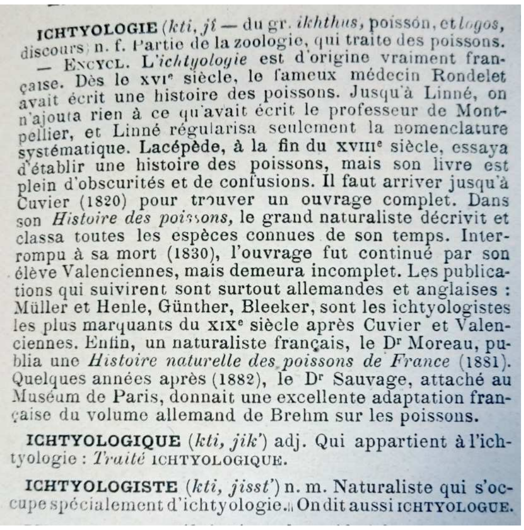


Figure 7 – Relation taille-poids des truites du Ru de Theuville en échelle log-log. La droite de régression du modèle linéaire $log(poids) = a + b * log(taille)$ est représentée.

seconde visite. Mr. Monod était un bien vieil homme à cette date et notre discussion fut courte et quelque peu décousue. J'en garde néanmoins un souvenir ému. Ce n'est pas tous les jours que l'on a l'occasion de rencontrer une telle personne.



ICHTYOLOGIE (*kti, jî* — du gr. *ikhthus*, poisson, et *logos*, discours) n. f. Partie de la zoologie, qui traite des poissons. — ENCYCL. L'*ichtyologie* est d'origine vraiment française. Dès le XVIe siècle, le fameux médecin Rondelet avait écrit une histoire des poissons. Jusqu'à Linné, on n'ajouta rien à ce qu'avait écrit le professeur de Montpellier, et Linné régularisa seulement la nomenclature systématique. Lacépède, à la fin du XVIIIe siècle, essaya d'établir une histoire des poissons, mais son livre est plein d'obscurités et de confusions. Il faut arriver jusqu'à Cuvier (1820) pour trouver un ouvrage complet. Dans son *Histoire des poissons*, le grand naturaliste décrivit et classa toutes les espèces connues de son temps. Interrompu à sa mort (1830), l'ouvrage fut continué par son élève Valenciennes, mais demeura incomplet. Les publications qui suivirent sont surtout allemandes et anglaises : Müller et Henle, Günther, Bleeker, sont les ichtyologistes les plus marquants du XIXe siècle après Cuvier et Valenciennes. Enfin, un naturaliste français, le Dr Moreau, publia une *Histoire naturelle des poissons de France* (1881). Quelques années après (1882), le Dr Sauvage, attaché au Muséum de Paris, donnait une excellente adaptation française du volume allemand de Brehm sur les poissons.

ICHTYOLOGIQUE (*kti, jik'*) adj. Qui appartient à l'ichtyologie : *Traité* ICHTYOLOGIQUE.

ICHTYOLOGISTE (*kti, jisst'*) n. m. Naturaliste qui s'occupe spécialement d'ichtyologie. || On dit aussi ICHTYOLOGUE.

FIGURE 8 – Extrait du Nouveau Larousse illustré, Tome cinquième, p. 221, Auge (Claude), pré 1928. Ouvrage, rare, d'une aire pré-wikipedia qui a bercé mon enfance...

Pour finir, suis-je ichthyologiste ou ichthyologue aujourd'hui ? Je ne le pense pas, mes activités ayant largement portées sur d'autre organismes que les poissons. Écologue et écologue aquatique, j'ai gardé en revanche cette fascination pour le monde subaquatique en général, avec une affection particulière pour la gent piscicole...

Première partie
Notice d'activité

1 C.V.

Date de naissance : 27/02/1975
Situation : Marié, trois enfants
Adresse : Irstea, UR Hydrobiologie, 3275 Route de Cézanne, CS 40061, 13182 Aix-en-Provence Cedex 05.
email : martin.daufresne@irstea.fr

1.1 Diplômes

- **2004 :** Thèse de doctorat en écologie, Université Claude Bernard Lyon 1, Lyon.
- **2000 :** DEA analyse et modélisation des systèmes biologiques, Université Claude Bernard Lyon 1, Lyon.
- **1999 :** Diplôme d'ingénieur agronome, ENSAIA, Nancy. Spécialisation **halieutique**, ENSAR, Rennes.

1.2 Expérience professionnelle

- **Depuis 2007 :** Chargé de recherche, Irstea, UR Hydrobiologie, Aix-en-Provence. *Étude de la dynamique des populations et des communautés d'organismes aquatiques continentaux, en particulier en milieu lacustre.*
- **2006–2007 :** Post-doctorat, IFM-GEOMAR, Kiel, Allemagne. *En charge des meta-analyses dans le cadre du projet de coordination d'un programme national de recherche portant sur les effets du changement climatique sur les organismes aquatiques (incluant 30 projets de recherches et plus de 100 chercheurs)*
- **2005 (3 mois) :** Post-doctorat, Cemagref, Lyon, France. *Impacts de la centrale nucléaire de production électrique de Bugey sur la communauté piscicole dans un contexte de réchauffement climatique.*
- **2005 (3 mois) :** Post-doctorat, Université de Constance, Constance, Allemagne. *Influence des facteurs trophiques et environnementaux sur la dynamique de la communauté piscicole du lac de Constance (1997-2004).*
- **2005 (3mois) :** Post-doctorat, Université d'Oslo, Department of Biology, Oslo, Norvège. *Stabilité temporelle des communautés planctoniques des lacs norvégiens.*

1.3 Outils

- **Langues :** Français, Anglais (courant).
- **Informatique :** OSS Windows, Linux ; LaTeX, R, SQL.

1.4 Divers

- **Expérimentation animale N1** (2013)
- **Stage conduite 4x4** (2010)
- **Permis bateau côtier & fluvial** (2008)
- **Habilitation pêche électrique** (2001)
- **Sport :** Escrime (7 ans, niveau international junior), ski, escalade, plongée bouteille (N2, RIFAP, TIV), apnée, pêche à la mouche.
- **Musique :** Guitare (bases).

- **Photo :** Prise de vue terrestre et sub-aquatique (photos & vidéo).
- **Activité associative :** Membre actif du Groupe d'Étude du Mérou (GEM), responsable "bio/photo" d'Aubagne Plongée Passion.

2 Travaux / Publications

2.1 Thèse de doctorat

Daufresne M. (2004). *Approche multi-échelles des relations dynamiques entre les organismes aquatiques et leur environnement.* Thèse de doctorat, Université Lyon 1, Villeurbanne, France, 175 pp.

2.2 Articles (nombre de citations au 15 janvier 2018 d'après le Web of Science, WoS)

Nombre total de citations (WoS, all databases) : 1224 au 8 septembre 2017.
H facteur (WoS, all databases) : 13 au 8 septembre 2017.

Les articles numéroté en gras et ceux précédés du signe "*" dans la liste suivante étaient cités respectivement plus de 50 et 100 fois au 8 septembre 2017 (source WoS, all databases). Les articles "**Highly cited paper**" sont les articles appartenant aux 1% des articles les plus cités pour l'année et la discipline d'après le WoS. Le nombre de citations de deux articles non référencés dans le WoS ont été obtenus dans Google Scolar.

[21] **Daufresne M.**, J. Veslot, H. Capra, G. Carrel, A. Poirel, J.M. Olivier and N. Lamouroux (2015). Fish community dynamics (1985–2010) in multiple reaches of a large river subjected to flow restoration and other environmental changes. *Freshwater Biology.* **6**, 1176–1191. (10)

[20] Mouthon J. and **M. Daufresne** (2015). Resilience of mollusc communities of the River Saone (eastern France) and its two main tributaries after the 2003 heatwave. *Freshwater Biology.* **12**, 2571–2583. (2)

[19] Reyjol Y., Argillier C., Bonne W., Borja A., Buijse A.D., Cardoso A.C., **Daufresne M.**, Kernan M., Ferreira M.T., Poikane S., Prat N., Solheim A.L., Stroffek S., Usseglio-Polatera P., Villeneuve B. and W. van de Bund (2014). Assessing the ecological status in the context of the European Water Framework Directive : Where do we go now ? *Science of the Total Environment.* **497–498**, 332–344. (33)

[18] Hemmer-Brepson C., L. Replumaz, C. Romestaing, Y. Voituron and **M. Daufresne** (2014). Non-stressful temperature effect on oxidative balance and life history traits in adult fish (Oryzias latipes). *Journal of Experimental Biology.* **217**, 274–282. (3)

[17] Clavel J., N. Poulet, E. Porcher, S. Blanchet, G. Grenouillet, S. Pavoine, A. Biton, N. Seon-Massin, C. Argillier, **M. Daufresne**, P. Teillac-Deschamp and R. Julliard (2013). A new freshwater biodiversity indicator based on fish community assemblages. *Plos One.* **8**, e80968. (3)

[16] Comte L., L. Buisson, **M. Daufresne**, and G. Grenouillet (2013). Climate-induced changes in the distribution of freshwater fish : observed and predicted trends. *Freshwater Biology.* **58**, 625–639. (68) **Highly cited paper**

[15] Hessen D.O., **M. Daufresne**, and H. Leinaas (2013). Temperature- size relations from the cellular-genomic perspective. *Biological Reviews.* **88**, 476–489. (29)

[14] Schleuter D., **M. Daufresne**, J. Veslot, N.W.H. Mason, C. Lanoiselée, S. Brosse, O. Beauchard and C. Argillier (2012). Geographic isolation and climate govern functional diversity of native fish communities in European drainage basins. *Global Ecology and Biogeography.* **21**, 1083–1095. (17)

[13] Mouthon J. and **M. Daufresne** (2011). Mollusc survey of the Ognon river (eastern France), decline of native bivalve populations (Unionidae and Sphaeriidae) between 1977 and 2007. *Malaco.* **7**, 391–397. (1, Google Scholar)

[12] Mouthon J. and **M. Daufresne** (2010). Long-term changes in mollusc communities of the Ognon river (France) over a 30-year period. *Fundamental and Applied Limnology.* **178**, 67–79. (6)

[*11] Schleuter D., **M. Daufresne**, F. Massol and C. Argillier (2010). A user's guide to functional diversity indices. *Ecological Monographs.* **80**, 469–484. (195) **Highly cited paper**

[*10] **Daufresne M.**, K. Lengfellner and U. Sommer (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences of the United States of America.* **106**, 12788–12793. (423) **Highly cited paper**

[9] **Daufresne M.** (2009). Impacts of climatic and non-climatic pressures on fish communities in large French rivers. *Hydroécologie Appliquée.* **16**, 109–134. (2, Google Scholar)

[8] Mouthon J and **M. Daufresne** (2008). Population dynamics and life cycle of Pisidium amnicum (Muller) (Bivalvia : Sphaeriidae) and Valvata piscinalis (Muller) (Gastropoda : Prosobranchia) in the Saone river, a nine-year study. *International Journal of Limnology.* **44**, 241–251. (6)

[*7] **Daufresne M.** and P. Boët (2007). Climate change impacts on structure and diversity of fish communities in rivers. *Global Change Biology.* **13**, 2467–2478. (103)

[6] **Daufresne M.**, P. Bady and J.F. Fruget (2007). Impacts of global changes and extreme hydro-climatic events on macroinvertebrate community structures in the French Rhône River. *Oecologia.* **151**, 544–559. (66)

[5] Jensen T.C., T.R. Anderson, **M. Daufresne** and D.O. Hessen (2006). Does excess carbon affect respiration of the rotifer Brachionus calyciflorus Pallas ? *Freshwater Biology.* **51**, 2320–2333. (15)

[4] **Daufresne M.** and O. Renault (2006). Population fluctuations, regulation and limitation in stream-living brown trout. *Oikos.* **113**, 459–468. (17)

[3] Mouthon J. and **M. Daufresne** (2006). Effects of the 2003 heatwave and climatic warming on mollusc communities of the Saône : a large lowland river and of its two main tributaries (France). *Global Change Biology.* **12**, 441–449. (89)

[2] **Daufresne M.**, H. Capra and P. Gaudin (2005). Downstream displacement of post-emergent brown trout : Effects of development stage and water velocity. *Journal of Fish Biology.* **67**, 599–614. (16)

[*1] **Daufresne M.**, M.C. Roger, H. Capra and N. Lamouroux (2004). Long-term changes within the invertebrate and fish communities of the Upper Rhône River : Effects of climatic factors. *Global Change Biology.* **10**, 124–140. (123)

2.3 Rapports scientifiques

[18] Boutrif M., **M. Daufresne** and J.M. Baudoin (2016) *Indicateurs fonctionnels des plans d'eau : les émissions de gaz à effet de serre des plans d'eau.* Rapport d'étape. ONEMA–Irstea, 30 pp.

[17] Marchand C., **M. Daufresne** and J.M. Baudoin (2016) *Développement d'indicateurs fonctionnels en plan d'eau.* Rapport Final. ONEMA–Irstea, 76 pp.

[16] Marchand C., T. Péroux, J. Dublon, **M. Daufresne** and J.M. Baudoin , (2016) *Protocole de conception et de déploiement de dispositifs pour l'étude de la décomposition de matière organique en plan d'eau.* Guide méthodologique et scientifique. ONEMA–Irstea, 41 pp.

[15] Rebière D., P.A. Danis, J. Dublon, J.M. Baudoin and **M. Daufresne** (2015) *Réseau de suivi pérenne de la température des plans d'eau : synthèse de la phase de test.* Rapport d'étape. ONEMA–Irstea, 54 pp.

[14] Baudoin J.M. and **M. Daufresne** (2014) *Rapport d'activité 2013 Pôle d'études et de recherches Onema-Irstea "Hydroécologie des Plans d'eau".* ONEMA–Irstea, 14 pp.

[13] Marchand C., **M. Daufresne** and J.M. Baudoin (2014) *Indicateurs fonctionnels des plans d'eau : mesure de la vitesse de décomposition de la matière organique – Phase de test –.* Rapport d'étape. ONEMA–Irstea, 50 pp.

[12] Rebière D., P.A. Danis, **M. Daufresne**, T. Péroux and J.M. Baudoin (2013) *Mise en place d'un réseau de suivi thermique lacustre à l'échelle nationale : phase de test.* Rapport d'étape. ONEMA–Irstea, 65 pp.

[11] Marchand C., **M. Daufresne** and J.M. Baudoin (2013) *Indicateurs fonctionnels des plans d'eau : processus et méthodes de mesure.* Rapport d'étape. ONEMA–Irstea, 39 pp.

[10] Rondel C., **M. Daufresne** and P.A. Danis (2012) *Typologie thermo-mictique - Application à une classification des lacs français.* ONEMA-Irstea, 60 pp.

[9] **Daufresne M.** and G. Lacroix (2012) *Vers de nouveaux indicateurs du fonctionnement des écosystèmes aquatiques.* ONEMA-Irstea, 17 pp.

[8] Rondel C., **M. Daufresne** and P.A. Danis (2011) *Réflexion sur la mise en place d'un réseau de mesure thermique en plans d'eau.* ONEMA-Cemagref, 50 pp.

[7] **Daufresne M.**, M. Coudel and D. Schleuter (2008). *Influence des CNPE sur la diversité fonctionnelles des communautés piscicoles dans un contexte de changement climatique.* EDF-Cemagref, 27 pp.

[6] **Daufresne M.** (2007). *Influence des CNPE sur la structure en taille des communautés et populations piscicoles dans un contexte de changement climatique.* EDF-Cemagref, 21 pp.

[5] De Bortoli J., **M. Daufresne** and C. Argillier (2007). *Définition des conditions de référence sur les plans d'eau, paramètre chlorophylle-a.* Cemagref, 19 pp.

[4] Fruget J.F., P. Bady, J.M. Olivier, G. Carrel, Y. Souchon, B. Villeneuve, **M. Daufresne** and H. Capra (2006). *Etude thermique globale du Rhône phase III, Lot 2 : Etude à l'échelle du Rhône des compartiments biologiques.* ARALEP/Cemagref/CNRS, 80 pp.

[3] **Contributing author** in : Moreno J., E. Galante and M.A. Ramos (2005). *Impact on animal biodiversity. in : A preliminary assessment of the impacts in Spain due to the effects of climate change* (Moreno J. eds). Final Report, Project ECCE, Ministry of the Environment and the University of Castilla La Mancha, pp 243. (Evaluated by external reviewers).

[2] **Daufresne M.** and P. Boët (2005). *Evolution temporelle de la structure des peuplements piscicoles de grands fleuves français.* EDF-Cemagref, 19 pp.

[1] **Daufresne M.**, Y. Souchon, B. Villeneuve and H. Capra (2005). *Influence du rejet thermique de la centrale nucléaire de Bugey sur la communauté de poissons du Rhône dans un contexte de réchauffement climatique.* EDF-Cemagref, 21 pp.

2.4 Mémoires d'élève

[13] Bouvier, E. (2016). *Analyse des liens température-organismes (phytoplancton, poisson) existant dans la base de données plan d'eau du pôle Onema-Irstea d'Aix-en-Provence à l'aide de modèles linéaires.* M1, UCO Angers. 31 p.

[12] Muret, E. (2015). *Impact de la température sur la balance oxydative et les traits d'histoire de vie des organismes aquatiques à l'échelle multigénérationnelle.* M2, Université Toulouse III. 35 p.

[11] Hemmer-Brepson C. (2013). *Effet de la température sur la balance oxydative de vertébrés ectothermes aquatiques à différentes échelles spatio-temporelles et conséquences sur les traits d'histoire de vie –Modèle poisson–.* Thèse de doctorat, Université Aix-Marseille. 350 p.

[10] Richez B. (2013). Rapport de stage de l'UE "Travaux Personnels Encadrés". L3, Université Aix-Marseille. 20 p.

[9] Replumaz, L. (2012). *Gestion du stress oxydant et acclimatation à la température. Étude préliminaire sur un vertébré ectotherme poisson : Oryzias latipes.* Master 1, Université Claude Bernard Lyon I. 17 p.

[8] Degut, A. (2012). *Effet de la pêche sur le stress oxydant du gardon (Rutilus rutilus) et de la perche commune (Perca fluviatilis).* IUT, Université Claude Bernard Lyon I. 38 p.

[7] Dutordoir, S. (2010). *Modélisation thermodynamique de la retenue Bimont à l'aide du modèle DYRESM.* Master 2 Sciences de l'Univers, Université Pierre et Marie Curie Paris. 48 p.

[6] Lefol, E. (2010). *Influence de la température et de la compétition interspécifique sur la structure en taille des communautés piscicoles lacustres.* Master 2 GILE Gestion Intégrée du Littoral et des Écosystèmes, Université de Corse. 36 p.

[5] Roux, M. (2009). *Structure en taille des populations et communautés piscicoles en milieux lacustres.* Master1 BGAE, Montpellier 2. 15 p.

[4] Coudel, M. (2008). *Impacts of global change on functional diversity of stream fish communities.* Licence, ENS Paris. 27 p.

[3] Guiserix, M. (2003). *Notice d'utilisation. Tests d'adéquation sous S-Plus.* Ecole Centrale Lyon. 24 p.

[2] Ferrier, V. (2003). *Détermination d'un stade sensible pour différents âges d'émergents de truite en réponse à une variation de débit.* DU, Univ. Clermont-Ferrand. 68 p.

[1] Pradel, S. (2002). *Etude de la sensibilité des émergents de truite commune (Salmo trutta L.) à une augmentation de débit.* DU, Univ. Claude Bernard Lyon I. 35 p.

2.5 Communications à des congrès

[15] **M. Daufresne**, A. Loisel, C. Hemmer-Brepson, C. Romestaing, and Y. Voituron (2016). *Resource allocation trade-offs and senescence may explain the temperature-size rule in aquatic organisms.* Sfecologie 2016, Marseille.

[14] Mari L., **M. Daufresne**, Y. Voituron, J. Guillard, G. Evanno, and E. Lasne (2016). *Response of a cold-water-adapted species, the arctic charr Salvelinus alpinus, to thermal changes : an ecophysiological perspective.* Sfecologie 2016, Marseille.

[13] Lasne E., I. Percelay, **M. Daufresne**, A. Isla, Y. Voituron, and G. Evanno (2015). *Arctic charr on the southern edge : thermal reaction norms for embryonic traits and adaptive potential in a warming climate.* 8th International charr symposium, Tromsø.

[12] Olivier J.–M., N. Lamouroux, E. Sivade, M. Zylberblat, E. Castella, H. Piégay, M. Forcellini, S. Mérigoux–Lhopital, J. Riquier, A. Paillex, S. Dolédec, L. Vaudor, and **M. Daufresne** (2015). *Restauration hydraulique et écologique du Rhône : retours d'expérience et enseignements.* I.S.Rivers, Lyon.

[11] Rebière D., P.A. Danis, J. Dublon, J.M. Baudoin and **M. Daufresne** (2014). *Choix du pas de temps et d'espacement des thermistors dans le cadre de la mise en place d'un réseau de suivi thermique des lacs français : application aux lacs sentinelles.* 5èmes rencontres scientifiques et techniques, lacs sentinelles, Aix-en-Provence.

[10] **Daufresne M.**, M. Coudel and D. Schleuter (2009). *Impact of global change on functional structure and diversity of large rivers fish communities.* ASLO Meeting, Nice.

[9] Schleuter D., **M. Daufresne**, F. Massol and C. Argillier (2009). *A user's guide to functional indices : are functional diversity indices consistent and complementary ?* ASLO Meeting, Nice.

[8] Schleuter .D, N. Mason, **M. Daufresne**, C. Lanoiselée, S. Brosse, O. Beauchard, C. Argillier (2008). *Consequences of introduced species for the functional diversity of fish communities in European drainage basins.* 38th GfÖ Annual Conference, Konstanz.

[7] **Daufresne M.** and U. Sommer (2007). *Impacts of climate warming on aquatic organisms : Are the smallest the strongest ?* 4th AQUASHIFT workshop / 37th GfÖ Annual Conference, Marburg.

[6] Sandow M. and **M. Daufresne** (2006). *Kiel : The Aquashift-Wiki productivity enhancement platform.* 3rd AQUASHIFT workshop, Kiel.

[5] **Daufresne M.** (2006). *About combining the AQUASHIFT projects.* 3rd AQUASHIFT workshop : Meta-analysis working group, Kiel.

[4] **Daufresne M.**, H. Capra and P. Gaudin (2004). *Dévalaison des alevins post-émergents de truite commune (Salmo trutta L.) : effet des vitesses de courant et du stade de développement.*

A La Truite Dans tous ses Habitats, Saint Flour.

[3] **Daufresne M.** and O. Renault (2004). *Effet des débits hivernaux sur la taille des populations de truite commune.* A La Truite Dans tous ses Habitats, Saint Flour.

[2] **Daufresne M.**, H. Capra and P. Gaudin (2003). *Influence of discharge increase on downstream dispersal of young brown trout (Salmo trutta, L.) at different stages of development.* At Symposium for European Freshwater Sciences 3, Edinburgh.

[1] **Daufresne M.**, M.C. Roger and H. Capra (2001). *Long term changes within the invertebrate and fish communities of the Upper Rhône River : effect of a climatic warming ?* At Symposium for European Freshwater Sciences 2, Toulouse.

2.6 Conférences invitées

[10] **Daufresne, M.** (2012). *Changement climatique et écosystèmes aquatiques.* Journée connaissance eau et changement climatique – Agence de l'eau RMC. Lyon, 19/09/ 2012.

[9] **Daufresne, M.** (2010). *Impact of climate warming on body size in aquatic ecosystems.* International workshop Climate change impacts on estuarine and coastal ecosystems : a zooplankton perspective, 28/06/2010 - 30/06/2010, Boulogne sur Mer.

[8] **Daufresne, M.** (2010). *The small story : Impact of climate warming on body size in aquatic ecosystems.* Sizemic, Barcelona, 21/06/2010-25/06/2010.

[7] **Daufresne, M.** (2010). *The small story : Impact of climate warming on body size in aquatic ecosystems.* UCBL1, Lyon, 03/04/2010.

[6] **Daufresne, M.** (2010). *The small story : Impact of climate warming on body size in aquatic ecosystems.* DIMAR/COM, Marseille, 23/04/2010.

[5] **Daufresne, M.** (2010). *The small story : Impact of climate warming on body size in aquatic ecosystems.* IMEP, Marseille, 10/08/2010.

[4] **Daufresne, M.** (2009). *Réchauffement vs. perturbation : impacts écologiques du changement climatique. L'exemple des ecosysèmes aquatiques.* Séminaire d'écologie et d'évolution, CEFE, Montpellier, 29/05/2009.

[3] **Daufresne, M.** (2009). *Warming vs. disturbance : The ecological impacts of climate change on aquatic ecosystems.* Aquashift, Kiel, 7/09/2009-9/09/2009.

[2] **Daufresne M.** (2005). Climate change and stream ecosystems. Lecture to the IFM-GEOMAR, Kiel.

[1] **Daufresne M.** (2005). *Dynamical and multi scale approach of the relationships between the stream organisms and their environment.* Lecture to the Institute of Biology, University of Oslo.

2.7 Ouvrages

[2] **Daufresne M.** and T. Daufresne (2011). *40 poissons d'eau douce. Où les trouver, comment les reconnaître.* Editions Glénat, Grenoble, France. 96 pp.

[1] Carrel G. and **M. Daufresne** (2008). *Les poissons et la pêche du Rhône. Peut-on prévoir les effets du changement climatique sur les poissons du Rhône ?* Le Rhône en 100 questions. Bravard, J.P., Clémens, A., Villeurbanne, p. 208 – 209.

2.8 Posters à des congrès

[9] Marchand C., **M. Daufresne**, P. Gibert, J. Dublon, F. Colas, and J.–M. Baudoin (2015). *Spatial distribution of leaf breakdown in two french mediterranean lakes.* At Sfecologie 2016, Marseille.

[8] Marchand C., M. Boutrif, J. Dublon, P. Gibert, T. Péroux, **M. Daufresne** and J.–M. Baudoin (2015). *Organic matter breakdown as functional indicator in lake ecosystems : first study in two French lakes.* At SEFS, Genève.

[7] Boutrif M., C. Marchand C., J. Dublon, T. Péroux, **M. Daufresne** and J.–M. Baudoin (2015). *Developpement of functional indicators for lentic ecosystems.* At SEFS, Genève.
[6] Rebière D., P.A. Danis, **M. Daufresne**, T. Peroux and J.M. Baudoin (2014). *Mise en place d'un réseau national de suivi thermique des lacs : phase de test des prototypes d'enregistrement in situ.* At JILO, Marseille.
[5] Marchand C., J.M. Baudoin, **M. Daufresne** and J. Dublon (2014). *Développement d'indicateurs du fonctionnement écosystémique en milieu lentique.* At JILO, Marseille.
[4] **Daufresne M.**, M. Coudel and D. Schleuter (2012) *Shrinking species : directional and non directional impact of climate warming.* At Colloque CNRS / Ecology Letters "Impacts écologiques des changements environnementaux". Paris.
[3] Hemmer C. and **M. Daufresne** (2011). *Toward a better understanding of the ecological impacts of global warming : a conceptual framework based on size variability.* At Thermadapt, Tartu.
[2] Hemmer C. and **M. Daufresne** (2011). *Toward a better understanding of the ecological impacts of global warming : a conceptual framework based on size variability.* At Sizemic, Hambourg.
[1] Schleuter D., N.W.H. Masson, **M. Daufresne**, C. Lanoiselée, S. Brosse, O. Beauchard, and C. Argillier. (2009). *Impact of introduced fish species on the functional diversity of European fish communities.* At ASLO meeting, Nice.

2.9 Expertises pour des revues

American Nauralist, Diversity and Distribution, Ecography, Ecology Letters, Global Change Biology, Global Ecology and Biogeography, Hydrobiologia, Journal of Environmental Monitoring, Journal of Fish Biology, Journal of North American Benthological Society, Marine Biology, Marine Ecology Progress Series, Nature Climate Change, Oecologia, Oikos, New Zeland Journal of Marine and Freshwater Research.

2.10 Expertises pour des agences nationales de recherche

National Science Foundation (NSF, USA).

2.11 Enseignement et formation

2.11.1 Encadrement de thèse

[1] **Ayala Loisel** (2015-2018), Irstea Aix-en-Provence, Université Aix-Marseille.
Depuis novembre 2015, une thèse, co-financée par la direction générale d'Irstea et la région Provence-Alpes-Côte d'Azure (50%-50%), vise à approfondir ces résultats et en particulier à étudier les patrons de sénescence et de sélection sous contrainte thermique dans le cadre d'une fiche action Onema. Ce travail se base sur des expériences de type "common-garden" sur des lignées de poissons élevés dans des conditions chaudes ou froides depuis une dizaine de générations (médakas) et sur des poissons issus d'environnements thermiquement contrastés (Ombles chevaliers des lacs Léman, d'Allos, Pavin, de Constance et de Ste Croix).

[2] **Lisandrina Mari** (2015-2018), INRA Thonon-les-Bains, Université de Savoie, thèse co-encadrée avec E. lasne (INRA Thonon les Bains).
Depuisoctobre 2015, une thèse financée par l'Université de Savoie, étudie l'impact de la température sur la physiologie et les traits d'histoire de vie des stades précoces d'omble chevalier. Ce travail se base sur des expériences de type "common-garden" sur des lignées de poissons issus d'environnements thermiquement contrastés (lacs Léman, d'Allos, Pavin, de Constance et de Ste Croix) mais ayant une origine génétique commune (lac Léman).

[3] **Claire Hemmer-Brepson** (2010-2013), Irstea Aix-en-Provence, Université Aix-Marseille. Claire a exploré les liens température-métabolisme et leurs conséquences en terme de modification des traits d'histoire de vie chez les ectothermes soumis à un réchauffement modéré. Son travail a particulièrement focalisé sur la sensibilité thermique de la balance oxydative (c.-à-d. le rapport entre les dégâts dus aux radicaux-libres (ERO) issus du métabolisme et les défenses mises en place par l'organisme pour les contrer). Deux approches ont été utilisées : (i) une étude de terrain sur deux poissons ubiquistes (*Perca fluviatilis* & *Rutilus rutilus*) et (ii) une expérimentation de laboratoire sur un poisson modèle (*Oryzias latipes*). Les résultats de cette thèse suggèrent un lien entre la balance oxydative et les stratégies de vie adoptées sous des environnements thermiques spatialement et/ou temporellement contrastés. Ainsi, des stratégies de vie de type "*r*" ont été observées dans les environnements les plus chauds (terrain et laboratoire). En effet, la maturité sexuelle semble atteinte plus tôt au chaud mais une mortalité précoce y est observée. Cette mortalité semble liée à un plus fort investissement dans les processus antioxydants, probablement pour contrer la production accrue d'ERO. Il est apparu que les défenses antioxydantes concernaient principalement les cellules germinales, au dépend du soma. Toutefois, certains résultats obtenus sur le terrain nous indiquent que d'autres facteurs environnementaux, d'origine naturelle ou anthropique, peuvent être responsables d'une augmentation du métabolisme, et donc de la production d'ERO, pouvant également jouer sur les stratégies de vie. Enfin, même si les stratégies de vie ne sont pas les mêmes entre individus élevés au chaud et au froid (laboratoire), le succès reproducteur (un proxy de la fitness) semble très similaire entre ces deux groupes soulignant la plasticité des organismes aux variations thermiques modérées.

2.11.2 Jury et comité de pilotage de thèse

[3] Membre du comité de pilotage de la thèse de Florian Holon (Université Montpellier II).
[2] Membre du comité de pilotage de la thèse de Mathieu Chevalier (Université Toulouse III).
[1] Membre du jury de thèse de Clément Tisseuil (Université Paul Sabatier Toulouse III).

2.11.3 Autres encadrements

[14] Boutrif M. (2015). **CDD - IR**, co-encadrement (70%). *Mise en place de méthodes de mesure du métabolisme écosystémique en milieu lentique.*

[13] Marchand C. (2014–2015). **CDD - IE**, co-encadrement (20%). *Mise en place de méthodes de mesure de la dégradation de le matière organique en milieu lentique.*

[12] Rebière D. (2013–2015). **CDD - IE**, co-encadrement (20%). *Mise en place d'un réseau de mesures thermiques en plan d'eau.*

[11] Muret E. (2015). **Master 2**, Université Toulouse III. *Impact de la température sur la balance oxydative et les traits d'histoire de vie des organismes aquatiques à l'échelle multigénérationnelle.*

[10] Richez B. (2013). **Licence 3**, Université Aix-Marseille. *Impact de la température sur la balance oxydative et les traits d'histoire de Oryzias latipes.*

[9] Rondel C. (2011–2012). **CDD - IE**, co-encadrement (30%). *Mise en place d'un réseau de mesure thermique en plan d'eau.*

[8] Dutordoir, S. (2010). **Master 2**, Sciences de l'Univers, Université Pierre et Marie Curie Paris. *Modélisation thermodynamique de la retenue Bimont à l'aide du modèle DYRESM.*

[7] Lefol, E. (2010). **Master 2**, GILE Gestion Intégrée du Littoral et des Écosystèmes, Université de Corse. *Influence de la température et de la compétition interspécifique sur la structure en taille des communautés piscicoles lacustres.*

[6] Schleuter, D. (2008–2009). **Post-doc**, ANR, co-encadrement (80%). *Diversité fonctionnelle, développement d'indicateurs et analyse de patron de variabilité dans les bassin versant européens.*

[5] Roux, M. (2009). **Master 1**, BGAE, Université Montpellier 2.*Structure en taille des populations et communautés piscicoles en milieux lacustres.*
[4] Coudel, M. (2008). **Licence 3**, ENS Paris. *Impacts of global change on functional diversity of stream fish communities.*
[3] Guiserix, M. (2003). **Seconde année d'école d'ingénieur**. Ecole Centrale Lyon. *Notice d'utilisation. Tests d'adéquation sous S-Plus.*
[2] Ferrier, V. (2003). **DU – niveau master 2**, Univ. Clermont-Ferrand. *Détermination d'un stade sensible pour différents âges d'émergents de truite en réponse à une variation de débit.*
[1] Pradel, S. (2002). **DU – niveau master 2**, Université Claude Bernard Lyon I. *Etude de la sensibilité des émergents de truite commune (Salmo trutta L.) à une augmentation de débit.*

2.11.4 Enseignement

- **2009-2014**. Master 1 HSE, Avignon, 4 heures de cours par an. *Risques associés aux hydrosystèmes.*
- **2004**. DEA Science de l'Eau dans l'Environnement Continental, Montpellier, 6 heures de cours. *Introduction à l'hydroécologie.*

2.12 Animation et diffusion de la recherche

2.12.1 organisation et participation à des séminaires de laboratoires, internes ou externes

T. Daufresne (INRA – CEFS), Laetitia Buisson (Univ. Paul Sabatier) et Wendell Haag (USDA) ont été invités pour présenter leurs travaux à l'UR HYAX, de même que P.A. Danis avant son intégration dans le pôle ONEMA. D. Atkinson, et G. Lacroix devraient également donner des interventions très prochainement. Localement nous avons organisé avec F. Massol (lorsqu'il faisait partit de l'UR HYAX) un certain nombre de séminaires internes portant sur la présentation des travaux des membres de l'UR.

2.12.2 organisation de congrès

- **2010**. WISER, Aix-en-Provence, Février. Participation à l'organisation et animation. 3 jours, rayonnement international.
- **2008**. Groupe d'inter-étalonnage européen, Aix-en-Provence, Avril. Participation à l'organisation et animation. 3 jours, rayonnement international.
- **2007**. Aquashift Workshop - Size structure, Kiel, Décembre. Participation à l'organisation et animation du groupe de travail. 15 jours, rayonnement international.

2.12.3 Responsabilités administratives

Je suis depuis 2014 co-directeur du pôle Onema-Irstea.

2.12.4 Rédaction, animation et gestion de projets

Les projets marqué d'un * ont obtenu un financement.

- ***ONEMA – Fiche action 61** 2013-2015. Mise en place d'un réseau de suivi thermique des milieux lacustres. Aide obtenue : 138 k€. Co-porteur (co-rédaction, -animation, -gestion).
- ***ONEMA – Fiche action 29+64** 2013-2015. Mise en place d'indicateur de fonctionnement des écosystèmes. Aide obtenue : de l'ordre de 450 k€. Co-porteur (co-rédaction, -animation, -gestion).

— *__ONEMA – Fiche action 36__ 2015. Impact du réchauffement climatique sur la biodiversité : exemple d'une espèce sentinelle, l'omble chevalier *Salvelinus alpinus*. Aide obtenue : 43 k€. Co-rédaction, co-animation. Porteur : E. Lasne.
— *__OT–Med – DHEMISA__ 2014. Dynamics of heterotrophic microorganisms determined by in situ automated flow cytometry. Aide obtenue : 1 post-doc + 10k€. Co-rédaction, co-animation. Porteur de projet : M. Denis.
— **OT–Med – REDMEC** 2014. Underlying mechanisms of body size decrease in aquatic ectotherms under global warming : phenotypic plasticity vs. genetic adaptation. Aide demandée : 141 k€. Non financé. Co-porteur (ie co-rédaction).
— *__ANR blanc – ARSENIC__ 2014. Adaptation and Resilience of Spatial Ecological Networks to human-Induced Changes. Aide obtenue : 498 k€. Co-rédaction. Porteur de projet : F. Massol.
— **ANR blanc – ClimSize** 2012, 2013, 2014. Temperature-Size Rule : patterns, causes and consequences within the context of global warming. Aide demandée : de l'ordre de 600 k€. Non financé, pré-selectionné en 2014. Porteur de projet (rédaction).
— **ANR blanc – METASpIN** 2013. Modélisation de l'évolution des associations de traits au sein des réseaux d'interaction spatiaux. Aide demandée : 500 k€. Non financé. Co-rédaction. Porteur de projet : F. Massol.
— **NORKLIMA** 2011–2014. PLankton size responses to increased Ocean Temperature (PLOT). Non financé. Co-rédaction. Porteur de projet : Dag Hessen.
— **AO Région PACA** 2011. Sélection naturelle ou plasticité phénotypique ? Etude de la nature et des mécanismes impliqués dans la réduction de la taille des ectothermes avec la température. Aide demandée : 21 k€. Non financé. Co-porteur de projet (co-rédaction avec Claire Hemmer).
— *__HYNES (EDF)__ 2010–2013. Déterminismes physiologiques de la TSR (Temperature-Size Rule). Aide obtenue : 150 k€. Porteur de Projet (rédaction, animation, gestion).
— *__ONEMA – Fiche action 13__ 2011. Synthèse bibliographique sur les indicateurs fonctionnels de qualité des milieux aquatiques lacustres. Aide obtenue : 4 k€. Porteur de Projet (rédaction, animation, gestion).
— *__ONEMA – Fiche action 9/12__ 2010–2011. Mise en place du réseau de mesures thermiques en plan d'eau. Aide obtenue : 48 k€. Porteur de Projet (rédaction, animation, gestion).
— **ANR Jeunes chercheurs – TaGGs** 2010. Thermal and geographic gradients in body size of ectotherms : from cells to communities. Aide demandée : 246 k€HT. Non financé. Porteur de projet (rédaction).
— **Ingénierie écologique** 2009. Modélisation des régimes thermiques des plans d'eau français. Aide demandée : 25 k€. Non financé. Porteur de projet (rédaction).
— *__AOI ECOREV__ 2009. Modélisation des régimes thermiques des plans d'eau français. Aide obtenue : 4 k€. Porteur de projet (rédation, animation, gestion).
— *__AQUAQHIFT__ 2006 – 2007 (14 mois). IFM-GEOMAR, Kiel, Allemagne. Post-doctorat. Animation des travaux de meta-analyses dans le cadre du projet de coordination d'un programme national de recherche portant sur les effets du changement climatique sur les organismes aquatiques (incluant 30 projets de recherches et plus de 100 chercheurs).

2.12.5 Accueil de chercheurs étrangers

— **Alejandro Isla** 2013-2016. Accueil de haut niveau. Financement Irstea. Métabolisme individuel et température, approches expérimentales.
— **Wendell Haag** 2014. 1 mois. Convention d'accueil Irstea – US Forest Service. Étude des relations entre variables environnementales et communautés biologiques sous contraintes anthropiques – Effet du réchauffement climatique sur la croissance des organismes.

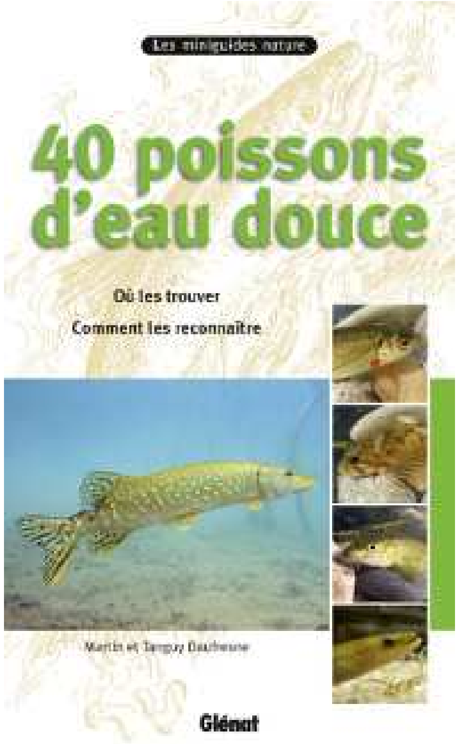
2.12.6 Collaborations

- Internes à l'UR :
  Les travaux relatifs aux effets du changement climatique et des CNPE sur les organismes des fleuves français ont été l'occasion de collaborations avec Irstea Lyon (UR BELY) et avec G. Carrel, aboutissant concrètement à ce jour à l'écriture d'un chapitre d'ouvrage (c.f. partie 2.7 ouvrage n° 1) et d'un article dans Freshwater Biology (c.f. partie 2.2, article n° 21). Mes autres activités ont fait l'objet de collaborations avec F. Massol (aujourd'hui CNRS) et C. Argillier (article n °11 et 14). Je travaille également de manière étroite avec les partenaires Onema du pôle, en particulier J.M. Baudoin sur les aspects relatifs aux indicateurs de fonctionnement des écosystème et avec P.A. Danis concernant l'évaluation des conditions thermiques en plan d'eau (voir partie 2.3, rapports n° 8, 10, 11, 12, 13, 14).
- Externes à l'UR :
  Au niveau national, le travail sur la diversité fonctionnelle a amené à des rapprochements avec l'Université Paul Sabatier de Toulouse (S. Brosse et O. Beauchard, article n° 14). J'ai de plus participé à l'écriture d'un article portant sur les impacts du changement climatique avec G. Grenouillet, L. Buisson et L. Comte (article n° 16). G. Lacroix, E. Thébault, E. Edeline et N. Loeille (IEES Paris), F. Massol (CNRS) sont impliqués dans le projet ARN Arsenic et ont participé à la rédaction du projet ClimSize. Un rapport à été rédigé avec G. Lacroix (n° 9) et ces collaborations devraient déboucher sur des productions scientifiques dans un avenir proche. Différents travaux portant sur l'impact de la température sur la typlologie des réseaux trophiques devraient en particulier être initiés en 2016 dans le cadre du pôle (financement Onema). Les thèses de C. Hemmer et A. Loisel ont été l'occasion de collaborations avec Y. Voituron et C. Romestaing (Univ. Lyon I, article n° 18 et co-rédaction de ClimSize), C. Delattre (EDF) et E. Edeline (IEES Paris). Des projets sont en cours de réalisation (fiche action Onema n°36, communication n° 13) ou d'écriture (prolongement fiche action Onema n° 36) avec E. Lasne (INRA Thonon les bains) et un livre sur l'identification des poissons d'eau douce a été publié avec T. Daufresne (INRA Montpellier, ouvrage n°2). Un article à été co-écrit avec P. Gaudin (INRA St Pée sur Nivelle). Enfin, je garde une certaine proximité avec le personnel d'Irstea Lyon (H. Capra, N. Lamouroux, J. Mouthon en particulier, voir articles n° 1, 2, 3, 8, 12, 13, 20, 21). Dans un passé plus éloigné, j'ai également collaboré avec P. Boët (aujourd'hui Irstea Bordeaux, article n° 7).
  Au niveau international, les travaux relatifs aux structures en taille ont été l'occasion de collaborations avec les laboratoires impliqués dans le projet AQUASHIFT. Dans ce sens, un article a été co-écrit avec U. Sommer et K. Lengfellner (IFM GEOMAR, article n° 10) et un autre avec D.O. Hessen (Univ. Oslo, Norway, article n° 15 et voir article n° 5 pour une collaboration plus ancienne). N. Mason (Landcare Research, New Zealand) et D. Schleuter (Univ. Konstanz) ont participé aux travaux sur la diversité fonctionnelle (article n° 11 et 14). D. Atkinson (Univ. Liverpool, UK) et D. Glazier (Juniata College, USA) ont participés à la thèse de C. Hemmer. A titre plus anecdotique, D. Gravel (Univ Rimouski, Quebec) et L. de Meester (KU Leven, Belgique) ont été impliqués dans la rédaction du projet ANR ClimSize.

2.12.7 Diffusion vers le grand public

- Conférencier invité (sur le thème des impacts écologiques du changement climatique) :
  - 2015. Réalisation de 3 panneaux portant sur les impacts du changement climatique pour l'exposition tournante "Des poissons et des hommes" réalisée par Image & Rivière (20 panneaux).
  - 2012. Les ≪ mardis du climat ≫, Ville d'ANNECY, Annecy.
  - 2009. Les ≪ mardis de l'environnement ≫, fondation d'entreprise Ricard, Paris.
  - Participation à la fête de la science.

  - Intervention à ENSAR (Rennes) en tant que conférencier à un meeting portant sur le changement climatique et la biodiversité organisé dans le cadre d'un travail de groupe d'étudiants de troisième année.
- Ouvrages, films et cédérom pour grand public :
  Daufresne M. and T. Daufresne (2011). 40 poissons d'eau douce. Où les trouver, comment les reconnaître. Editions Glénat, Grenoble, France. 96 pp.



- Interviews, participations à des émissions de radio ou de télévision :
  Mes activités et en particulier l'article publié dans PNAS ont été l'objet de nombreuses communications auprès du grand public. Je ne donne ici que quelques exemples notables : De nombreuses interventions radiophoniques : France Inter (la tête au carré, journaux radiophoniques), Radio Canada (les années lumières, journaux radiophoniques), France info, RFI, RMC, BBC... Des interventions dans la presse ecrite : AFP, Le Monde, L'Est républicain, Der Spiegle, Science Daily, Nature Climate Change.... Un article dans l'Encyclopedia Britannica : Book of the year 2010, en tant que fait marquant de l'année 2010 dans le domaine des sciences de la vie. Une participation à un documentaire sur le réchauffement des rivière produit par lfdbproduction. En cours de réalisation en 2016 pour la chaîne de TV Seasons.

Deuxième partie

Synthèse scientifique et projets

– Le Porto s'écoule sous le soleil estival. Corse, Aout 2015. –

1 Introduction

– Herbier de posidonie. Le soleil apporte aux milieux aquatiques sa lumière mais aussi sa chaleur. La Ciotat, Décembre 2010. –

Il est désormais clairement établi que la température atmosphérique du globe a tendance à se réchauffer. 1983-2012 sont probablement les 30 années les plus chaudes des derniers 1400 ans dans l'hémisphère nord. Le réchauffement de la température moyenne de la planète (calculée sur des données atmosphériques et de la surface des océans) a été de l'ordre de 0.85 °C au cours de la période 1880-2012 (Fig. 9, IPCC 2014), même si il existe une forte hétérogénéité spatiale et temporelle dans les patrons de réchauffement des eaux et de l'atmosphère.

Outre les températures océaniques, ce réchauffement atmosphérique a également impacté la température des écosystèmes aquatiques continentaux (Webb 1996; Straile et al. 2003; Webb et al. 2008; Adrian et al. 2009). Les données de température qui ont pu être récoltées sur les différents cours d'eau sur lesquels nous avons mené nos analyses ont pu confirmer ces patrons. Le Rhône, la Saône et ses afluents, la Seine ou la Loire se sont par exemple réchauffées au cours des 20 à 30 dernières années (Fig. 10, Fig. 11, Daufresne et al. 2004; Mouthon & Daufresne 2006; Daufresne & Boët 2007; Mouthon & Daufresne 2010, 2015; Daufresne et al. 2015). Le climat impacte également les régimes hydrologiques mais les patrons sont plus complexes (Stahl et al. 2010). En Europe par exemple, des tendances négatives des débits moyens ont été observés entre 1962 et 2004 dans les régions les plus méridionales et orientales tandis que ces tendances étaient positives ailleurs. Des tendances à l'augmentation des débits étaient en général observées en hiver dans la plupart des bassins versants alors qu'ils avaient tendance à diminuer au printemps et l'été (Stahl et al. 2010). Dans le cas de la Saône et de ses affluents, nous avons effectivement pu mettre en évidence une diminution des débits moyens annuels sur la période 1977-2011 avec des débits particulièrement bas depuis 2003 (Mouthon & Daufresne 2015).

Concernant les plans d'eau, le réchauffement climatique se fait également ressentir (Straile et al. 2003; Adrian et al. 2009). Cependant, la thermodynamique de ces masses d'eau est très dépendante de facteurs hydromorphologiques (ex : bathymétrie, nombre et régime hydrologique des tributaires, orientation) et climatiques (ex : vent) non thermiques et les relations entre températures de l'air et de l'eau sont moins évidentes. Les températures de surface semblent se réchauffer (Fig. 12, Adrian et al. 2009) mais ces changements peuvent être variables en fonction de la profondeur et de la saison et certaines couches peuvent même montrer des tendances au refroidissement à certaines époques de l'année (Fig. 13, Straile et al. 2003).

En dehors de ce réchauffement graduel, il est très probable que le globe ait été soumis à une augmentation de la fréquence des épisodes climatiques extrêmes (extrêmes thermiques, niveaux océaniques extrêmes ou épisodes pluviométriques extrêmes, IPCC 2014). La fréquence

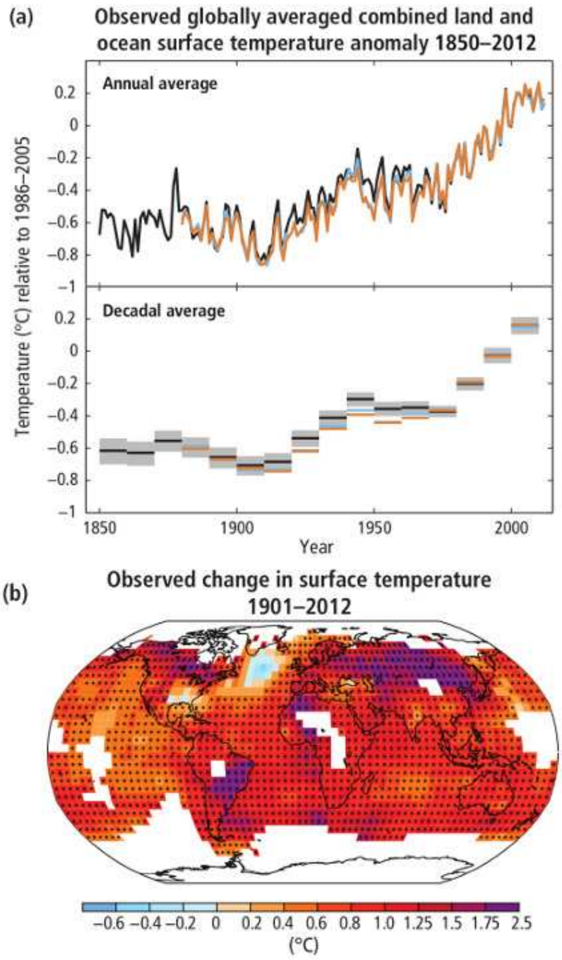


FIGURE 9 – Modifications temporelles et spatiales observées des températures à l'échelle du globe (IPCC 2014).

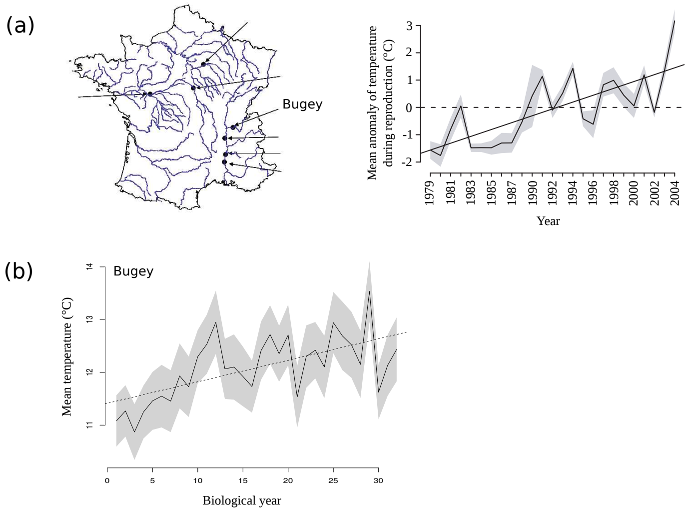


FIGURE 10 – Exemple de réchauffements observés sur la Loire, le Rhône et la Seine. (a) Anomalie moyenne des temperatures de reproduction des cyprinidés (avril-juin) d'après Daufresne & Boët (2007). (b) Température moyenne annuelle à Bugey (d'après Daufresne & Boët 2007; Daufresne et al. 2015). Les années sont calées sur le cycle biologique de la plupart des espèces piscicoles présentes et s'étendent de début juillet à fin juin. L'année biologique "1" désigne la période 01/07/1978-30/06/1979. Les données ont été récoltées par EDF.

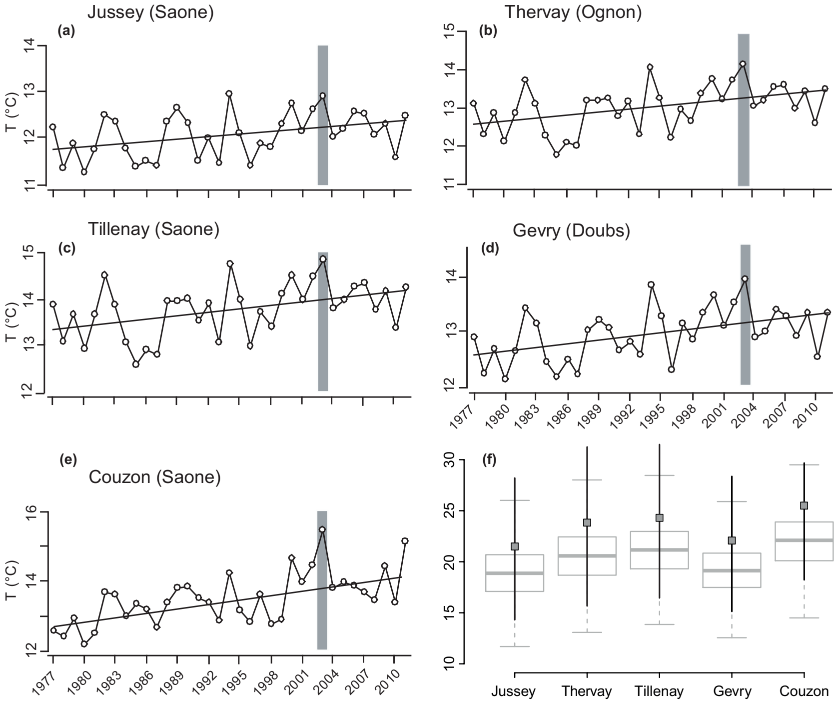


Figure 11 – (a–e) Chroniques des températures moyennes annuelles sur la Saône à Jussey, Tillenay et Couzon et sur ses affluents, l'Ognon à Thervay et le Doubs à Gevry. Les tendances linéaires sont figurées, les axes des ordonnées sont ajustées aux données et l'année de la canicule de 2003 est figurée en gris. (f) Boîtes à moustaches des températures moyennes estivales (juillet-septembre) sur les différents sites entre 1977 et 2011. les limites supérieures et inférieures représentent le premier et le troisième quartile des données. La barre horizontale en gris représente la médiane. Pour chaque boîte, les moustaches s'étendent entre les points le plus extrêmes pour lesquels l'écart entre les valeurs représentent plus de 1.5 fois l'interquartile. L'étendue des valeurs observées en 2003 (ligne verticale en gras) et la médiane de 2003 (carré gris) sont indiquées. Les données ont été modélisées à partir de données de température de l'eau et de l'air fournies par l'Onema et météo-france.

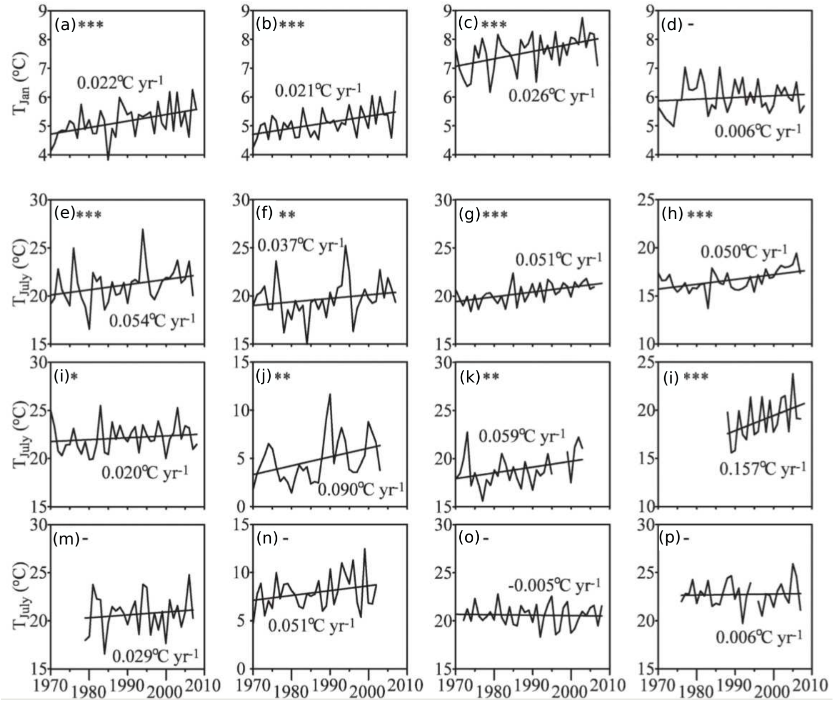


FIGURE 12 – Chroniques de températures moyennes mensuelles de surface depuis 1970 en janvier (T Jan, a–d) et juillet (T July, e–p) dans différents lacs de l'hemisphère nord (d'après Adrian et al. 2009, Fig. 1). (a) et (e) lac de Zürich (Suisse) ; (b) et (f) Lac de Constance (Allemagne, Suisse, Autriche) ; (c) et (g) lac Washington (Washington) ; (d) et (h) lac Tahoe (Californie, Nevada) ; (i) Greifensee (Suisse) ; (j) lac Vättern, bassin de l'Edeskvarva (Suède) ; (k) Lac Mäleren, bassin du Görvaln (Suède) ; (l) Stensjön (Suède) ; (m) Müggelsee (Allemagne) ; (n) Lac Baikal (Russie) ; (o) Lac Champlain (Vermont) ; (p) Blue Chalk Lake (Canada). Pour chaque lac, le niveau de significativité du test de tendance de Mann–Kendall est indiqué par un, deux, ou trois astérisques ($P < 0.1$, $P < 0.05$, and $P < 0.01$, respectivement) ou un tiret (pas de tendance au seuil $P < 0.1$). Le taux de changement moyen de T Jan ou T July est également donné et la tendance linéaire est figurée.

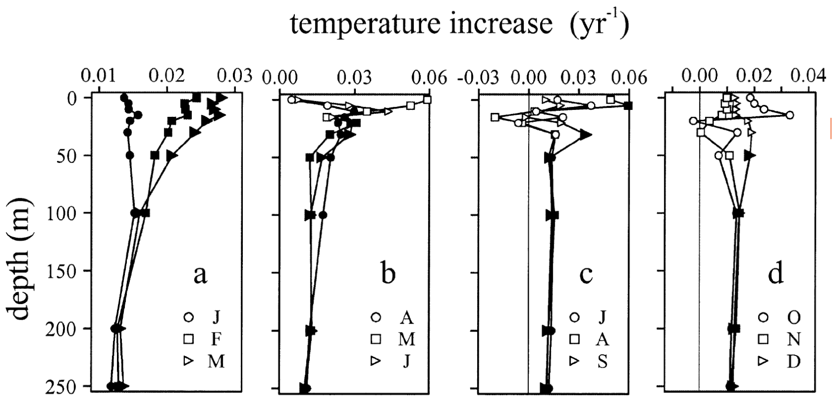


FIGURE 13 – Modification de la température entre 1962 et 1998 sur le lac de Constance (Allemange) en fonction du de la profondeur et du mois (en °C an^{-1}) (d'après Straile et al. 2003, Fig. 4). (a) janvier-mars (b) avril-juin (c) juillet-septembre (d) octobre-decembre. Les symboles pleins indiquent des changements significatifs ($P < 0.05$).

des canicules a par exemple très probablement augmenté en Europe, en Asie et en Australie et augmentera sans doutes dans l'avenir (IPCC 2014).

La variabilité climatique, tant spatiale que temporelle, et à quelque échelle que ce soit, est une force motrice évidente des variables écologiques en général (Begon et al. 2005). Plus spécifiquement, on sait depuis longtemps que les dérives graduelles des conditions environnementales sont supposeées impacter les dynamiques des communautés et des populations de deux manières principales. Les modifications les plus évidentes sont des dérives graduelles des structures des communautés. Ces phénomènes sont à la base des théories des successions écologiques (Hutchinson 1941, 1959, 1961; Wilson 1990; Sommer et al. 1993). L'idée de base est de considérer qu'à un moment *t*, une communauté est constituée d'espèces adaptées à la situation environnementale présente (avec une taux de croissance positif ou nul) et d'espèces adaptées aux conditions passées (avec un taux de croissance négatif ou nul). Ces phénomènes ont été largement démontrés tant théoriquement qu'empiriquement.

Outre ces changements graduels, des modifications plus brutales des variables d'état des populations, communautés ou écosystèmes peuvent être observées quand les conditions environnementales changent de manière brutale ou graduelle. On parle de "regime shits" (Scheffer et al. 2001a). Si ce type de phénomène a surtout été étudié de manière théorique (Scheffer et al. 2001a; van Nes & Scheffer 2004), des observations empiriques en lien avec le réchauffement climatique ont pu être rapportées, en particulier dans les écosystèmes lacustres (Scheffer et al. 2001a,b; Scheffer & Carpenter 2003). Les mécanisme sous-jacent à ces modifications d'états d'équilibres ont été bien vulgarisés par Scheffer et ses collaborateurs (voir Fig. 14).

Si les impacts écologiques des changements environnementaux ont déjà été théoriquement et empiriquement largement étudiés, les études relatives au réchauffement global sont plus récents. Les travaux ont d'abord été essentiellement descriptifs, mais la tendance actuelle consiste à favoriser des approches plus prédictives. Cela sous-entend de mieux connaître les mécanismes sous-jacents aux patrons écologiques observées. Mes travaux s'inscrivent dans ce cadre et ont d'abord porté (i) sur la description des dynamiques de populations & de communautés d'organismes aquatiques face au réchauffement graduel (section 2) et (ii) face à des évênements climatiques extrêmes (section 3). Des premieres analyses de compréhension des interactions entre impacts des contraintes climatiques vs. non climatiques ont été initiées (section 4). Dans un deuxième temps, mes approches se sont plus portées sur la recherche de règles générales d'influence du réchauffement graduel (section 5, section 6). Ce travail a principalement consisté, et consiste encore, à revisiter des règles d'écologie générale sous un angle d'écologie thermique. Ces travaux et leurs contextes sont présentés ici.

2 Dérives climatiques et dérives biologiques

2.1 Introduction

La prise de conscience du réchauffement global et de ses conséquences sur l'environnement au sens large (agriculture, écosystèmes, ressources en eau, glacier, niveau de la mer, activités humaines divers) a commencé à s'imposer au niveau mondial dans les années 1990 (IPCC 1990) et des programmes d'étude de chroniques de données écologiques ainsi que des suivis long-terme spécifiques ont commencé à se mettre en place. Dès lors, les impacts écologiques du réchauffement climatique les plus connus et documentés ont été et sont (i) un avancement des évênements printaniers (modification des phénologies) et (ii) une modification des aires de distribution des espèces vers des altitudes et des latitudes (sud ou nord) élevées (Walther et al. 2002; Parmesan & Yohe 2003; Root et al. 2003). Concernant les écosystèmes aquatiques, si la prise de conscience de l'importance de l'étude des impacts écologiques du réchauffement s'est également imposée dans les années 90 (Regier et al. 1990), les études empiriques montrant ces impacts ont été rares jusque dans le début des années 2000 et le restent quelque peu du fait d'un manque de chroniques long-terme. Dans les eaux courantes continentales, une inertie particulière à ce type d'étude tenait au

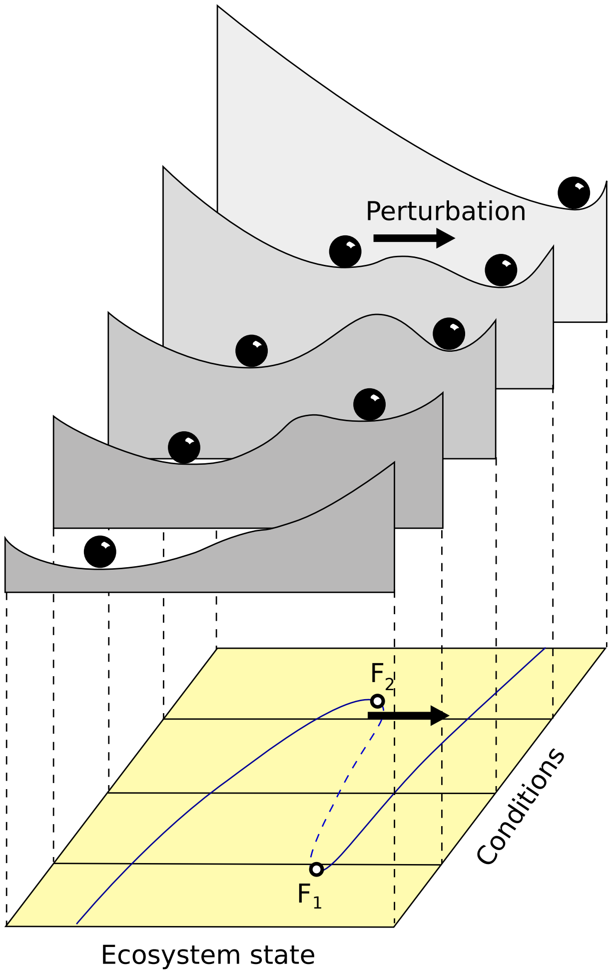


FIGURE 14 – Conséquences des états stables alternatifs en écologie (d'après Scheffer et al. 2001a; van Nes & Scheffer 2004). Un paysage de stabilité est utilisé pour illustrer ces conséquences. Les vallées représentent des bassins d'attraction, c'est à dire les états qui vont avoir tendance à retourner vers l'état d'équilibre au fond de la vallée. Le plan du bas représente les états d'équilibre du système en fonction des conditions environnementales. Quand un systèmes est dans un état de la courbe de gauche et que les conditions environnmentales changent (y compris graduellement) suffisamment pour atteindre un seuil (point de bifurcation *F2*) un changement d'état drastique vers la courbe de droite s'opère brutalement. Pour revenir à l'état d'équilibre initial, il n'est pas suffisant de restaurer l'environnement aux conditions *F2*. Il est nécessaire de les restaurer au niveau du point de birfurcation *F1*. On appelle cette “résistance” au retour à l'équilibre initial “hystéresis”. Notez que dans les conditions pour lesquelles il existe des états stables alternatifs (c-à-d entre *F1* et *F2*) une perturbation ponctuelle suffisamment sévère peut entraîner un passage d'un état à l'autre. Ces transitions sont d'autant plus faciles que les bassins d'attractions (la résilience) sont petits/peu profonds.

fait que les contraintes majeures portant sur les populations et communautés étaient considérées comme étant principalement hydrologiques (Poff & Ward 1989; Poff et al. 1997; Poff 1997; Lytle & Poff 2004), ce qui est très certainement pertinent dans un environnement thermiquement stable sur le long-terme. C'est dans ce contexte que j'ai commencé à mener mes premières analyses de chroniques de données biologiques récoltées sur les grandes rivières et grands fleuves français. Ces travaux ont consisté (i) à étudier d'éventuelles dérives des structures des communautés de poissons et de macro-invertébrés sous l'influence du réchauffement climatique, (ii) à étudier l'impact d'évènements climatiques extrêmes et (iii) à explorer les interactions entre pressions environnementales climatiques et non climatiques.

2.2 Modification des structures de communautés

Publications associées : Daufresne et al. (2004); Daufresne & Boët (2007).

2.2.1 Cas du Rhône à Bugey

Des données piscicoles et de macro-invertébrés ont été récoltées depuis 1979 dans le cadre du suivi des impacts des rejets thermiques du centre nucléaire de production électrique (CNPE) de Bugey sur le Rhône. Ces données constituaient une occasion unique d'étudier l'impact d'un réchauffement déjà visible au début des années 2000 sur ce milieu (Fig. 10b). L'utilisation de techniques d'ordination “classiques” (analyse en composantes principales, ACP ; analyse factorielle des correspondances, AFC) et de méthodes d'analyse de tendances prenant en compte les autocorrelations temporelles (test de Mann-Kendall modifié ; Hamed & Rao 1998, encadré 2.2.1) ont mis en évidence des changements graduels de structures des communautés.

Encadré 2.2.1 : test de tendance

Pour une série d'observations $X = x_1, x_2, ..., x_n$, le test de Mann-Kendall consiste à calculer la statistique S :

$$S = \sum_{i<j} sgn(x_j - x_i)$$

où

$$sgn(x_j - x_i) = \begin{cases} +1 & \text{si} \quad x_i < x_j \\ 0 & \text{si} \quad x_i = x_j \\ -1 & \text{si} \quad x_i > x_j \end{cases}$$

La significativité de la tendance est testé en comparant la statistique $Z = S/[var(S)]^{0.5}$, où $var(S)$ est la variance de S, à une loi normale. D'après (Kendall 1955), $var(S)$ est donnée par :

$$var(S) = \frac{n(n-1)(2n+5)}{18}$$

Hamed & Rao (1998) ont montré théoriquement que les autocorrélations temporelles modifiaient la variance de S. Les auteurs proposent d'appliquer une correction au calcul de var(S) prenant en compte ces autocorrélations. Pour cela, une estimation non paramétrique de la tendance Sen (décrite par 1968) est d'abord soustraite de la série X et les autocorrélations entre les rangs de cette nouvelle série sont calculées. Les coefficients d'autocorrélation significativement différents de zéro ($\rho_s(i)$ au pas de temps i) sont alors utilisés pour calculer la variance corrigée de S, $V^*(S)$:

$$V^*(S) = var(S)Cor$$

où Cor est la correction due aux autocorrélations

$$Cor = 1 + \frac{2}{n(n-1)(n-2)} \sum_{i=1}^{n-1} (n-i)(n-i-1)(n-i-2)\rho_s(i)$$

Concernant les poissons, le chevaine (*Squalius cephalus*) et le barbeau (*Barbus barbus*) ont vu leurs effectifs relatifs augmenter au cours de la période d'étude (1979-1999) tandis que la vandoise (*Leusciscus leusciscus*) et l'ablette (*Alburnus alburnus*) montraient des tendances inverses (Fig. 15). Ces changements semblent traduire une domination graduelle du peuplement par des espèces méridionales. En effet, les espèces sont assez bien ordonnées sur F2 suivant leur indice de “méridionalité” ($x = L_{Bugey} - L_{Inf}/L_{sup} - L_{Bugey}$, où L_{Bugey}, L_{Inf} et L_{sup} sont les latitudes (°N) du site d'étude, de la limite sud d'aire de répartition de l'espèce et de la limite nord d'aire de répartition, Daufresne et al. 2004). Le même type de résultat a été trouvé pour les invertébrés du site. Des analyses de corrélation adaptées aux séries temporelles ont pu montrer des liens interannuels significatifs avec des variables thermiques.

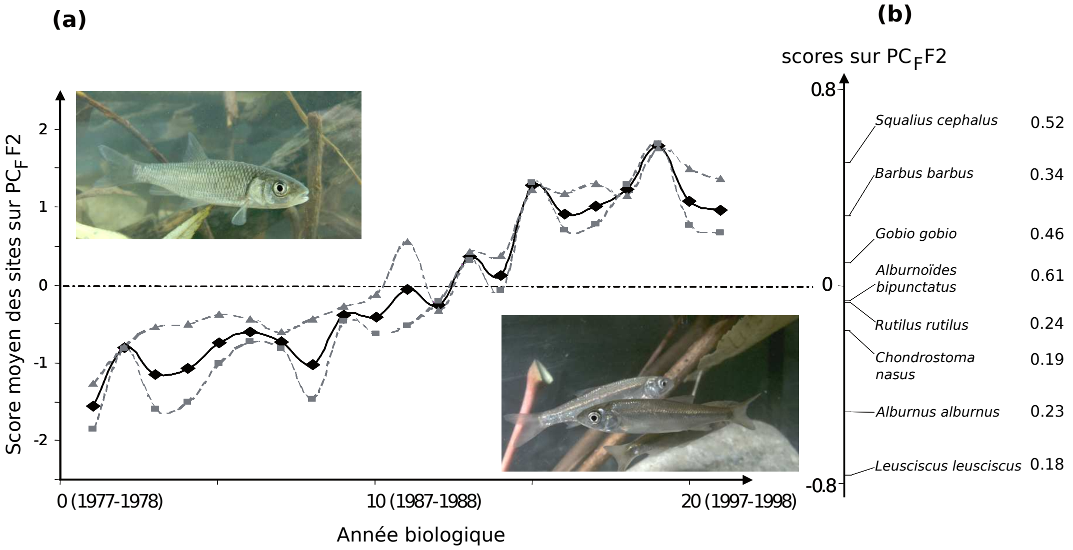


FIGURE 15 – Modifications de la structure du peuplement de poissons à Bugey (d'après Daufresne et al. 2004). (a) Les changements sont résumés par les coordonnées factorielles des stations d'échantillonnage sur l'axe F2 d'une ACP réalisée sur des données moyennes de capture par unité d'effort des espèces (CPUE, transformées en $ln(x+1)$) par année biologique et par site. les coordonnées sont moyennées pour toutes les stations (ligne noire), pour les stations non influencées (triangles, ligne pointillée grise) ou influencées (carrés, ligne pointillée grise) par le rejet du CNPE. Les années biologiques sont calées sur le cycle biologique des espèces principales et s'étendent du premier juillet au 30 juin. (b) La contribution des espèces principales sur F2 et leur indice de “méridionalité” sont donnés. Les chroniques de CPUE sont visibles dans Daufresne et al. (2004). Photos : chevaine et vandoise en haut et en bas respectivement.

Il est intéressant de noter que l'analyse de ces données actualisées révèle des successions dans les patrons de dominations du peuplement par certaines espèces, conformément aux hypothèses d'Hutchinson (Fig. 16). Les populations de vandoise et d'ablette restent à des niveaux d'abondances relatives très bas tandis que celles de barbeau et de chevaine tendent à décroitre, au profit du spirlin (*Alburnoïdes bipunctatus*) qui possède l'indice de méridionalité le plus haut de ces espèces (Fig. 15). Si cette domination des communautés par des espèces méridionales ayant une certaine affinité pour les températures douces parait aujourd'hui trivial, cela n'était pas encore le cas au début des années 2000. Dans ce sens, l'article de Daufresne et al. (2004) était relativement précurseur, au moins concernant les milieux d'eau courante, ce qui lui vaut encore aujourd'hui un assez bon taux de citation (8.69 citations/an au 8 septembre 2017).

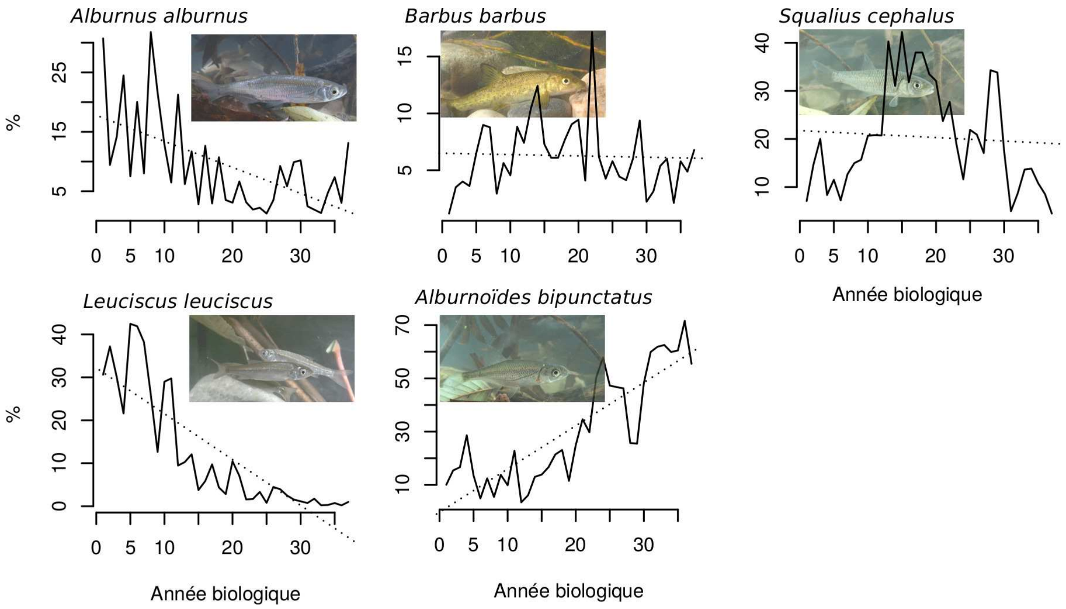


FIGURE 16 – Chroniques de pourcentages d'abondance des espèces principales pêchées à Bugey. Les années biologiques sont calées sur le cycle biologique des espèces principales et s'étendent du premier juillet au 30 juin.

2.2.2 Cas des Grands fleuves français

L'étude sur le site de Bugey a nécessité de discuter de manière approfondie l'impact des facteurs confondants (ex : mise en place de barrages amonts et chasses d'eau associées, interactions trophiques, qualité d'eau) pour essayer de conforter l'hypothèse d'un impact climatique sur les patrons écologiques observés (voir Daufresne et al. 2004). Naturellement, le moyen le plus efficace pour conforter cette hypothèse a été de regarder, à l'échelle nationale, les modifications de structures de communautés sur un ensemble de sites ayant une histoire climatique globale similaire mais subissant par ailleurs des pressions anthropiques non climatiques variées (Daufresne & Boët 2007). Nous avons utilisés des données récoltées sur 24 stations d'échantillonage réparties dans 7 sites d'études (Fig. 17).

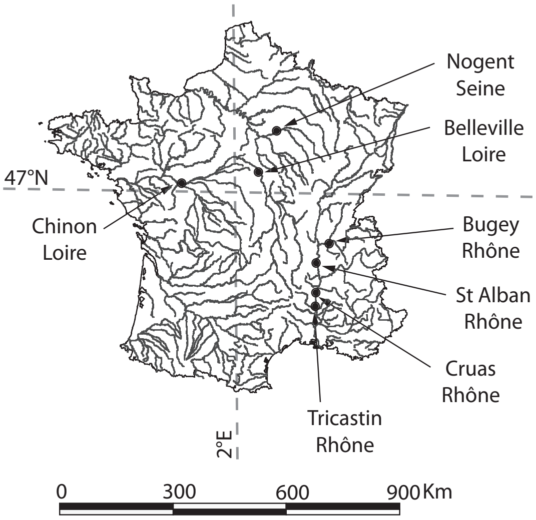


FIGURE 17 – Localisation des sites de suivi à long terme des populations de poissons sur les grands fleuves français utilisés dans Daufresne & Boët (2007).

Nous avons étudié les tendances d'un ensemble de variables biologiques dont (i) la proportion d'espèces à tendance thermophile et (ii) la proportion d'espèces méridionales (dont l'appartenance était basée sur l'indice de méridionalité x). La significativité globale des tendances était évaluée par des techniques de méta-analyse (encadré 2.2.2).

Encadré 2.2.2 : Méta-analyse

La réalisation d'une méta-analyse consiste à évaluer la significativité globale d'une statistique calculée sur plusieurs individus ("effect size" ou taille d'effet) et éventuellement un effet de groupe. La réalisation d'une méta-analyse avec pondération par la variance et utilisation d'un modèle mixte se déroule selon différentes étapes décrites en détails d'un point de vue théorique par Gurevitch & Hedges (1993) et les références associées à leur article. Par soucis d'homogénéité avec les notations utilisées par Gurevitch & Hedges (1993), nous noterons ici d_{ij} les effect sizes du groupe i et individus j et w_{ij} leurs pondérations associées (c.-a-d. l'inverse de leur variance). D'un point de vue pratique, il convient dans un premier temps de calculer les effect sizes moyens par groupes pour le modèle fixe selon :

$$d_{i+} = \frac{\sum_{j=1}^{k_i} w_{ij} d_{ij}}{\sum_{j=1}^{k_i} w_{ij}} \tag{1}$$

et leurs variances

$$s^2(d_{i+}) = \frac{1}{\sum_{j=1}^{k_i} w_{ij}} \tag{2}$$

où k_i est égal au nombre d'effect sizes dans le groupe i. Nous calculons ensuite une homogénéité intra-groupe Q_w de l'analyse à effets fixes selon :

$$Q_w = \sum_{i=1}^{m} \sum_{j=1}^{k_i} w_{ij} (d_{ij} - d_{i+})^2$$

où m est le nombre de groupes. Ensuite, nous calculons pour chaque groupe i une constante c_i donnée par :

$$c_i = \sum_{j=1}^{k_i} w_{ij} - \frac{\sum_{j=1}^{k_i} w_{ij}^2}{\sum_{j=1}^{k_i} w_{ij}}$$

Nous calculons alors une estimation de la variance intra-groupe totale σ^2_{pooled} donnée par :

$$\sigma^2_{pooled} = \frac{Q_w - \sum_{i=1}^{m} (k_i - 1)}{\sum_{i=1}^{m} c_i}$$

qui nous permet de calculer les variances inconditionnelles du modèle mixte, et donc de nouveaux poids w^*_{ij} selon :

$$w^*_{ij} = \frac{1}{\frac{1}{w_{ij}} + \sigma^2_{pooled}}$$

On peut alors calculer les nouveaux effect sizes moyens par groupes (d^*_{i+}) et leurs variances ($s^2(d^*_{i+}) = \frac{1}{w^*_{i+}}$) selon les équations (1) et (2) mais en utilisant les nouveaux poids w^*_{ij}. L'effet des groupes sur les effect sizes est évalué en calculant l'homogénéité inter-groupe Q^*_b selon :

$$Q^*_b = \sum_{i=1}^{m} w^*_{i+} d^*_{i+} - \frac{(\sum_{i=1}^{m} w^*_{i+} d^*_{i+})^2}{\sum_{i=1}^{m} w^*_{i+}}$$

et qui suit une loi de χ^2 à $(m-1)$ degrés de liberté. Si Q^*_b est significatif, c'est à dire si les effect sizes varient plus entre les groupes qu'espéré par hasard, on interprète les résultats en analysant les effect sizes moyens par groupe et leurs intervalles de confiance à 95% ($d^*_{i+} \pm 1.96\sqrt{s^2(d^*_{i+})}$). Si en revanche Q^*_b n'est pas significatif on interprète alors les résultats en analysant l'effect size moyen global d^*_{++} calculé selon :

$$d^*_{++} = \frac{\sum_{i=1}^{m} w^*_{i+} d^*_{i+}}{\sum_{i=1}^{m} w^*_{i+}}$$

et sa variance

$$s^2(d^*_{++}) = \frac{1}{\sum_{i=1}^{m} w^*_{i+}}$$

Dans tous les cas, on considère comme significatif (c.-à-d. significativement non nul au risque de 5%) un effect size dont l'intervalle de confiance à 95% n'inclue pas zéro.

L'étude a confirmé une tendance significative à l'augmentation des proportions d'espèces à tendance thermophile sur les sites d'étude (Fig. 18). Concernant les espèces méridionales, nous avons pu montrer que leurs proportions avaient tendance à augmenter sur les stations les plus septentrionales mais pas sur les stations méridionales. Une des hypothèses pour expliquer un tel phénomène est de considérer que la mer méditerranée est une barrière naturelle à la migration d'espèces méridionales vers des plus hautes latitudes. Il convient toutefois de garder à l'esprit que ces stations étaient également localisées le long des aménagements hydroélectriques du Rhône (voir section 4).

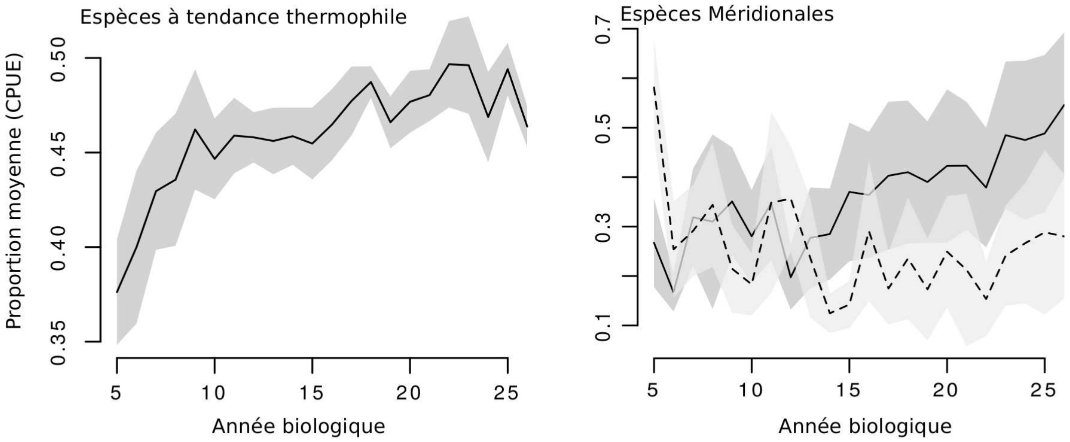


FIGURE 18 – Modification des proportions moyennes d'espèces à tendance thermophile et méridionales dans les grands fleuves français dans un contexte de réchauffement climatique (d'après Daufresne & Boët 2007). Les intervalles de confiance à 95% sont figurés en gris. Pour les espèces méridionales, les modifications sont figurées par une ligne pointillée sur les stations les plus méridionales (et localisées le long d'ouvrages hydroélectriques) et par une ligne continue sur les autres.

2.3 Impact sur la diversité spécifique et fonctionnelle

Publications associées : Daufresne & Boët (2007) ; Daufresne *et al.* (in prep).

Outre des modifications des structures des communautés, les successions écologiques associées aux modifications graduelles des conditions environnementales ont également des impacts sur la diversité biologique (Hutchinson 1941; Connell 1978; Wilson 1990). Si il n'est ni trop rapide ni trop lent, le changement climatique va permettre la co-existence d'espèces adaptées à des environnements différents mais qui peuvent être en compétition pour la même ressource. Il existe en ce sens des similitudes entre la théorie des perturbations intermédiaires (ou Intermediate disturbance hypothesis, IDH) et l'hypothèse de changement climatique graduel (Gradual Climate Change, GCC (Connell 1978; Wilson 1990)). Cette co-existence est transitoire mais permet d'avoir une biodiversité supérieure à celle d'un état stable d'un point de vue environnemental. Dans une situation où le climat commence à changer, on devrait donc noter des augmentations de biodiversité, au moins concernant les indices liés à la richesse spécifique. Pour ces derniers, l'augmentation devrait être d'autant plus marquée qu'il est toujours plus rapide de "gagner" une espèce que de la "perdre". D'un point de vue des stratégies écologiques, il est également probable que les espèces aux stratégie de type “K” (relativement à la communauté en place) soient défavorisées au profit des espèces de type “r” (Connell 1978).

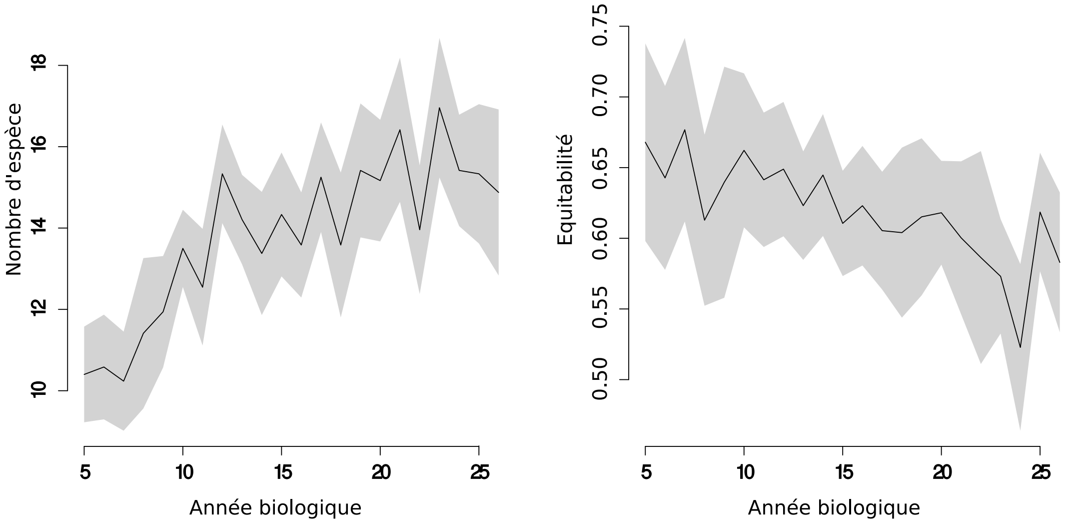


Figure 19 – Modification de la richesse spécifique et de l’équitabilité de Shannon dans les grands fleuves français dans un contexte de réchauffement climatique (d’après Daufresne & Boët 2007). Les intervalles de confiance à 95% sont figurés en gris.

En ce qui concerne les communautés de poissons, l’augmentation de la richesse spécifique a été confirmée par l’étude de Daufresne & Boët (2007) sur les grands fleuves français (Fig. 19). Ce phénomène a par ailleurs depuis été observé en milieu marin (Hiddink & ter Hofstede 2008) et modélisé sur les cours d’eau français (Buisson & Grenouillet 2009). Une étude des tendances de la richesse et de l’équitabilité fonctionnelle sur les communautés de poissons étudiées par Daufresne & Boët (2007) a été réalisées et donne des résultats similaires. Pour ce faire, nous avons utilisé des traits morphologiques reflétant (i) des comportements alimentaires et (ii) d’utilisation d’habitat et (iii) des traits liés aux stratégies de reproduction. Pour chacun de ces axes de niche (nutrition, habitat, reproduction) la richesse et l’équitabilité fonctionnelle ont été calculées pour chaque année et chaque station d’après Schleuter et al. (2010), en utilisant l’indice FRI_s pour la richesse et l’indice multi-dimensionnel FE_m (Villeger et al. 2008) pour l’équitabilité. Pour une année et une station, FRI_s représente l’étendue des valeurs de traits observés dans la communauté relativement à l’étendue maximum qui peut être observée quand toutes les années sont considérées. FE_m utilise la distance euclidienne, pondérée par l’abondance, entre toutes les paires d’espèces pour calculer le réseau de taille minimum qui lie toutes les espèces dans l’espace multidimensionnel des traits. L’indice mesure ensuite l’homogénéité des longueurs des branches du réseau. Une haute valeur de FE_m signifie que les valeurs de traits et leurs abondances sont équitablement distribuées dans la communauté. Une méta-analyse des statistiques de tendances calculées sur les valeurs de FRI_s et FE_m sur les stations d’échantillonnage de Daufresne & Boët (2007) a montré des augmentations des richesses fonctionnelles mais des diminutions des équitabilités fonctionnelles pour les 3 axes de niche (voir Fig. 20A pour l’exemple de l’axe reproductif).

Outre ces modifications de diversité spécifique et fonctionnelle, nous avons pu montrer que les structures fonctionnelles avaient aussi tendance à changer. Les modifications sur les axes de nutrition et d’habitat tendent à montrer une domination graduelle du peuplement par des espèces assez généralistes, avec des tendances à la décroissance des espèces aux stratégies extrêmes. Ce dernier point a été étudié à l’aide d’un indice de variance fonctionnelle FD_Q qui est l’entropie quadratique de Rao (Rao 1982). Cet indice est basé sur l’indice de diversité de Simpson (Simpson 1949) et pondère la distance entre paires d’espèces dans l’espace des traits par le produit de leurs abondances relatives. Une faible valeur de FD_Q signifie que la variabilité des valeurs

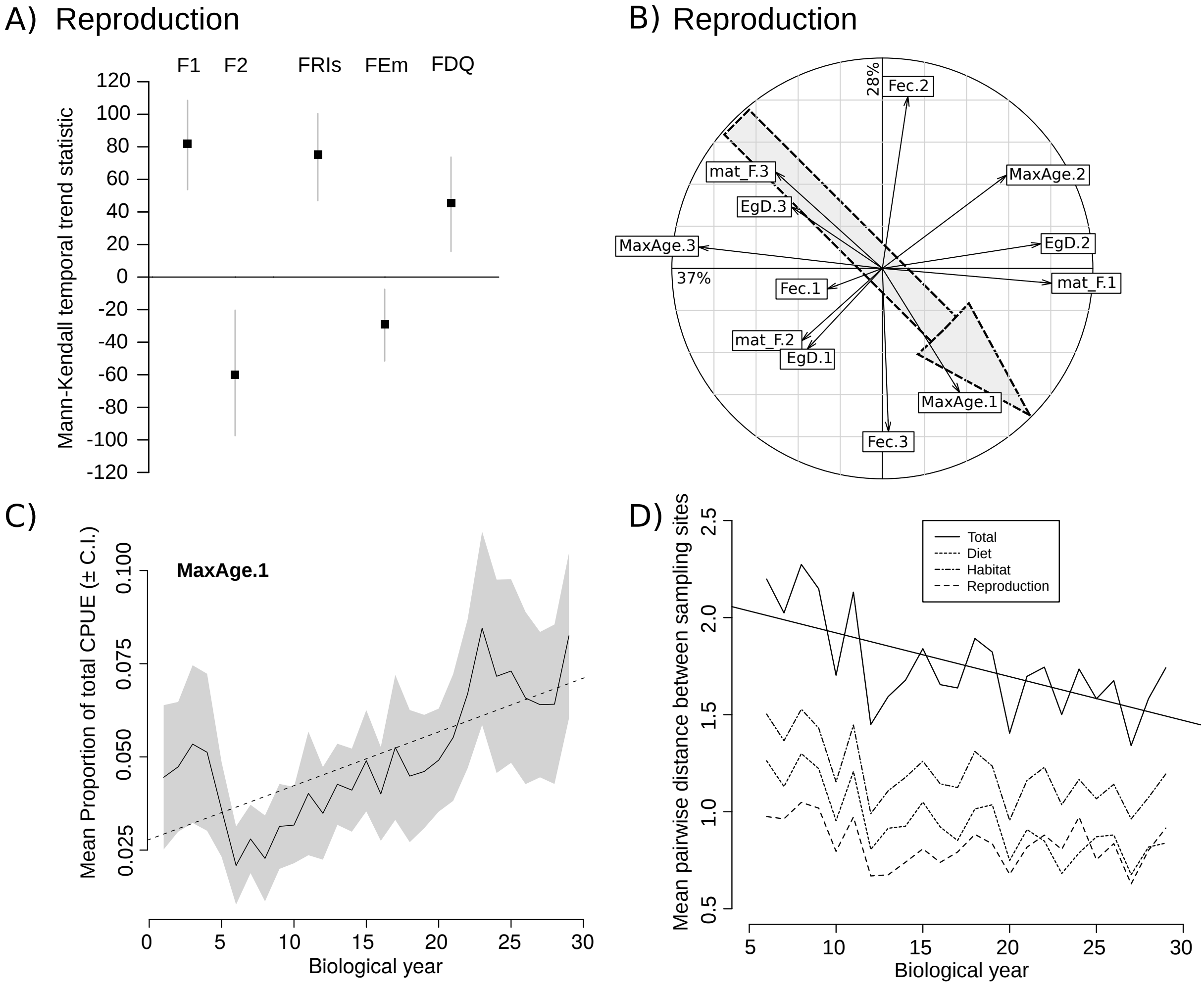


Figure 20 – Modification de la structure et de la diversité fonctionnelle des communautés de poissons des grands fleuves français dans un contexte de réchauffement climatique. A) Exemple de taille d'effet moyen des statistiques de tendance pour des variables calculées sur les traits reproductifs. Les carrés représentent des moyennes pondérées par la variance. Les barres grises représentent les intervalles de confiance à 95%. Un effet dont l'intervalle de confiance n'inclu pas 0 est considéré comme significatif au seuil de 5%. $F1$, $F2$ représentent les coordonnées factorielles des stations d'une ACP intra-sites réalisée sur des données de CPUE par modalité de traits. FRI_s est un indice de richesse fonctionnelle, FE_m un indice d'équitabilité fonctionnelle, FD_Q un indice de divergence fonctionnelle. Le signe des valeurs indique la direction des tendances (positif pour une croissance et négative pour une diminution). Des résultats similaires ont été observés pour les autres axes de niche (nutrition, habitat) B) Projection des coordonnées factorielles des modalités de trait sur le plan $F1$x$F2$ de l'ACP réalisée sur les données de reproduction. Chaque modalité de trait correspond à un tierstile des valeurs observées pour l'ensemble des espèces. EgD : diamètre des oeufs, Fec : fécondité absolue, $MaxAge$: longévité, mat_F : âge à maturité. La flèche représente la direction des changements temporels observés en A) (un accroissement des coordonnée des stations sur $F1$ et une diminution sur $F2$). C) Exemple de modification des proportions moyennes des espèces ayant les plus faibles longévités. D) Modification des distances euclidiennes moyennes entre paires de stations calculées sur les matrices des CPUE par modalité de trait (totaux ou par axe de niche) pour les sites ayant le plus d'années biologiques communes (Bugey, Cruas et Tricastin).

des traits dans une communauté est faible (ex : un groupe de valeurs intermédiaires et peu d'extrêmes). Une analyse des tendances de FD_Q sur nos stations a montré une décroissance marginalement significative pour les axes de nutrition et d'habitat, soulignant la domination graduelle de la communauté par des espèces aux stratégies intermédiaires. Concernant l'axe de reproduction, les stratégies de type "r", ou plus exactement les espèces ayant des durées de vie courtes, des maturités précoces et des fécondités élevées semblent également être favorisées par le réchauffement (Fig. 20B). La domination graduelle du peuplement par ces stratégies "extrêmes" tend à faire augmenter la divergence fonctionnelle FD_Q sur cet axe (Fig. reffig :CCDFA). Il est interessant de noter que ces changements, de même que ceux associés aux axes de nutrition et d'habitat, tendent à homogénéiser la structure fonctionnelle de la communauté comme en témoigne la diminution des distances euclidiennes entre les stations dans l'espace des traits au cours du temps (Fig. 20D). Ces patrons confirment les impacts observés sur d'autres écosystèmes marins ou terrestres (Munday 2004; Clavel et al. 2011).

Notez que cet article a été soumis à la revue Ecology en 2012 et a recu deux évaluations très positives, les référés ayant explicitement donné un avis favorable à sa publication. Malheureusement, l'éditeur à refusé cette publication pour des raisons qui restent relativement obscures. Cette HDR me donne l'occasion de présenter ces résultats à défaut de les publier, au moins pour l'instant.

3 Impact des évênements climatiques extrêmes

– Le Rhône au barrage de Valbrègues, 9 octobre 1993. Le pic de crue est pratiquement atteint (9800 $m^3.s^{-1}$). © Georges Carrel. –

Le changement climatique s'exprime principalement par des changements environnementaux graduels. Ce type de perturbation est dénommé "ramp disturbance" (Lake 2000) et se distingue des perturbations d'intensité constante ("press disturbances") ou ponctuelles ("pulse disturbances") par une intensité croissante. Si cet aspect du changement climatique est le plus documenté actuellement, on sait qu'il devrait être accompagné d'une augmentation de la fréquence des évênements climatiques extrêmes (IPCC 2012, 2014). Les organismes se trouvent donc soumis à deux régimes de pressions distincts l'un de type "ramp" et l'autre de type "press". On sait, en particulier depuis les travaux de Scheffer et collaborateurs que ces pressions climatiques peuvent, individuellement, entrainer des changement brutaux dans la structure des écosystèmes ("regime shifts", Fig. 14). Leur synergie pourrait diminuer d'autant plus la résilience des états d'équilibre originels et donc (i) augmenter les occurences de shifts de structure et (ii) si ces shifts se produisent, compliquer les possibilités de retour à l'équilibre initial (hystéresis). Je présente ici des travaux qui ont consisté à étudier la réponse des structures de communautés à des évênements climatiques extrêmes (crues, canicules) dans le cadre du réchauffement climatique. Ces travaux ont porté sur des chroniques long-terme de données de macroinvertébrés du Rhône et de la Saône et de ses affluents. Nous nous sommes attaché à étudier la dérive des structures de communauté à la fois graduelles et brutales (Mouthon & Daufresne 2006; Daufresne et al. 2007) et tenté, pour les mollusques de la Saône, d'étudier la résilience de ces communautés aux évênements extrêmes (Mouthon & Daufresne 2015).

3.1 Canicule de 2003 et mollusques du bassin de la Saône

Publications associées : Mouthon & Daufresne (2006, 2015)

FIGURE 21 – Site d'étude et localisation des stations d'échantillonnage de mollusques (M) et des stations de mesure du débit (Q), de la température de l'eau (T_w) et de la physico-chimie de l'eau (PC).

Un des évènements climatiques majeurs récent en Europe est la canicule de 2003 (Schar et al. 2004; Stott et al. 2004; Trigo et al. 2005; Twardosz & Kossowska-Cezak 2013). Des canicules de cette envergure sont extrêmement rares, se produisant seulement une fois toutes les centaines de milliers ou millions d'années (Schar et al. 2004). Pourtant, les études portant sur les conséquences écologiques de cette évènement sont assez rares, en particulier en milieu aquatique (voir Mouthon & Daufresne 2006, 2015). Dans ce cadre, les données de mollusques aquatiques récoltées à l'échelle mensuelle par J. Mouthon (Isrtea Lyon) sur la Saône à Lyon représentent une occasion unique d'étudier l'impact de cette canicule et cela d'autant plus que J. Mouthon a également pu récolter plus ponctuellement des données sur les affluents principaux de la Saône avant et après la canicule (Fig. 21).

Globalement, les stations présentent sur la période 1977-2011 des diminutions de leurs débits moyens annuels tandis que leurs températures moyennes annuelles ont eu tendance à augmenter (Mouthon & Daufresne 2006, 2015). Cette augmentation des températures a cependant été principalement du à la tendance sur la période 1977-2003, avec une pose dans le réchauffement entre 2003 et 2011, un phénomène plus largement observé à l'échelle planétaire (IPCC 2014). Pour toutes les stations, l'année 2003 présente les débits les plus bas et les températures les plus chaudes, les valeurs sortant très clairement des distributions des données des autres années. Les années 2010 et 2008 sont relativement fraiches, tandis que les débits de 2006, 2007 et 2010 sont assez comparables aux situations initiales.

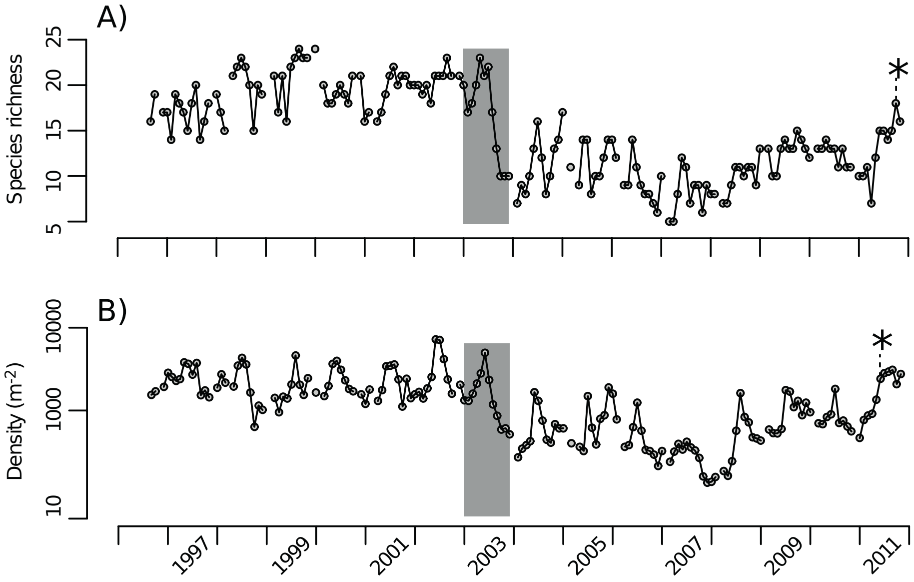


FIGURE 22 – Chroniques mensuelles de la richesse spécifique (A) et de la densité (B) des mollusques de la Saône à Lyon. La canicule de 2003 est figurée en gris. Les dates de retour aux situations initiales sont indiquées par les étoiles. Pour identifier ces dates, nous avons regroupé les données en deux groupes distincts avant/après canicule et avons réattribués les données les plus actuelles au groupe pré-canicule pas à pas (du plus ancien au plus récent) jusqu'à optimiser la variance inter-groupe.

Concernant la communauté de mollusques de la Saône à Lyon, il apparaît très clairement que la canicule a très fortement diminué la richesse spécifique et la densité totale et ce pendant près de 10 ans (Fig. 22). La structure de la communauté a aussi été profondément changée comme l'atteste les chroniques des coordonnées factorielles des échantillons du le plan F1xF2 d'une ACP réalisée sur les données (Fig 23). Les modifications de structure de la communauté se traduisent par une première dérive pré-canicule, où des espèces telles que *Corbicula fluminea* ou *Physa acuta* ont vu leurs densités diminuer au profit d'espèces telles que *Valvata piscinalis* ou *Musculium lacustre* (Fig 23, Fig 24). La canicule est particulièrement néfaste pour ces dernières espèces mais aussi pour d'autres telles que *Potamopyrgus antipodarum*. Elle est en revanche très ponctuellement favorable à *Dreissena polymorpha*. S'en suit une nouvelle dérive qui voit certaines espèces, comme *Potamopyrgus antipodarum* ou *Physa acuta* retrouver des densités comparables à celles de la fin des années 90, alors que *Dreissena polymorpha* tend à marquer le pas. Ces modifications ne traduisent en revanche pas un retour à l'équilibre initial, certaines espèces ne parvenant pas à recoloniser le milieu (ex *Valvata piscinalis* ou *Musculium lacustre*, Fig 24). Même si il existe encore un écart aux conditions originelles, il est intéressant de noter que les années plus fraiches et humides des années 2007-2011 tendent à être bénéfiques à la résilience de la communauté (Fig 23).

Les analyses réalisées sur les données de Lyon (des ANOVA prenant en compte les autocor-

Tableau 1 – Date (année ou mois et année en fonction de la fréquence d'échantillonnage) à laquelle un retour à l'état initial a été observé suite à la canicule pour la densité totale de mollusques, la richesse spécifique et la structure de communauté.

			Structure de communauté	
Station (rivière)	Densité	Richesse spécifique	F1	F2
Port/Saône	2008	Pas de retour	2008	Pas de shift/Chang. graduel
Gray (Saône)	2008	2009	2008	Pas de retour
Pontailler/Saône	2009	Pas de retour	2008	pas de retour
Allériot (Saône)	2011*	2010	2006*	pas de retour
Lyon (Saône)	06/2011	10/2011	06/2011	Pas de shift/Chang. graduel
Broye-les-Pesmes (Ognon)	2008	2008	2008	Pas de retour
Saunière (Doubs)	2005	2004	2004	Pas de shift/Chang. graduel

relations temporelles, voir Mouthon & Daufresne 2015, pour plus de détails) n'ont pas pu être réalisées sur les autres stations du fait du manque d'échantillons. Les patrons de modification des structures des communautés ont donc été appréhendés de manière très descriptive en mettant simplement en oeuvre des ACP. Néanmoins, il apparaît que la canicule a eu un impact négatif sur la densité totale de mollusques et la diversité spécifique de toutes les autres stations. Les structures des communautés étaient également différentes avant et après la canicule. Si un retour à l'état initial n'a été observé sur aucune des stations (en terme de combinaison densité totale x richesse spécifique x structure de communauté) la résilience des communautés apparaît très variable en fonction des stations (Tab. 1). Ainsi, des signes clairs de retour à l'équilibre initial étaient observés à Saunière, la station la moins impactée par des pressions anthropiques non climatiques. Cependant, des retours rapides ont aussi été observés à Allériot, un site pourtant particulièrement dégradés. Encore une fois, cela est sans doute du au fait que ce site est localisé en aval de zones particulièrement favorables aux mollusques dans le Doubs et a probablement pu bénéficier d'un effet source. Pour les autres sites, la résilience des communautés apparaissait relativement faible.

Les communautés d'invertébrés sont connus pour récupérer, en terme de densité totale et de richesse spécifique, en moins de trois ans de perturbations ponctuelles (Resh et al. 1988; Niemi et al. 1990; Yount & Niemi 1990; Lake 2000; Watanabe et al. 2005). Il apparaît que la résilience des communautés de mollusques à une canicule est très inférieure à ces valeurs. Même si tous les sites semblent avoir bénéficié des conditions climatiques clémentes des dernières années, le réchauffement climatique futur et l'augmentation probable des évènements climatiques extrêmes associés pourraient mettre en péril les communautés de mollusques qui apparaissent particulièrement sensibles aux canicules.

3.2 Grandes crues du bassin du Rhône

Publications associées : Daufresne et al. (2007)

Considérant l'importance d'une perturbation telle que la canicule de 2003 il n'est pas très étonnant qu'elle ait provoqué des changements majeurs dans la structure et la diversité des communautés d'éctothermes aquatiques. Cependant, sous la contrainte du réchauffement graduel et en accord avec les prédictions de van Nes & Scheffer (2004), des "regime shifts" pourraient également être observés spontanément ou en présence de perturbations jugées jusque là insignifiantes (Fig. 14).

Le régime hydrologique d'une rivière est un des facteurs les plus structurant des communautés de ces milieux (Poff & Ward 1989; Poff et al. 1997; Poff 1997; Lytle & Poff 2004). La fréquence, l'intensité des crues et des étiages sont connues pour influencer les structures et di-

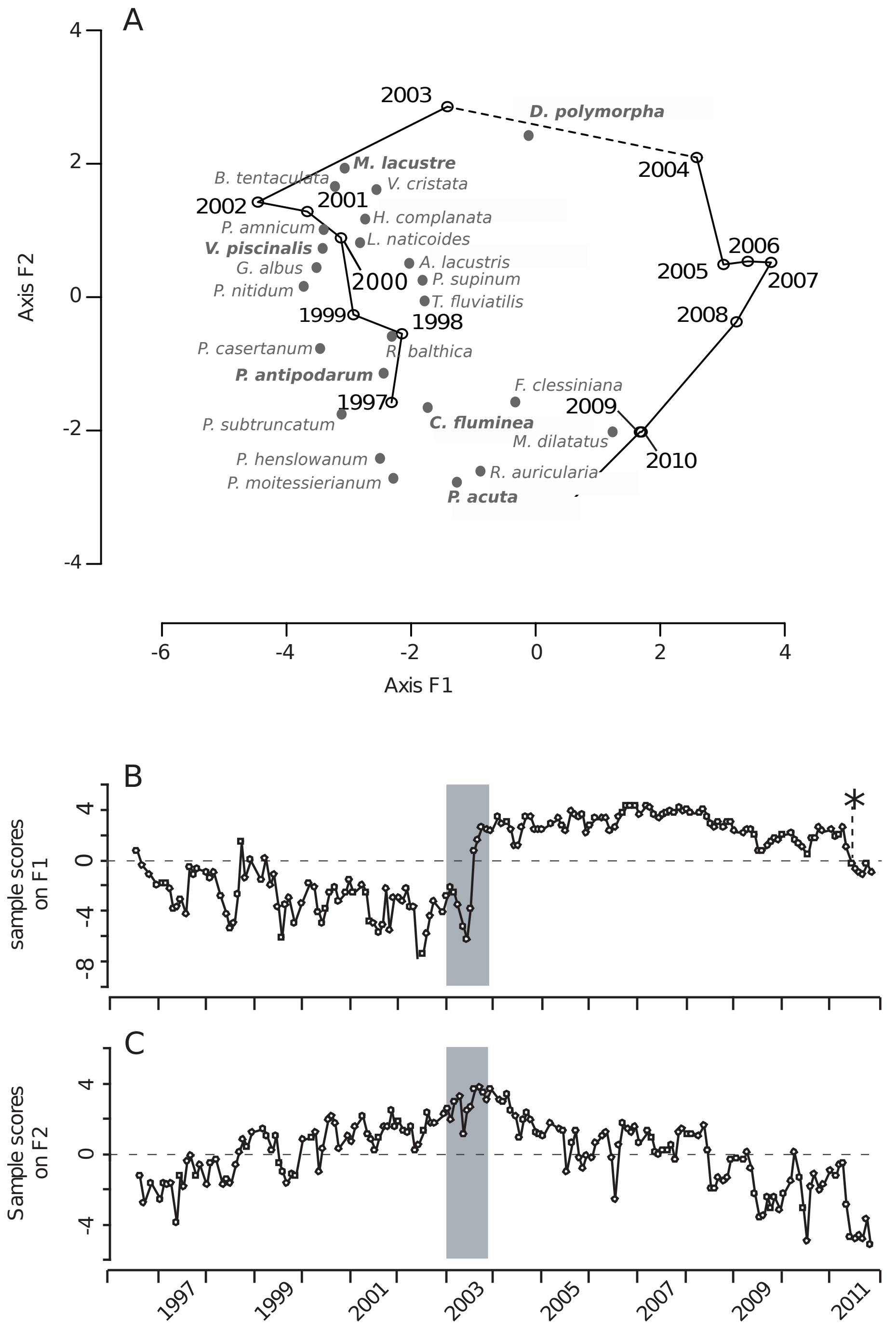


FIGURE 23 – Résultats d'une ACP réalisée sur les données de mollusques de la Saône à Lyon. A) Chronique des coordonnées factorielles moyennes annuelles des échantillonnages et des espèces sur le plan F1xF2 de l'ACP. Les coordonnées des espèces ont été multipliées par 4 pour une meilleure lisibilité. Les chroniques de densité des espèces en gras sont données en Fig 24. B),C) Chroniques mensuelles de coordonnées factorielles. La canicule de 2003 est figurée en gris. Les dates de retour aux situations initiales sont indiquées par les étoiles.

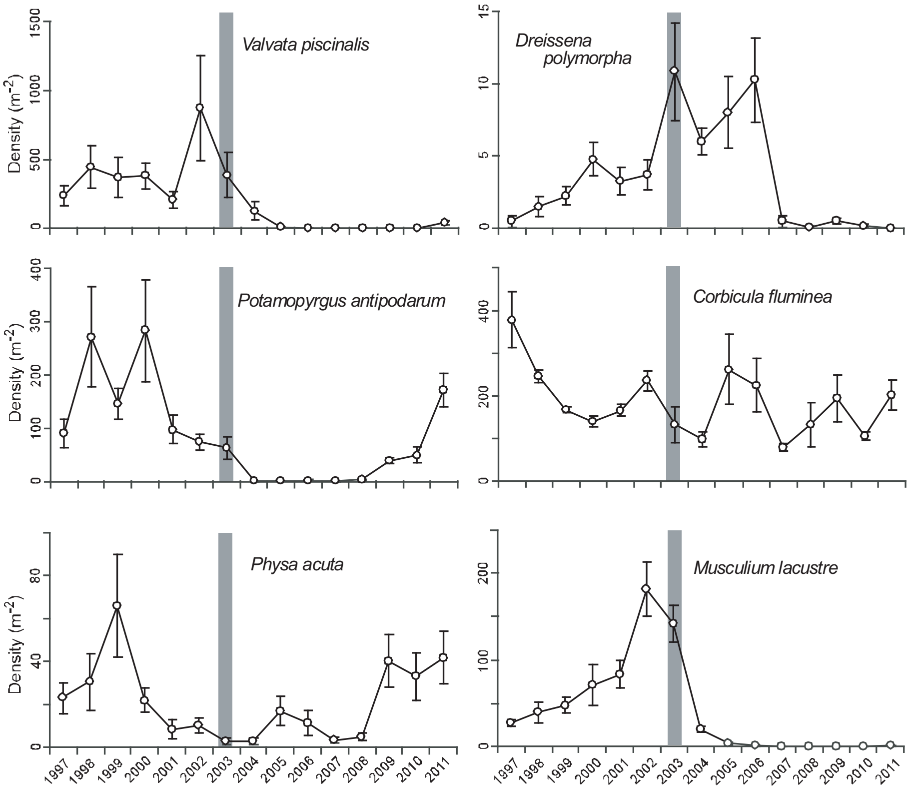


Figure 24 – Chroniques de densités moyennes annuelles (± écart type) de six espèces clefs de mollusques de la Saône à Lyon. Les ordonnées sont ajustées aux données. La canicule de 2003 est figurée en gris.

versités spécifiques et fonctionnelles. Dans un milieu donné et dans un cadre environnemental stable en dehors de la variabilité hydrologique, il est souvent assez clair d'identifier, par exemple en terme de période de retour, les perturbations hydrologiques susceptibles de modifier fortement et durablement les communautés (Resh et al. 1988; Poff & Ward 1989; Niemi et al. 1990; Yount & Niemi 1990; Poff et al. 1997; Poff 1997; Lake 2000; Lytle & Poff 2004; Watanabe et al. 2005). Cependant, sous contrainte climatique, on peut s'attendre a une modification importante des seuils à partir desquels les évènements hydrologiques pourraient destructurer les communautés. Une étude menée sur les changements à long terme des structures de communautés de macro-invertébrés du Rhône tend à supporter cette hypothèse (Daufresne et al. 2007).

Malgré des différences évidentes de composition spécifique, il apparaît qu'il existe des similitudes dans les patrons des dynamiques temporelles des structures des communautés le long du gradient amont-aval du Rhône Francais entre 1985 et 2004. Toutes les structures montrent des dérives graduelles ponctuées de différents changements brutaux (shifts) survenant de manière synchrone entre 1991 et 1992, 1993 et 1994, 2001 et 2002, 2002 et 2003 et enfin 2003 et 2004 (Fig. 25). Nous avons pu montrer que ces dynamiques biologiques étaient liées aux dynamiques physico-chimiques globales mais les causes de ces shifts et leurs intensités restent plus ou moins difficile à interpréter. Dans la mesure ou nous avons travaillé sur des données moyennées par an, les shift de 2003 et 2004 correspondent à l'effet de la canicule de l'été 2003 que j'ai décrit précédemment (sous section 3.1). Le shift de 1991 pourrait être attribué à des conditions sèches et chaudes et à une pause dans l'amélioration des conditions d'oxygénations des eaux qui pourrait être attribué à une compensation des efforts d'épuration par le réchauffement émergeant. Le shift de 1993 est plus facilement attribuable aux grandes crues qui ont touché le Rhône en 1993 et 1994. Il est intéressant de noter que les crues de 2001-2002, d'une instensité comparable, ont eu une effet bien plus marqué sur la structure des communautés. La résilience des communautés à ce type d'événements a donc visiblement eu tendance à décroître avec le temps, ce qui tend à confirmer les hypothèses de van Nes & Scheffer (2004).

Au final, il apparaît que les communautés sont soumises à un ensemble de pressions ponctuelles (pulse, ex : crues, canicules) qui modifient leurs structures. Cependant, ces pressions ponctuelles interviennent dans un contexte de changement global des conditions environnementales, en particulier thermiques. Outre leurs effets propres, ces perturbations graduelles modifient la qualité des réponses des communautés aux perturbations ponctuelles compliquant de manière drastique la prédiction des trajectoires écologiques et, corollairement, la gestion des ces systèmes.

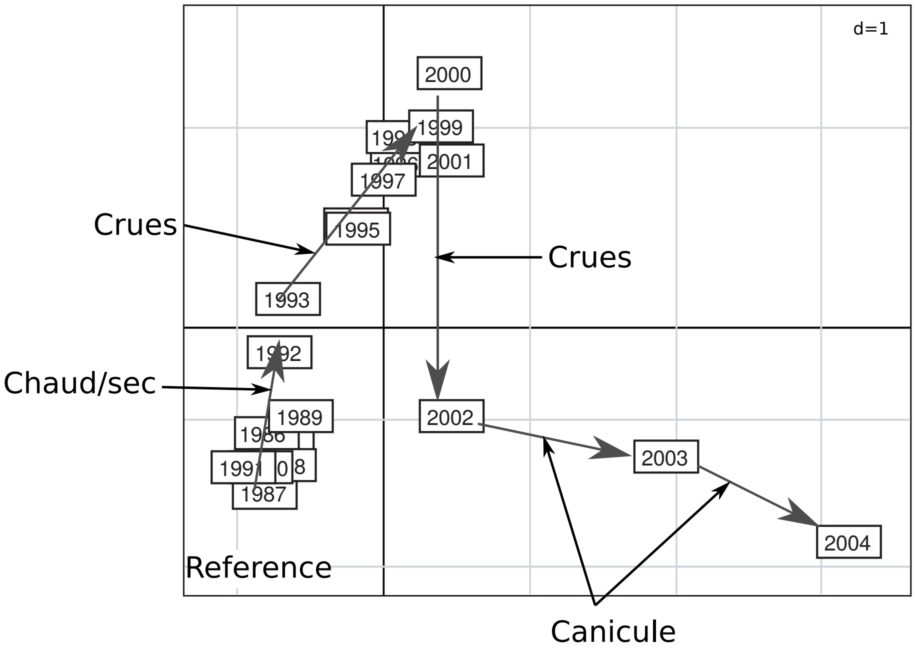


FIGURE 25 – Structure temporelle de référence d'une analyse de coinertie multiple (ACOM) réalisée sur des abondances moyennes (transformées en ln(x+1)) par famille de macro-invertébrés échantillonnées sur 9 sites répartis le long d'un tronçon d'environ 250 km du Rhône Français (F1xF2). Les cosinus carrés des angles entre les axes de référence et des analyses par sites varient entre 0.62 et 0.95, révélant une bonne adéquation. De même les RV varient entre 0.63 et 0.91 suggérant un lien fort entre la référence et les structures par site.

4 Interactions entre perturbations climatiques et non climatiques

Publications associées : Daufresne & Boët (2007); Daufresne et al. (2015)

– L'eau du haut Rhône refroidit les réacteurs nucléaires du CNPE. Bugey, 20 Mai 2003. –

Nous avons pu voir que les communautés réagissaient de manière importante aux contraintes climatiques graduelles et ponctuelles et que les interactions entre contraintes compliquaient passablement la prédiction des trajectoires de ces systèmes et leur gestion. Cette question de la gestion des hydrosystèmes se pose assez clairement dans le cadre des aménagements hydroélectriques. En dehors de l'analyse des effets du changement climatique en tant que tel, les jeux de données que j'ai eu à ma disposition m'ont aussi permis d'évaluer dans quelle mesure ces aménagements et leur gestion pouvaient interagir avec le réchauffement global, même si cela n'a finalement représenté qu'une faible partie de mes activités.

4.1 Impact des rejets thermiques des Centres Nucléaires de Production Electrique (CNPE)

La première pression anthropique non climatique que j'ai pu étudier était l'impact des rejets des CNPE. C'est autour de ces centres que les données utilisées dans l'étude des dérives à long terme des structures de communautés de poissons et d'invertébrés dans les grands fleuves français présenté ici ont été pour la plupart récoltées (Daufresne et al. 2004; Daufresne & Boët 2007; Daufresne 2007; Daufresne et al. 2008, 2009; Daufresne 2009; Daufresne et al. 2015). Je ne détaillerais pas ici leurs impacts dans la mesure où ils avaient une influence très limitée sur la structure des communautés, tant en termes de composition spécifique que d'un point de vue fonctionnel (Fig. 26). Notez cependant (i) que des effets marginaux ont pu être montrés, comme par exemple la présence d'espèces particulières (ex : silure) dans les rejets et (ii) que cela n'implique pas que les rejets n'ont pas d'impact sur les organismes. Cela signifie simplement que sur les données récoltées et pour les variables analysées, les effets étaient faibles. Il est extrêmement probable que de simples inventaires ne sont pas suffisant pour montrer ces impacts. Des analyses complémentaires plus fines, portant sur l'étude de la variabilité de traits écologiques (démographie, croissance) et de variables physiologiques (ex : marqueurs de stress) seraient probablement pertinentes. Dans ce sens, une étude portant sur les structures en taille a pu montrer des effets sur le site de Bugey où le réchauffement dû au CNPE est particulièrement marqué. Si les tendances temporelles sont les mêmes dans les stations réchauffées ou non par le CNPE,

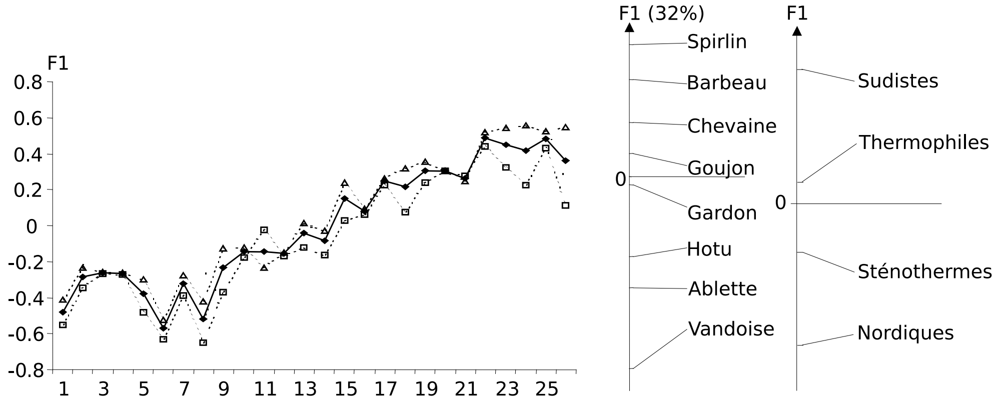


FIGURE 26 – Exemple de chroniques de coordonnées factorielles des échantillons (moyennées pour toutes les stations) et contribution des espèces sur l'axe F1 d'une AFC réalisées sur les données piscicoles de Bugey. L'interprétation des axes en termes de traits écologiques est donnée. Les évolutions sont également représentées pour les stations situées en amont du CNPE (carrés ouverts) et en aval du CNPE (triangles ouverts). Des résultats similaires sont observés autour des autres CNPE (Loire : Belleville, Chinon ; Rhône : Cruas, Saint Alban, Tricastin ; Seine : Nogent ; Daufresne 2009)

.

la taille moyenne des poissons était plus faible dans les stations réchauffées, particulièrement en début de période d'étude, et la proportion d'espèce de petite taille était supérieure dans ces mêmes stations (Fig. 27).

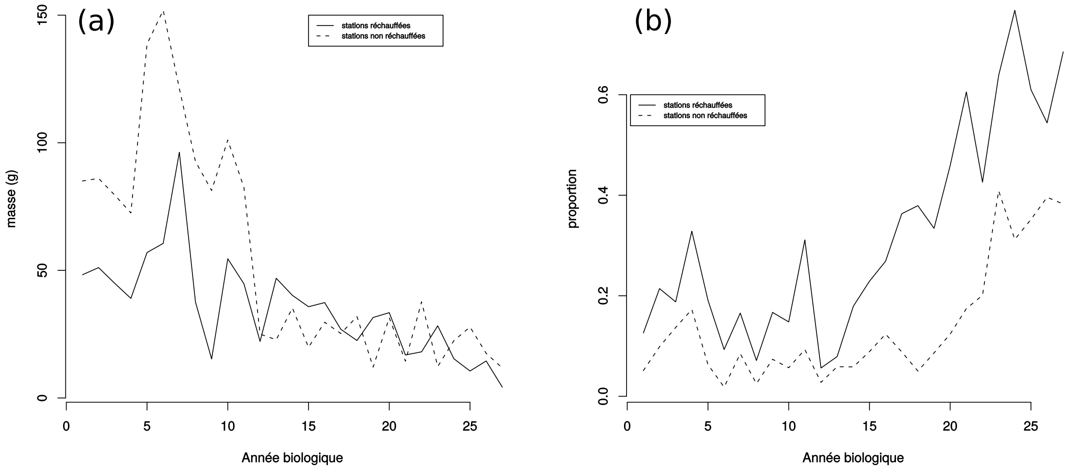


FIGURE 27 – Exemple de tendances à long terme des structures en taille de la communauté de poissons à Bugey sous contrainte du réchauffement global dans des stations réchauffées et non réchauffées par le CNPE. (a) Taille moyenne des individus à l' échelle de la communauté. (b) Proportion des espèces de petite taille (Daufresne 2007).

4.2 Impacts des ouvrages hydroélectriques

Les grands fleuves français sont très fortement soumis aux contraintes des ouvrages hydroélectriques. Par exemple, le Rhône francais (c-à-d depuis le lac Léman jusqu'à la méditerranée) a été soumis au cours des dernières décennies aux impacts de 22 barrages hydroélectriques (Daufresne et al. 2015). Les régimes hydrologiques influencent la plupart des processus écologiques dans les grands cours d'eau et les modifications des ces régimes associés aux ouvrages vont nécessairement impacter ces systèmes (Poff et al. 1997; Bunn & Arthington 2002). Une des premières spécificités des stations localisées le long d'ouvrage hydroélectrique est qu'elle n'ont pas vu leur proportion d'espèces méridionales augmenter ces dernières années (Fig. 18). Si nous avons déjà évoqué l'hypothèse selon laquelle ce phénomène pourrait être dû à la proximité d'une barrière naturelle à la migration (la mer méditerranée), nous ne pouvons pas exclure l'impact des barrières d'origine anthropique que représentent les barrages.

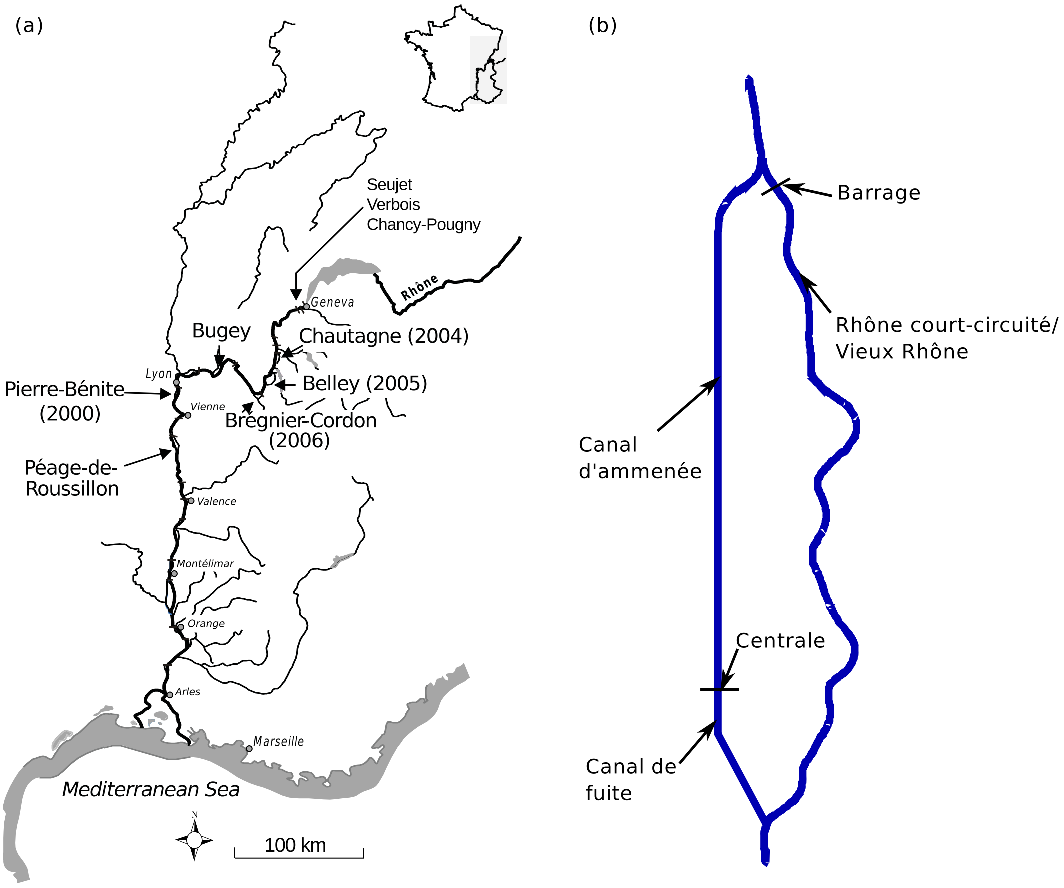


FIGURE 28 – Sites ayant été utilisés pour l'étude de l'impact de la restauration des débits dans les Rhône court-circuités. (a) Localisation de l'ensemble des sites. Les dates de changement des régimes hydrologiques sont indiqués entre parenthèses. (b) Caractéristiques d'un ouvrage hydroélectrique.

Pour toutes les autres variables étudiées, il apparaît que les changements biologiques sont communs à tous les sites, indiquant que les effets du réchauffement climatique sont perceptibles dans la plupart des environnements, même ceux perturbés par des contraintes anthropiques non climatiques.

Plus récemment, nous avons analysé les réponses des communautés de poissons à des travaux de restauration des débits dans les parties du Rhône court-circuités par le canal d'amenée et de fuite des ouvrages hydroélectriques (Fig. 28). Ce travail à consité à étudier les modifications de structure des communautés dans des sites soumis à différents nivaux de restauration (c'est-à-dire des hausses de débits jusqu'à un facteur 10 par rapport aux débits d'origines) et des sites non directement localisés le long d'ouvrage hydroelectriques (Bugey, Péage-de-Roussillon) entre 1985 et 2010. En fonction des contraintes pesant sur ces communautés et des dégrés de restauration nous avons tester différentes hypothèses de changements de leurs structures. Comme attendu, la communauté de Chautagne, la plus proche du dernier barrage construit sur le Rhône et soumise à une augmentation de débit d'un facteur 5 depuis 2004 a d'abord montré une décroissance des proportions des espèces préférant les milieux profonds et courants puis un accroissement de ces proportions depuis 2004 (Fig. 29). Ces proportions ont aussi augmenté à Pierre Bénite, où

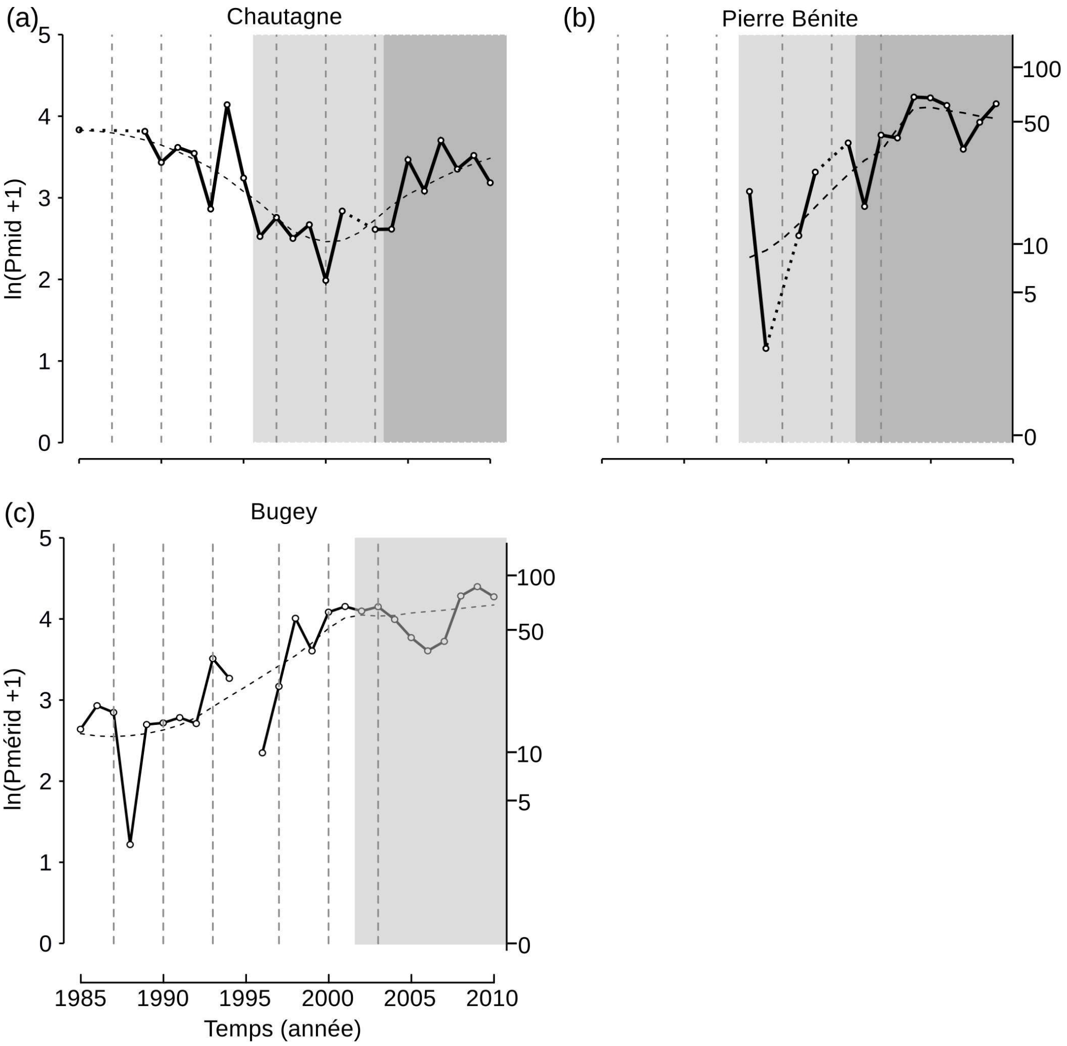


Figure 29 – Exemple de changements temporels dans les structures de communautés piscicoles du Rhône. (a)-(b) Modification des proportions d'espèces préférant les eaux profondes et courantes (espèces dites "midstream", Pmid). (c) Modification des proportions d'espèces méridionales à Bugey. Les pourcentages sont donnés à droite des panneaux. Les périodes groupant les 8 dernières années avant restauration et les années post-restauration sont indiquées par des rectangles gris clairs et gris foncés respectivement. Les lignes verticales indiquent les chasses utilisées pour nettoyer les surplus de sédiments dans les retenues. Les pourcentages sont donnés à droite des panneaux. one reservoirs.

l'augmentation de débit a été d'un facteur 10. Aucun des sites n'a montré de signes évidents d'impact du réchauffement climatique, à part Bugey et Péage-de-Roussillon où des réponses ont pu être partiellement observées (Fig. 29). Ces réponses partielles sont probablement dû au fait que la période considérée n'est pas la période où le réchauffement a été le plus marqué. Cela souligne cependant que le réchauffement ne masque pas les effets des autres pressions et en particulier celles liées aux modifications des régimes hydrologiques qui restent des déterminants forts de la dynamique des communautés.

5 Vers des approches plus mécanistes

– L'onde d'un gravillon métamorphose le reflet de l'écosystème terrestre sur l'écosystème aquatique.
L'Huveau, Roquevaire, Février 2016. –

5.1 Ecologie, taille individuelle et température

Publications associées : Daufresne (2009); Hessen et al. (2013).

5.1.1 Introduction

Une grande partie de mes travaux décrits dans les chapitres précédents ont porté sur une "simple" mise en évidence des impacts du réchauffement climatique. Même si des extrapolations sont possibles, il n'est pas évident de tirer des conclusions fermes quant aux changements qui devraient être observés dans l'avenir sous tels ou tels scénario climatique. Aujourd'hui les modèles prédictifs se limitent souvent à des modèles de distribution des espèces (Guisan & Thuiller 2005), souvent dit de "niche". Ces modèles sont éssentiellement basés sur des approches macroécologiques qui visent à connaître les présences/absences des espèces dans les environnements abiotics actuels et d'inférer des modifications de ces distributions en fonction des modèles de modification des environnements suivant les scenarii climatiques (Thuiller et al. 2005, 2008; Buisson et al. 2008). Je ne discuterai pas ici en détail la notion de niche mais cette appellation me parait ici erronée dans la mesure où la niche est, sinon intrinsèquement conceptuelle, au moins difficilement délimitable (Begon et al. 2005; Chase & Leibold 2003). Cette approximation souligne à elle seule les limites de ces modèles, qui ne sont probablement que très rarement et grossièrement capables de modéliser les niches des espèces. Ainsi, leur capacité de prédiction semble finalement assez limitée (Comte et al. 2013; Comte & Grenouillet 2013). Cette remarque ne remet pas en cause l'intérêt de ces approches et la plupart des auteurs eux-mêmes ont bien conscience de ces limites et tentent actuellement d'y pallier, au moins en partie, en approfondissant nos connaissances sur les processus de colonisation et de disparition et leurs déterminants, à la fois biotiques (interaction inter-spécifiques) et abiotiques (ex : impacts des pressions anthropiques non climatiques) (Thuiller et al. 2008; Conti et al. 2015; Comte & Grenouillet 2015).

Parallèlement à ces approches par modélisation des distributions, des modèles plus mécanistes ont été développés (Morin & Thuiller 2009). Ces modèles restent cependant plus rares et sont souvent espèce-spécifiques. Ils intègrent des processus biologiques calibrés sur des données et permettent souvent des prédictions plus quantitatives. Ils peuvent également potentiellement également intégré de la variabilité génétique (Morin et al. 2007; Morin & Thuiller 2009). Ces approches souffrent cependant de leur spécificité et les changements d'échelles biologiques sont naturellement compliqués à mettre en oeuvre.

Il apparaît donc que les outils prédictifs des impacts du réchauffement climatique sont limités et le manque de règles générales décrivant ces impacts manquent encore cruellement (Thuiller et al. 2008; Daufresne et al. 2009). Outre le développement de règles spécifiques à “l'écologie thermique”, il convient dans un premier temps d'intégrer des règles d'écologie générale dans les approches climatiques (Thuiller et al. 2008; Daufresne et al. 2009). Les ponts que j'ai tenté d'établir entre les réponses des organismes aux perturbations en générales sont intégrés dans cette démarche générale (voir 2.3, 3). Sans rejeter les approches décrites précédemment (on ne peut pas tout faire), mes choix se portent aujourd'hui sur le développement d'approches mécanistes globales qui pourraient s'appliquer à de nombreux organismes et permettre des changements d'échelle plus aisés. Dans ce sens, mes travaux actuels intègrent des questions liant taille, température et métabolisme.

5.1.2 Allométries

La taille est une caractéristique biologique essentielle en écologie pour des raisons à la fois pratiques (c'est un paramètre fréquemment mesuré par les écologues) et théoriques. De nombreuses fonctions biologiques et écologiques sont en effet dépendantes de la taille : métabolisme, fécondité, mortalité des individus, taux de croissance d'une population, compétition entre espèces, production, relations prédateurs proies etc (Peters 1983; Calder 1984; Brown et al. 2004). L'étude des structures en taille est donc un outil intéressant pour évaluer les fonctions écologiques d'un individu, d'une population, d'une communauté ou d'un écosystème. Elle présente en outre l'avantage de s'affranchir de la taxonomie, ce qui permet l'étude comparative de phénomènes et mécanismes entre différents écosystèmes.

La relation entre une variable biologique ou écologique Y et la masse M d'un organisme peut en général être exprimée selon l'équation dite “allométrique” ou de “scaling” (Peters 1983; Calder 1984; Brown et al. 2004) :

$$Y = Y_0 M^b \tag{3}$$

où Y_0 est une constante caractéristique du type d'organisme considéré et b désigne l'exposant allométrique. Notez que si l'on utilise en général la masse dans ce type d'équation, utiliser la taille ne change pas le type de relation dans la mesure où masse et taille sont elles mêmes liées par une relation allométrique dont l'exposant est en général proche de 3. Ces relations sont facilement linéralisables par transformation logarithmique et leur coefficient de détermination est généralement très élevé (Fig. 30). Bien connaître la variabilité des tailles et la cause de cette variabilité offre donc des pistes intéressantes dans l'appréhension de la variabilité des variables écologiques.

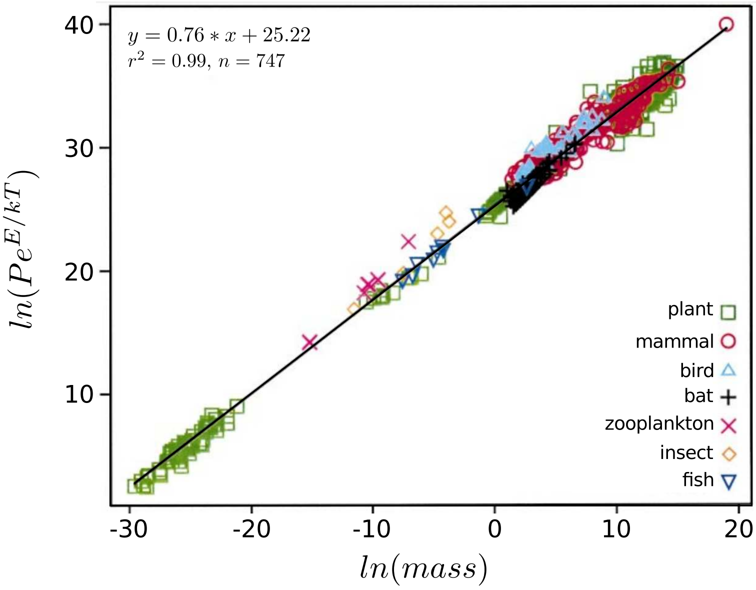


FIGURE 30 – Exemple de relation allométrique, liant la masse des organismes à la production de biomasse (P, mesurée en gramme par individu et par an) (d'après Brown et al. 2004). k représente la constante de Boltzmann ($8.62 * 10^{-5} eVK^{-1}$), T la température en kelvin, et E désigne l'énergie d'activation de la voie métabolique considérée (respiration ou photosynthèse).

5.1.3 Les règles taille-température

Il existe deux "règles" principales concernant les relations entre la taille (ou la masse) des organismes et la température : la règle dite de "Bergmann" (Bergmann 1847), initialement énoncée pour les endothermes, et la règle taille-température chez les ectothermes (temperature-size-rule, ou TSR ; Atkinson 1994). Globalement, ces deux règles prédisent des réductions de la taille individuelle (au moins à l'âge adulte) avec l'augmentation de la température, même si elles ont chacune des spécificités propres (Fig. 31).

La règle de Bergmann est une des règles qui a été le plus étudiée en écologie (même si je consens que cette affirmation repose plus sur mon ressenti que sur une analyse fine de la bibliographie). Telle qu'exposée par Bergmann, elle stipule que pour un groupe d'espèces phylogénétiquement proches, les espèces les plus petites/légères (vs. grandes/grosses) vont se trouver dans les environnements les plus chauds (vs. froids) (Bergmann 1847). Eco-géohraphique, cette règle désigne par exemple souvent les patrons de modification de taille le long de gradients latitudinaux.

Si la nature géographique de la règle est assez clairement définit par Bergmann, la définition de la proximité phylo-génétique des espèces est assez confuse. Afin de clarifier les choses, Blackburn et al. (1999) a supposé que la règle initiale valait pour un groupe d'espèces appartenant au même genre, même si cette décision reste quelque peut arbitraire. Pourtant, si on se base sur l'utilisation qui a été faite de la règle de Bergmann, il existe des digressions considérables concernant l'échelle biologique à laquelle elle est censée s'appliquer (Blackburn et al. 1999; Daufresne et al. 2009). En particulier, James (1970) fut le premier à souligner qu'il était important de différencier les patrons de modification des tailles observés à l'échelle inter-spécifique (la règle de Bergmann proprement dite) de ceux observés à l'échelle intra-spécifique, dans la mesure où les mécanismes sous-jacents pouvaient être passablement différents (en particulier les contraintes évolutives). Pour bien distinguer ces phénomène, Blackburn et al. (1999) a proposé de dénommer règle de James la version intra-spécifique de la règle de Bergmann. Pour une espèce donnée, cette règle stipule donc que les populations ayant les tailles/masses individuelles les plus faibles (vs. importantes) se trouvent dans les environnements les plus chauds (vs. froids).

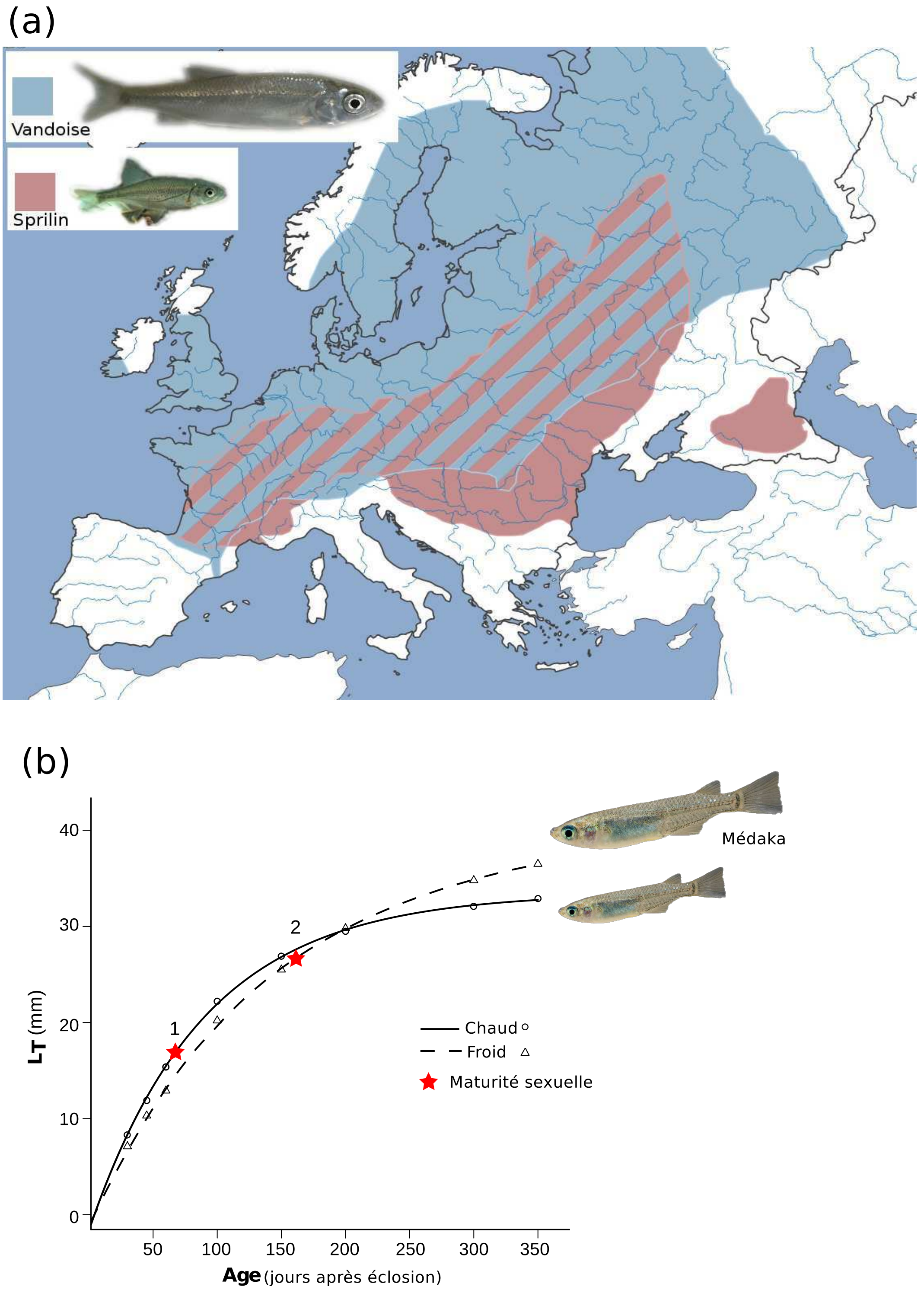


FIGURE 31 – Les règles taille-température.(a) Illustration de la règle de Bergmann. Au sein d'un groupe phylogénétiquement proche, les espèces les plus petites se trouvent dans les environnement les plus chauds. La règle est explicitement inter-spécifique (b) TSR, pour une espèces, les individus élevés dans les conditions les plus chaudes vont avoir un taux de croissance plus élevé mais une taille asymptotique plus petite et vont maturer plus tôt et à une taille inférieure (issu de Hemmer-Brepson 2013).

Les aspects spatiaux sont au coeur des règles de Bergmann et de James, même si la température a évidemment été considérée comme l'un des déterminants majeurs des gradients géographiques des structures en taille (voir par ex. Bergmann 1847; James 1970; Blackburn et al. 1999; Blackburn & Hawkins 2004). La règle de Bergmann à été initialement énoncée pour les endothermes et, même si cette règle peut s'appliquer aux ectothermes (Blackburn et al. 1999), ces derniers sont plutôt concernés par la TSR. Contrairement à la règle de Bergmann, la température est explicitement au coeur de la TSR. La différence est subtile mais suffisante pour avoir généré deux voies finalement assez indépendantes d'analyse des liens taille-températue, l'un concernant la règle de Bergmann (ex. Bergmann 1847; James 1970; Blackburn et al. 1999; Belk & Houston 2002; Blackburn & Hawkins 2004; Litzgus et al. 2004; Rodríguez et al. 2006; Meiri & Thomas 2007; Meiri et al. 2007; Rodríguez et al. 2008) et l'autre la TSR (ex. Atkinson 1994; Van der Have & deJong 1996; Atkinson & Sibly 1997; Angilletta & Dunham 2003; Atkinson et al. 2003; Angilletta et al. 2004, 2006; Angilletta 2006; Atkinson et al. 2006). En dehors de ces considérations, la spécificité principale de la TSR est de considérer que les individus élevés à des températures chaudes (mais non stressantes relativement à l'ectotherme considéré) vont (i) avoir un taux de croissance initial (k) plus élevé mais (ii) vont avoir une taille à maturité plus petite (en longueur ou en masse (m_{mat}) ; Fig. 31b). Corollairement, les individus vont en général également (iii) maturer plus tôt (si on considère qu'il existe une taille minimale pour se reproduire) et (iv) avoir une taille asymptotique (ou masse asymptotique, m_∞) plus faible (si on considère que la croissance décélère après la maturité sexuelle et qu'il n'existe pas de regain tardif de croissance). Cette règle a principalement été étudiée à l'échelle intra-spécifique et peut apparaître comme une explication à la règle de James pour les ectothermes. Cependant, l'échelle à laquelle la TSR pourrait s'appliquer en dehors de l'échelle intra-spécifique à déjà été discutée (Atkinson et al. 2006).

Si il apparaît assez évident qu'il existe des liens forts entre température et taille des organismes, les mécanismes impliqués dans ces liens sont toujours difficiles à identifier. Plusieurs explications ont été proposées pour expliquer les règles de Bergmann et de James. L'explication la plus populaire repose sur l'hypothèse de la conservation de la chaleur, qui stipule que les organismes sont plus gros dans les environnements froids à cause d'un meilleur ratio entre volume et surface corporelle (Blackburn et al. 1999). Plusieurs hypothèses non thermiques basées par exemple sur des capacités de migration plus importantes et une meilleure résistance aux périodes de disettes pour les gros organismes ont également été proposées mais semblent moins générales (Blackburn et al. 1999).

De la même manière, plusieurs hypothèses ont été proposées pour expliquer la TSR. Je détaillerai ces hypothèses dans la section 6.3 mais un premier groupe de mécanismes développés reposent sur des contraintes physiques pesant sur la taille des cellules. Ces mécanismes suggèrent que la taille des cellules doit être plus petite à forte température (Van der Have & deJong 1996; Woods 1999; Atkinson et al. 2006). Ces hypothèses ont diverses limites, en particulier le fait qu'elles ne permettent pas toutes d'expliquer les patrons de TSR observés sur les organismes dont la taille à l'âge adulte n'est pas dépendante du nombre de cellules (Arendt 2007; Angilletta et al. 2004). D'autres mécanismes portent sur des contraintes thermique limitant la taille maximale des organismes du fait de considérations métaboliques (Strong & Daborn 1980; Perrin 1995; Atkinson et al. 2006). Cependant ces différentes hypothèses ne permettent pas non plus, au moins prises individuellement, d'expliquer l'ensemble des patrons de la TSR (voir la section 6.3 pour plus de détails).

5.1.4 Vers une clarification des modifications des tailles à différentes échelles biologique

James (1970) et Blackburn et al. (1999) ont souligné l'importance de la clarification de l'échelle à laquelle sont étudiés les patrons de modification des tailles pour en analyser la généralité et les causes. Il existe effectivement une très grande confusion dans les patrons étudiés, et il est par exemple évident que les différents mécanismes proposés pour expliquer un compromis entre m_∞ et k dans les milieux aquatiques ne sont pas nécessairement appropriés pour étudier la règle de Bergmann. James (1970) et Blackburn et al. (1999) se sont limités à distinguer la variabilité des tailles à l'échelle inter-specifique (et intra-groupe faunistique phylogénétiquement proche ou intra-genre) de celle des tailles à l'échelle intra-spécifique. Cela constituait une étape fondamentale mais il me semblait nécessaire d'essayer de clarifier les patrons de modification des tailles à d'autres échelles et la manière dont ces patrons pouvaient s'emboîter avant toute étude de variabilité de ces tailles avec le réchauffement climatique. Cela a constitué le coeur de mes premiers travaux sur la taille (Daufresne et al. 2009; Hessen et al. 2013). J'ai tenté d'élaborer une représentation schématique des variations en taille en me basant sur l'idée qu'un ensemble donné variait en taille si la taille de ses sous ensembles et/ou le nombre de ses sous-ensembles variait (Fig. 32). La porté de ce schéma dépasse l'étude de l'impact de la température. Cependant, les hypothèses associées à un réchauffement implique que, pour cette contrainte particulière, le sens de variations des variables associées doit aller vers une décroissance des tailles. Plus explicitement, cela implique qu'un réchauffement devrait entraîner une baisse de la taille moyenne à l'échelle de la communauté, une augmentation de la proportion d'espèces de petites taille, une diminution des tailles moyennes des populations etc.

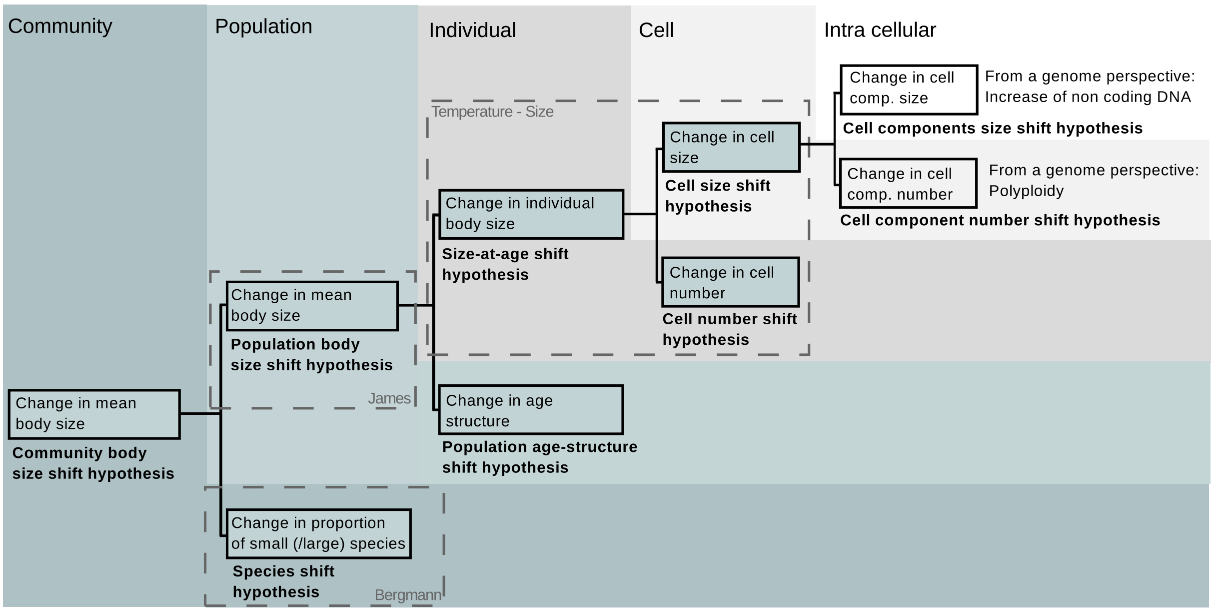


Figure 32 – Schéma conceptuel des patrons de variabilité en taille de l'échelle de la communauté à l'échelle intra-cellulaire (ou Bilogical scalE Explicit Framework (BEEF) to study changes in body-size; dérivé de Daufresne et al. 2009; Hessen et al. 2013). Concernant l'impact de la température, les modifications associées sont supposées toutes aller vers des diminutions des tailles.

Les hypothèses exposées de l'échelle de la communauté à l'échelle individuelle ont été proposées dans Daufresne et al. (2009) et inclue les règles de Bergmann et de James ainsi que la TSR (Fig. 32). Les autres hypothèses étaient naturellement déjà en place en 2009 mais je ne disposais pas de données pour travailler aux échelles cellulaire et intra-cellulaire et elles n'ont donc pas été présentées dans l'article. A l'échelle individuelle, il a déjà été évoqué à de nombreuses reprises que la variabilité en taille pouvait être dû à des variations du nombre et de la taille des cellules et Arendt (2007) a pu montrer que la température jouait principalement sur leur taille ("cell size shift hypothesis", Fig. 32). Les hypothèses exposées à l'échelle intra-cellulaire ont été développées pour les métazoaires ectothermes dans une revue bibliographique que nous avons récemment publiée (Hessen et al. 2013). Il apparait assez clairement que la taille des cellules est souvent liée à la taille du noyaux et qu'il existe des preuves de croissance de la taille du génome dans les environnements froids chez ces organismes. La taille du génome peut varier de deux manière principale : (i) par une variation de la taille des régions non codantes (accumulation de paires de bases dans les introns) et (ii) par copie des gènes, ou polyploïdie. L'accroissement des tailles cellulaire par polyploïdie semble particulièrement répandu chez les organismes ayant un nombre de cellules relativement fixe, comme probablement la plupart des nématodes et des rotifères. Dans des environnements froids, la polyploïdie pourrait compenser l'impact négatif de la température sur les cinétiques enzymatiques et la production de protéines (Xia 1995). Dufresne & Hebert (1998) et Van Geest et al. (2010) ont pu montrer que les individus polyploides de *Daphnia pulex* grandissaient plus rapidement et maturaient plus tôt que les individus diploïdes dans les environnements froids. La polyploïdie pourrait donc représenter un avantage adaptatif dans de tels milieux. L'accumulation de région non codantes est plus difficile à expliquer dans la mesure où elle a en générale plutôt tendance à nuire à la croissance du fait de temps de division cellulaire accrus. Il est possible que dans les environnements froids ce caractère ne soit simplement pas contre-sélectionné. Notez que ces arguments ne permettent pas d'expliquer la TSR en tant que telle (qui relève principalement de la plasticité phénotypique) mais qu'ils soulignent en revanche la cohérence du schéma conceptuel proposé concernant la variabilité des tailles à différentes échelles biologiques. La base de l'explication de la TSR donnée par Van der Have & deJong (1996) rentre aussi dans ce cadre, le nombre de composés cellulaires étant supposé être plus faible à forte température et donner des cellules plus petites ("cell components number shift hypothesis", voir le paragraphe 6.3.1 pour plus de détails).

5.1.5 Impact du réchauffement climatique sur les tailles

Les règles taille-température ont généralement été basées sur des analyses de variabilités spatiales (on regarde des tailles dans des milieux contrastés thermiquement) ou dans des milieux expérimentaux n'incluant la plupart du temps qu'une seule espèce. Dans ce sens, l'effet d'un réchauffement climatique sur la modification de la taille des organismes dans un système complet est très peu connue. Récemment, nous avons pu montrer que les structures en taille des organismes sous contraintes climatiques avaient cependant effectivement tendance à évoluer selon les règles taille-température (Daufresne et al. 2009), c'est-à-dire qu'on observait dans des milieux complexes naturels (rivières, océans) ou expérimentaux (mésocosmes) (i) une augmentation des proportions de petites espèces à l'échelle de la communauté, (ii) une augmentation des proportions de juvéniles à l'échelle de l'espèce et (iii) une décroissance des tailles à un âge donné à l'échelle de l'individu, et ce, des bactéries aux poissons en passant par le phyto- et le zoo-plancton (voir Fig. 33 pour des exemples de modifications des structures en taille des poissons dans les grands fleuves français au cours des trente dernières années).

Du point de vue des spectres de taille, ces modifications se traduisent par une augmentation des pentes des relations log densité - log de taille. Cela a en particulier pu être montré sur des communautés phytoplanctoniques dans des mésocosmes, à la fois en milieu marin (Fig. 34 ; Sommer & Lengfellner 2008; Daufresne et al. 2009) et d'eau douce (Yvon-Durocher et al. 2011).

On peut considérer la validation de toutes nos hypothèses comme un succès d'un point de vue scientifique. Sans renier la satisfaction éprouvée lors de la découverte de ces décroissances en taille lors de l'analyse des différents jeux de données je me dois ici d'exprimer également une certaine frustration. Une de mes espérances était en réalité de voir émerger une hiérarchie dans les patrons de variabilité en taille. Cela aurait énormément simplifié la recherche des mécanismes sous-jacents. La validation de l'ensemble des hypothèses milite pour l'existence d'une contrainte forte, mais qui reste difficile à appréhender et qui pourrait être la conséquence d'un, comme de nombreux, mécanismes.

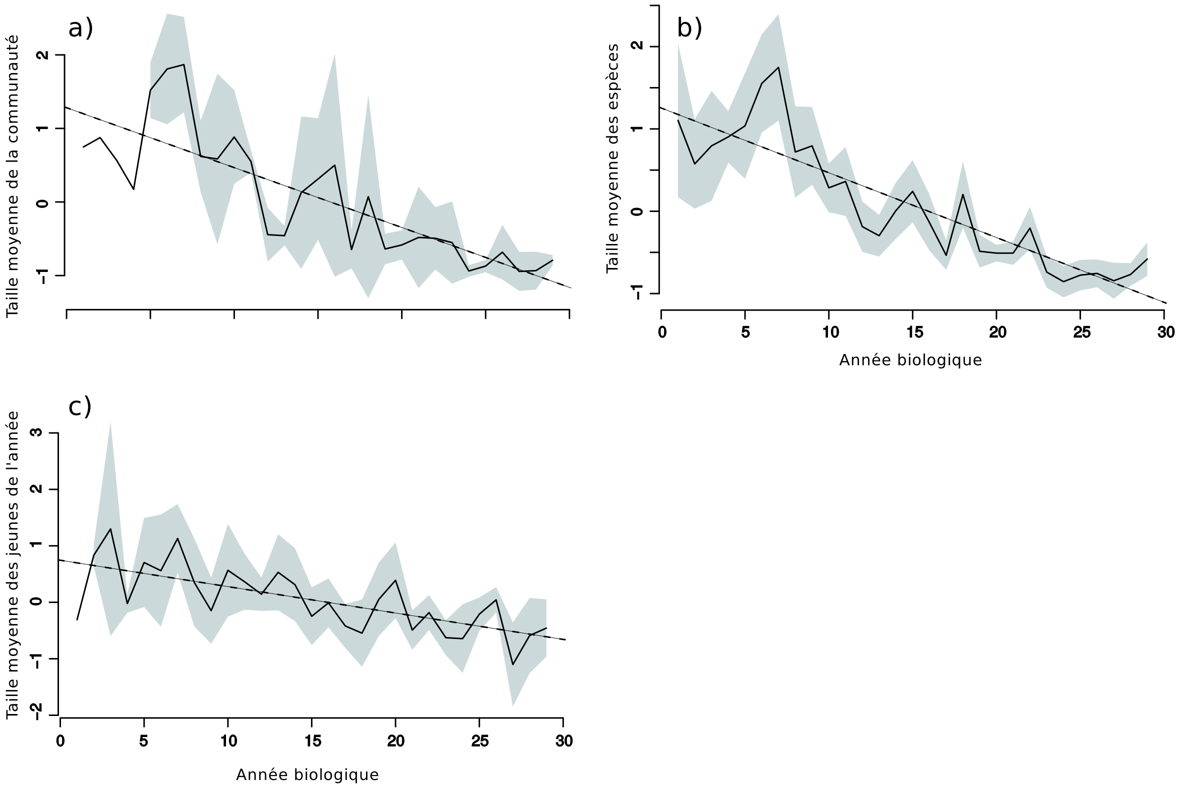


FIGURE 33 – Exemples de modification de structure en taille des poissons dans le Rhône (3 sites : Bugey, Cruas et Tricastin) et la Seine (à Nogent) sous contrainte de réchauffement climatique. a) Exemple relatif à l'hypothèse de “community body size shift” du BEEF. b) Exemple relatif à l'hypothèse de “population body size shift”. c) Exemple relatif à l'hypothèse de “population age-structure shift”. Les intervalles de confiance à 95% sont représentés en grisé. Les tendances linéaires sont figurées.

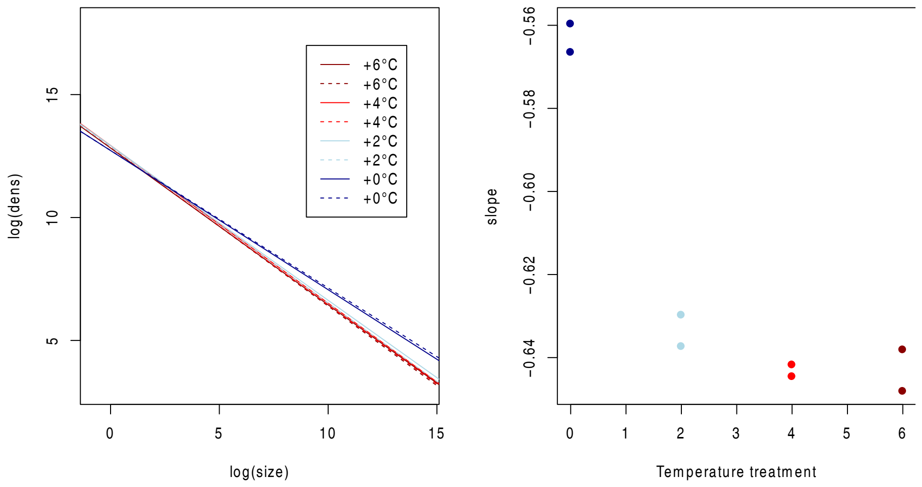


FIGURE 34 – Spectres de taille de communautés phytoplanctoniques de la mer baltique pendant le bloom printanier dans des mésocosmes soumis à différents régimes thermiques. +0 °C indique le régime thermique moyen observé au printemps dans la mer baltique. +2, +4 et +6 °C indiquent des régimes thermiques modifiés de 2, 4 et 6 °C par rapport au régime moyen observé.

6 Perspectives

– Changer de point de vue offre parfois d'étonnantes perspectives. L'Huveaune, Roquevaire, Février 2016. –

6.1 Introduction

Mes travaux ont porté sur les liens taille-réchauffement dans les écosystèmes aquatiques mais j'ai évoqué dans l'article publié dans PNAS en 2009 la possibilité que la diminution en taille puisse être la troisième règle générale d'impact du réchauffement climatique sur les ectothermes (après les changements d'aires de distribution et de phénologie ; Daufresne et al. 2009). Compte tenu de l'importance de la taille en écologie, ce travail a suscité de l'intérêt dans la communauté scientifique. Des revues bibliographiques ont pu par exemple montrer que le réchauffement pouvait être bénéfique aux petits individus dans de nombreux écosystèmes chez les ectothermes comme chez les endothermes (Gardner et al. 2011; Sheridan & Bickford 2011). Très rapidement sont néanmoins (ré)apparu des lacunes quant aux échelles biologiques considérées. Gardner et al. (2011), par exemple, visent à illustrer l'hypothèse d'une “troisième règle générale” mais ne considèrent finalement que notre “body size shift hypothesis” (Fig. 32). De plus, il existe toujours de nombreuses zones d'ombres quant aux mécanismes impliqués dans ces décroissances en taille aux différentes échelles biologiques. Enfin, les conséquences écologiques restent peu ou pas explorées.

Dans ce cadre, mes travaux actuels s'articulent autour de trois axes principaux (Fig. 35). Le premier axe consiste à mieux décrire les patrons de diminution des tailles à différentes échelles biologiques (ce que l'on peut décrire comme étant *le comment*). Le deuxième axe vise à étudier les impacts physiologiques du réchauffement et dans quelle mesure ces impacts peuvent expliquer les règles taille-température (ce que l'on peut décrire comme étant *le pourquoi*). Le troisième axe consiste à explorer les conséquences des règles taille-température sur les relations allométriques en générales et, plus spécifiquement, sur les structures des chaînes trophiques (ce que l'on peut décrire comme étant *les conséquences*).

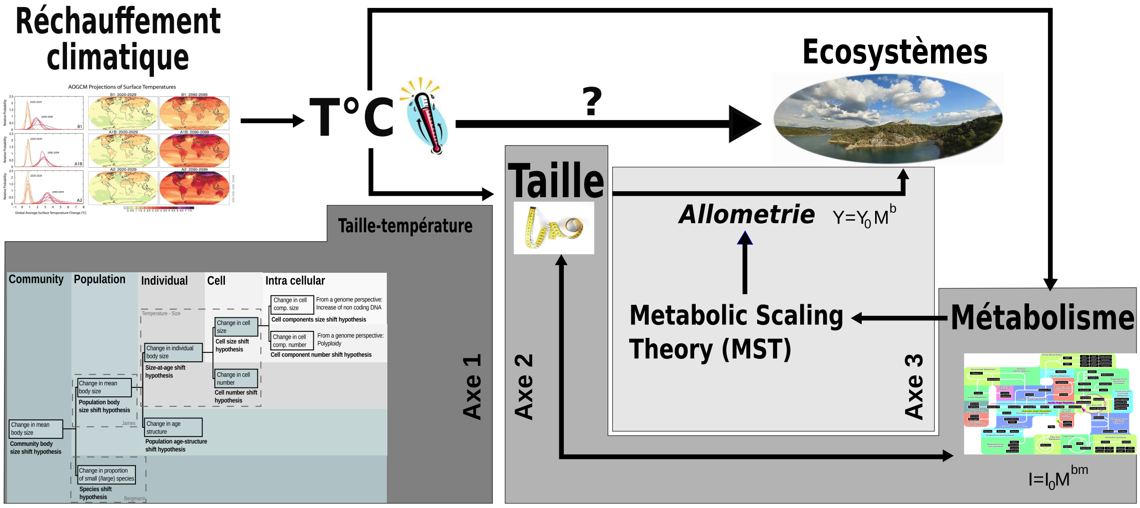


Figure 35 – Cadre général de mes travaux présents et futurs. Les différents axes de travail peuvent être décrit comme explorant *le comment*, *le pourquoi* et *les conséquences* des liens taille-température en écologie dans un contexte de réchauffement climatique.

Il m'est évidemment impossible de traiter tous ces axes de manière exhaustive. Je ne présenterai ici qu'un certain nombre d'actions menées dans ce cadre général. La plupart de ces actions ont été planifiée dans différentes versions d'un projet ANR (ClimSize). Ce projet n'a pas été financé par l'anr mais les actions qui le composent ont pu en grande partie être financées par l'Onema.

6.2 Axe1 – Règles taille-température et échelles biologiques

Cet axe est celui qui est le moins prioritaire à mes yeux ou plus précisément pour lequel j'ai le moins de temps à accorder et que je n'ai pas encore réussi à financer. Cela ne signifie évidemment pas qu'il ne présente pas d'intérêt intrinsèque ou que je n'éprouve pas le désire d'explorer la nature des liens taille-température à différentes échelles. Tel que décrit dans ClimSize cette étude reposait sur le test des hypothèses du BEEF dans les plans d'eau français par l'utilisation de données biologiques et thermiques. Outre leur simple test, un des buts ultimes est de quantifier, a chaque bifurcation du BEEF, l'importance relative des hypothèses “filles” pour expliquer l'hypothèse mère (par exemple dans quelle mesure les hypothèses de “species shift” et de “population body size shift” expliquent l'hypothèse de “community body size shift”, Fig. 32). Ce travail nécessite de disposer en particulier de données biologiques et thermiques de qualité sur un grand nombre de systèmes.

6.2.1 Caractérisation des thermodynamiques lacustres

Nous ne disposions à mon arrivée dans l'équipe (en 2007) que d'un nombre très réduit de données thermiques sur les plans d'eau et cela a très fortement pénalisé le développement de cet axe. La mise en place du réseau de surveillance dans le cadre de la DCE a partiellement résolu ce problème même si les données ne sont récoltées (i) que depuis 2006, (ii) sur un nombre réduit de plans d'eau et (iii) avec a minima 4 profils par an tous les 6 ans. A ce jour, nous disposons de 4 à 70 profils par plan d'eau sur 397 plans d'eau (Danis & Prats 2016). Dans le cadre de la mise en place d'un réseau de suivi des conditions thermiques dans les plans d'eau francais par l'Onema (Rondel et al. 2011, 2012; Rebière et al. 2013, 2014; Rebière & Danis 2015; Prats & Danis 2015), nous avons également équipé 9 lacs de chaînes de thermomètres avec un enregistrement en continu (Rebière et al. 2014; Rebière & Danis 2015, Fig. 36).

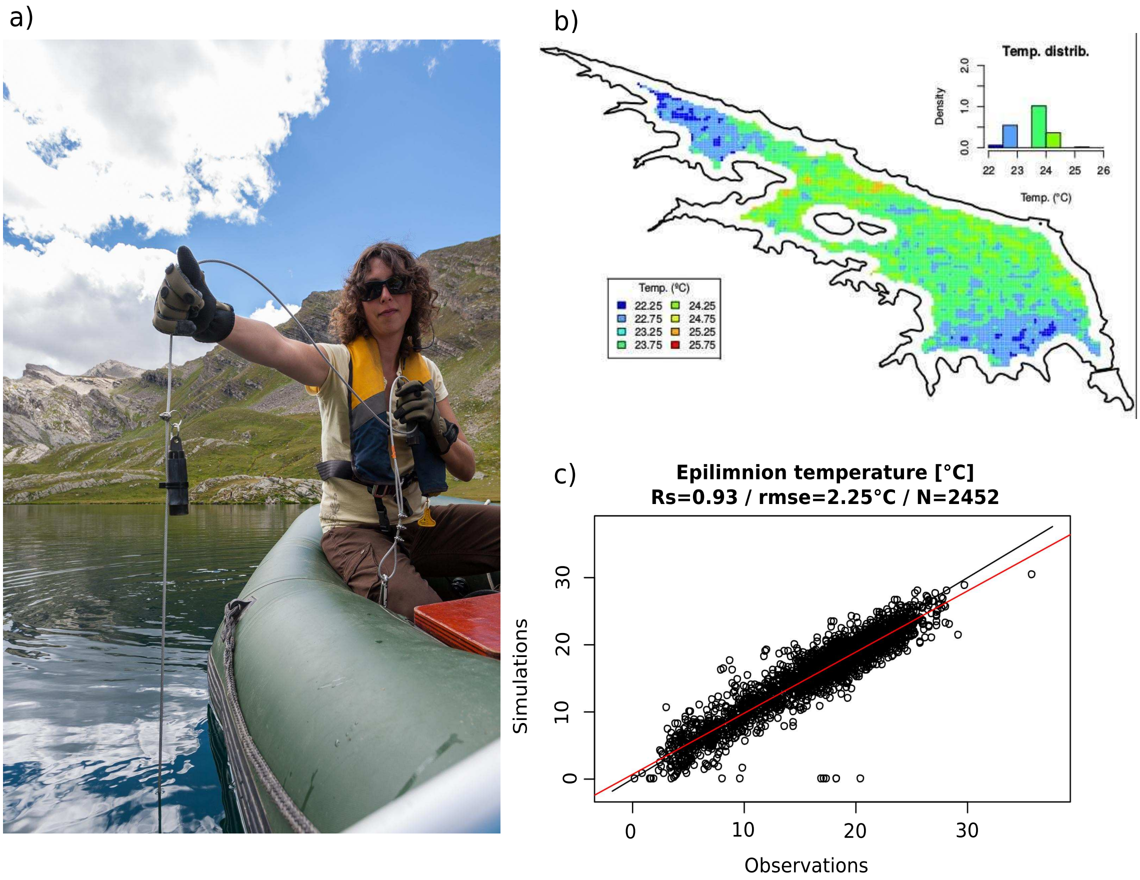


FIGURE 36 – Données thermiques en plan d'eau. a) Pose d'une chaîne d'enregistreurs thermiques en continu in situ. b) Exemple d'évaluation de la température de surface d'un plan d'eau (Naussac) à partir d'images satellites Landsat (03/08/2003). c) Performances d'un modèle dérivé de Ottosson & Abrahamsson (1998). Crédit photo : P.A. Danis.

Le réseau n'est pas opérationnel aujourd'hui mais des recherches sont développées à la fois sur l'utilisation des images satellites (Simon et al. 2014; Prats & Danis 2015; Danis & Prats 2016, Fig. 36) et sur l'utilisation d'approches de modélisation (Prats & Danis 2015; Danis & Prats 2016, Fig. 36). Les images satellites Landsat permettent d'évaluer la température de peau (Simon et al. 2014) qui est assez représentative de la température de l'épilimnion. Des travaux de comparaison des performances et d'améliorations éventuelles de modèles simples empiriques (Ottosson & Abrahamsson 1998), semi-empériques (Toffolon et al. 2014) ou mécanistes (Mironov et al. 2010; Hipsey et al. 2014) sont en cours (Prats & Danis 2015; Danis & Prats 2016). Aujourd'hui, le modèle qui semble le plus performant est un modèle dérivé du modèle Ottosson & Abrahamsson (1998), au moins concernant la modélisation des températures de l'épilimnion (Fig. 36), probablement du fait du calage du modèle sur les données existantes. L'utilisation de ce modèle laisse déjà percevoir un probable réchauffement des eaux de l'épilimnion dans les plans d'eau DCE au cours des trente dernières années (Fig. 37). Nous esperons dans l'avenir améliorer nos capacités de modélisation dans l'espace et dans le temps, notamment via l'utilisation du modèle GLM (Mironov et al. 2010; Hipsey et al. 2014). Ce travail sera porté par J. Prats dans le cadre du pôle, sous l'encadrement de P.-A. Danis sur la période 2016-2018.

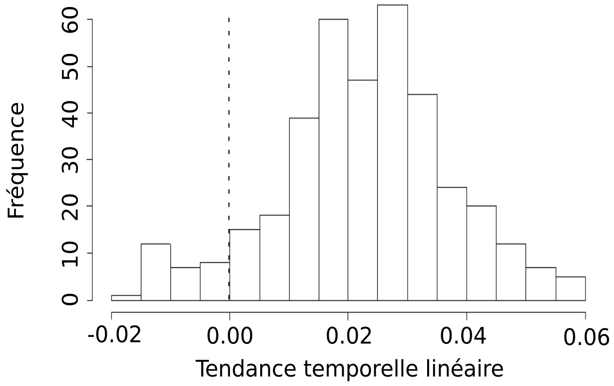


FIGURE 37 – Exemple de distribution des tendances temporelles (b, avec $temperature = a + b * an$) des températures moyennes annuelles de l'épilimnion modélisées à partir du modèle dérivé d'Ottosson & Abrahamsson (1998) sur les plans d'eau DCE au cours de la période 1980-2013.

Ces travaux ont été et sont principalement portés par P.-A. Danis, membre Onema du pôle, et je ne revendique pas leur paternité. J'ai néanmoins toujours soutenu ces actions et ce dès mon arrivée dans l'équipe. Ces démarches étaient déjà intégrées dans l'ANR jeune chercheur TaGGs soumis en 2010 et j'ai contribué au co-encadrement d'étudiants et de contractuels travaillant sur ces sujets ainsi qu'à la rédaction ou la relecture des rapports associés (voir la partie Notice d'activité).

6.2.2 Données biologiques

Nous disposons au pôle de divers jeux de données qui peuvent être utilisés pour tester les différentes hypothèses du BEEF. Concernant les données récoltées in situ, les plans d'eau DCE font l'objet d'échantillonnages poisson, phytoplancton, macrophyte tous les 6 ans. Ces données sont gérées au pôle et nous disposons aujourd'hui de données poisson et phytoplancton sur environ 250 plans d'eau (évolutif) et de données macrophyte sur environ 150 plans d'eau (évolutif). En outre, nous disposons également de données physico-chimiques (incluant les mesures thermiques citées dans la section précédente) sur 480 plans d'eau (évolutif). Concernant les données poisson, les individus sont identifiés à l'espèce et mesurés. Les hypothèses à l'échelle de la communauté et de la population peuvent donc être envisagées. Concernant le phytoplancton, les individus sont identifiés à l'espèce quand cela est possible et un bio-volume fixe pour l'espèce leur est attribué. En l'état seul les hypothèses énoncées à l'échelle de la communauté peuvent être testées.

Outre les données récoltées in-situ, nous disposons également d'installations expérimentales dans lesquelles sont élevés des médakas (*Oryzias latipes*) à deux température différentes (20°C et 30°C) depuis une dizaine de générations (Hemmer-Brepson 2013; Hemmer-Brepson et al. 2014).

6.2.3 Résultats préliminaires et développements

Deux étudiantes de master 1 (Roux 2009) et 2 (Lefol 2010) ont réalisé des analyses exploratoires de la variabilité en taille des communautés et populations piscicoles en utilisant des données de température atmosphérique et (i) une version primaire de la base de données poisson (Roux 2009) et (ii) des données récoltées à l'échelle européennes et disponibles ponctuellement durant la réalisation du projet WISER (Lefol 2010). Ces analyses ont pu souligner des décroissances significatives avec la température moyenne estivale (juillet - septembre) des tailles moyennes individuelles à l'échelle de la communauté et pour deux espèces communes dans nos plans d'eau, la perche et le gardon à l'échelle européenne (Fig. 38). A ce stade, ces analyses sont encore grossières et l'amélioration de la qualité des données thermiques devraient en particulier permettre d'aller un peu plus loin dans leurs développements.

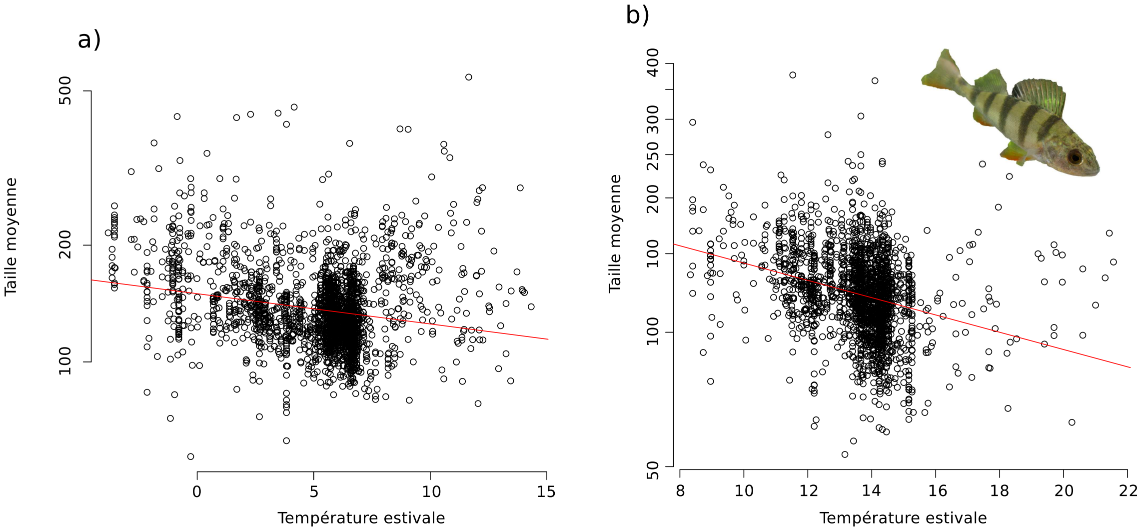


FIGURE 38 – Exemple de liens taille-température dans les communautés piscicoles de 1482 lacs européens (données issues du projet WISER). La température considérée est la température atmosphérique journalière moyenne sur la période juillet-septembre. a) Taille moyenne à l'échelle de la communauté. b) Taille moyenne des individus des populations de perche (*Perca fluviatilis*). Les tendances linéaires (significativement non nulles, $p < 0.01$) sont figurées en rouge.

Les données récoltées sur les médakas ont par ailleurs pu mettre en évidence des patrons de TSR (Fig.31). Même si ils illustrent l'existence de relations taille-température conformes aux règles existantes et à nos hypothèses, l'ensemble de ces travaux ne sont pas aboutis et il reste de nombreux développement à mettre en place pour mieux évaluer la prévalence des différents patrons décrit par le BEEF. Il était dans ce sens proposé dans ClimZise d'échantillonner des tissus (i) de gardons et de perches dans les plans d'eau français thermiquement contrastés et (ii) de médakas dans nos installations expérimentales afin d'évaluer les patrons de croissances (par scalimétrie et otolithométrie) et le nombre et la taille des cellules et du génome (en collaboration avec Dag Hessen).

Ces perspectives ne sont pas abandonnées même si elles n'ont pour l'instant pas reçu de financements et que mon planning pour les trois prochaines années au moins laisse peut de place à leur développement. Cependant, les avancées concernant la caractérisation des conditions thermiques passées, présentes et futures dans les plans d'eau de notre base m'ont poussé à commencer à animer un groupe de travail au sein de l'équipe sur les liens température-biologie en générale. Dans la mesure ou de nombreux travaux de l'équipe ne peuvent s'affranchir de la prise en compte de ce facteur, au moins en terme de facteur confondant, il est tout a fait possible que l'émergence de collaborations internes puisse faciliter ces travaux malgré mon emploi du temps chargé.

6.3 Axe2 – TSR, compromis ("trade-offs") d'allocation des ressources et stress oxydant

Publications associées : Hemmer-Brepson (2013); Hemmer-Brepson et al. (2014) ; Daufresne et al. (in prep).

L'objectif principal de cet Axe 2 réside dans la compréhension des mécanismes sous-jacents à la TSR (Fig. 35).

6.3.1 La TSR, un paradoxe évolutif ?

Les évidences d'une nature évolutive Les patrons de TSR tels qu'ils ont été initialement décrits sont dûs à de la plasticité phénotypique (deux individus issus de mêmes géniteurs et élevés à deux températures différentes vont montrer des patrons de TSR ; Atkinson 1994; Atkinson & Sibly 1997). Cependant, il existe deux types d'indices qui suggèrent la nature adaptative des relations négatives entre taille et température. Premièrement de nombreuses espèces d'ectothermes montrent des patrons géographiques de variabilité en taille, les individus des hautes altitudes ou latitudes présentant souvent des tailles supérieures, même lorsqu'ils sont élevés dans des conditions standardisées (Partidge & French 1996; Partridge & Coyne 1997). Deuxièmement, des expériences évolutives ont pu montrer que divers ectothermes produisaient par exemple des individus génétiquement plus grands après sélection à basse température (Partridge et al. 1994; Partidge & French 1996; Partridge & Coyne 1997). On ne peut donc pas exclure, au moins pour partie, la nature évolutive de la TSR, qu'elle soit liée à une pression de selection sur ses patrons et/ou sur leur plasticité.

Le paradoxe Si la nature évolutive de la TSR est très probable, la question de savoir pourquoi la sélection naturelle devrait favoriser une croissance plus rapide (plus lente) mais une maturité plus précoce/ à plus petite taille (plus tardive/ à plus grande taille) dans des environnements chauds (froids) reste entière. La plupart des modèles évolutifs d'optimisation de la fitness prédisent une taille à maturité plus grande dans les environnements qui favorisent la croissance (Berrigan & Charnov 1994; Sibly & Atkinson 1994; Atkinson & Sibly 1997). Cela a déjà été démontré pour la disponibilité de la ressource, les individus élevés dans des environnements où la ressource est abondante montrant des taux de croissances plus élevés et des maturités à plus grande taille.

Les modèles de Berrigan & Charnov (1994),Sibly & Atkinson (1994), et Atkinson & Sibly (1997) sont basés sur l'hypothèse d'un lien entre taille et fécondité, les grand individus produisant plus de juvéniles. Maturer plus gros dans un environnement où la croissance est faible signifie maturer beaucoup plus tard (tel que suggéré par la TSR). Cela accroît certes la fécondité mais le gain est en général trop faible par rapport au coût d'une probabilité de mortalité plus forte des juvéniles pour rendre cette stratégie optimale d'un point de vue évolutif (Berrigan & Charnov 1994; Sibly & Atkinson 1994; Atkinson & Sibly 1997). Différentes hypothèses ont été avancées pour expliquer cet apparent paradoxe évolutif, les plus nombreuses portant sur un effet négatif de la température sur la taille maximale des organismes. En effet, l'existence d'un compromis entre le taux de croissance initial k et la masse asymptotique m_∞ permet d'expliquer une maturité à plus grande taille dans les environnements où la croissance est lente et inversement (Berrigan & Charnov 1994; Perrin 1995).

De la taille des cellules et des organes Un premier groupe de mécanismes proposés pour expliquer la TSR repose sur des contraintes physiques pesant sur la taille des cellules à l'âge adulte. Ces mécanismes suggèrent que la taille des cellules doit être plus petite à forte température (i) à cause de différences de cinétique de croissance et de division cellulaire (Van der Have & deJong 1996), (ii) à cause de la décroissance de disponibilité d'oxygène dissous au centre de la cellule (Woods 1999) ou encore (iii) pour optimiser le ratio surface/volume et donc l'approvisionnement en oxygène (hypothèse MASROS, Atkinson et al. 2006).

Van der Have & deJong (1996) suggèrent que la réplication de l'ADN est plus sensible à la température ($Q_{10} \approx 2$) à cause d'une forte diffusibilité de l'ADN réplicase par rapport à celle des sous-unités ribosomiales qui sont beaucoup plus volumineuses. Cela implique une synthèse protéique moins sensible aux changement de températures ($Q_{10} \approx 1$). Dans les environnements chauds, la division cellulaire va donc être plus favorisée que la croissance cellulaire et les individus devraient être plus petits du fait de cellules plus petites (la "cell size shift hypothesis" du BEEF ; Fig. 32). Il est important de noter que cette hypothèse est purement physique et que Van der Have & deJong (1996) suggèrent clairement que les patrons de TSR ne sont pas de nature adaptative. Cela limite la porté de cette hypothèse. Il est reconnu que les ectothermes soumis à des environnements chauds sont en général plus petits à cause de cellules plus petites, même si il existe de nombreuses exceptions (Arendt 2007). Cependant, il est clair que la taille d'un organisme dépend aussi du nombre de cellules (Arendt 2007) et cette hypothèse ne permet pas d'expliquer le paradoxe adaptatif : pourquoi les individus ne maturent pas à des tailles plus grandes du fait d'un nombre plus important de cellules (même si leur taille est plus petite) ? En d'autres termes, cette hypothèse ne pourrait être valide que si la taille adulte était déterminée par un nombre fixe de cellules, ce qui n'est pas le cas pour la plupart des organismes (Atkinson & Sibly 1997; Angilletta et al. 2004).

L'hypothèse de Woods (1999) offre des perspectives évolutives différentes. Selon l'auteur, la température influence plus la consommation d'oxygène ($Q_{10} \approx 1.4$) que sa diffusion ($Q_{10} \in [1.5, 4]$) et des cellules trop grosses dans un environnement chaud devraient voir leur fonctionnement altéré. Les organismes ayant de petites cellules devraient donc être sélectionnés dans les environnements chauds, soit parce que leurs fonctions biologiques vont être moins, ou pas, altérées, soit parce que leurs oeufs vont montrer une meilleure survie. Cette dernière assertion repose sur l'hypothèse que des petits oeufs vont donner naissance (ou être produit) par des individus ayant des petites cellules. Si il existe des évidences de liens entre taille des oeufs et taille des adultes, il n'y a à ma connaissance cependant pas d'étude montrant un lien entre taille des oeufs et taille des cellules des adultes. De plus, comme l'hypothèse de Van der Have & deJong (1996), ces mécanismes ne permettent pas non plus de comprendre pourquoi les individus ne pourraient pas compenser cette décroissance en taille des cellules par une augmentation de leur nombre. De ce point de vue, la MASROS est plus subtile. Elle repose également sur l'hypothèse d'une limitation par l'oxygène mais ne se limite pas au problème de taille des cellules mais plus généralement à la taille des organes et de l'individu entier. La taille et la forme d'un organe doit être optimale pour assurer sa fonction et son approvisionnement en oxygène. De même la taille, la forme et le système respiratoire et circulatoire d'un organisme doivent être optimals pour assurer une distance minimale entre la source d'oxygène et les zones de consommation. Cette hypothèse n'est donc pas sensée s'appliquer de manière identique en fonction des tissus, des organismes et de l'ontogénie. Malheureusement, elle est de ce fait complexe, repose sur de nombreuses sous-hypothèses et est difficilement testable.

De manière générale, ces hypothèses ont donc des limites. Elles permettent en particulier difficilement d'expliquer la nature à la fois plastique et issue de divergence génétique des patrons de TSR. Cependant ces hypothèses me paraissent intéressantes et nous ne pouvons pas omettre le fait que les génomes, les cellules, et divers organes ont effectivement tendance à être plus petits quand la température augmente (Hessen et al. 2013, Fig. 32). De plus, ces hypothèses pourraient expliquer pourquoi les organismes aquatiques semblent plus sensibles à la TSR que les organismes terrestres (Forster et al. 2012). En effet, la solubilité de l'oxygène dans l'eau décroît avec la température et il existe une relation négative entre température et concentration en oxygène dissous. L'oxygène étant un élément de base du métabolisme, sa limitation dans le milieu impacte les capacités des organismes et en particulier leur croissance (Atkinson et al. 2006; Portner 2006; Verberk et al. 2011; Verberk & Bilton 2011). Dans ce sens, je n'exclue pas dans l'avenir de travailler sur ces questions même si je n'ai pas privilégié cette piste pour l'instant, plus par manque de temps que d'intérêt. La contrainte de l'approvisionnement des cellules en oxygène est incontestablement une contrainte forte pesant sur les organismes et en particulier les organismes aquatiques.

De l'anabolisme et du catabolisme En dehors de ces travaux portant sur l'échelle intra-individuelle, d'autres travaux ont en parallèle considéré des contraintes à l'échelle individuelle pesant sur la variabilité des coefficients de l'équation de croissance de von Bertalanffy (Strong & Daborn 1980; Perrin 1995). Von Bertalanffy suggère que le taux de croissance ($\frac{dW}{dt}$, où W est la masse d'un individu) peut être exprimé comme la différence entre le taux de conversion de matière et d'énergie en masse (ou l'anabolisme, aW^m) et le taux de perte d'énergie et de masse (catabolisme, bW^n) :

$$\frac{dW}{dt} = aW^m - bW^n \tag{4}$$

où a et b sont les coefficients d'anabolisme et de catabolisme et m et n sont des constantes. Von Bertalanffy (1960) suggère que la température doit peu impacter la disponibilité et l'acquisition d'énergie et de ressources mais beaucoup la consommation de ces dernières (ce qui rejoint les hypothèses de Woods (1999) ou Atkinson et al. (2006) mais à une échelle différente). Perrin (1995) considère que l'acquisition de matière et d'énergie est proportionelle à la surface de l'organisme ($m = \frac{2}{3}$) et que la consommation est isométrique ($n = 1$). Sous ces conditions d'invariance de m et n, Von Bertalanffy (1960) suppose que la sensibilité thermique relative de b doit être supérieure à celle de a. Sous cette hypothèse, Perrin (1995) démontre que l'équation 4 implique une relation négative entre k et l_∞. Comme je l'ai mentionné plus haut, il apparaît d'un point de vue adaptatif que la taille à maturité doit également être inférieure à haute température dans ces conditions (Sibly & Atkinson 1994; Atkinson & Sibly 1997).

Le travail de Perrin (1995) pourrait donc être une explication valable à la TSR. Cependant, l'hypothèse d'invariance de m et n est très discutable. Strong & Daborn (1980) considèrent par exemple que m décroît avec la température tandis que n croît et que ces exposants ne sont pas fixes. Au chaud, $m < n$ et il est intéressant de maturer avant l'atteinte d'une croissance trop faible (c.-à.-d. quand le gain de fécondité par la taille est inférieur au risque d'accroissement de la mortalité). Inversement, au froid, $m > n$ et la croissance croît avec l'âge. Il est alors intéressant de maturer plus tard. Strong & Daborn (1980) ont pu montrer une telle variabilité de m et n expérimentalement. Cependant, les cas observés de TSR correspondent par définition à des cas où la croissance décroît avec l'âge et donc où $m < n$.

Il est en réalité probable que ni les coefficients ni les exposants de l'équation 4 ne soient constants et qu'ils varient en fonction des conditions environnementales (Kozlowski et al. 2004). Il est également possible que l'équation 4 ne représente que mathématiquement les courbes de croissance mais ne soit pas liées aux processus sous jacents qui doivent inclure des compromis d'allocation des ressources vers la croissance et la reproduction et que ces compromis changent avec l'ontogénie et la mortalité (Czarnoleski & Kozlowski 1998).

Des compromis d'allocation des ressources Kozlowski et al. (2004) reprend les composants de l'équation 4 mais ne considère pas qu'ils décrivent le taux de croissance mais le taux de production $P(w)$, où :

$$P(w) = aW^m - bW^n \tag{5}$$

Il existe une taille optimale de production si $m \leq n$. Kozlowski et al. (2004) considère que ce taux de production est utilisé pour assurer la croissance et/ou la reproduction et il cherche à déterminer les stratégies optimales d'allocation de cette production vers ces deux fonctions. La taille optimale à maturité (w_{opt}, c'est à dire la taille à laquelle la production doit être attribuée à la reproduction pour optimiser le succès de reproducteur R_0) doit satisfaire la condition :

$$\frac{d[\frac{P(w_{opt})}{m(w_{opt})}]}{dw_{opt}} = 1 \tag{6}$$

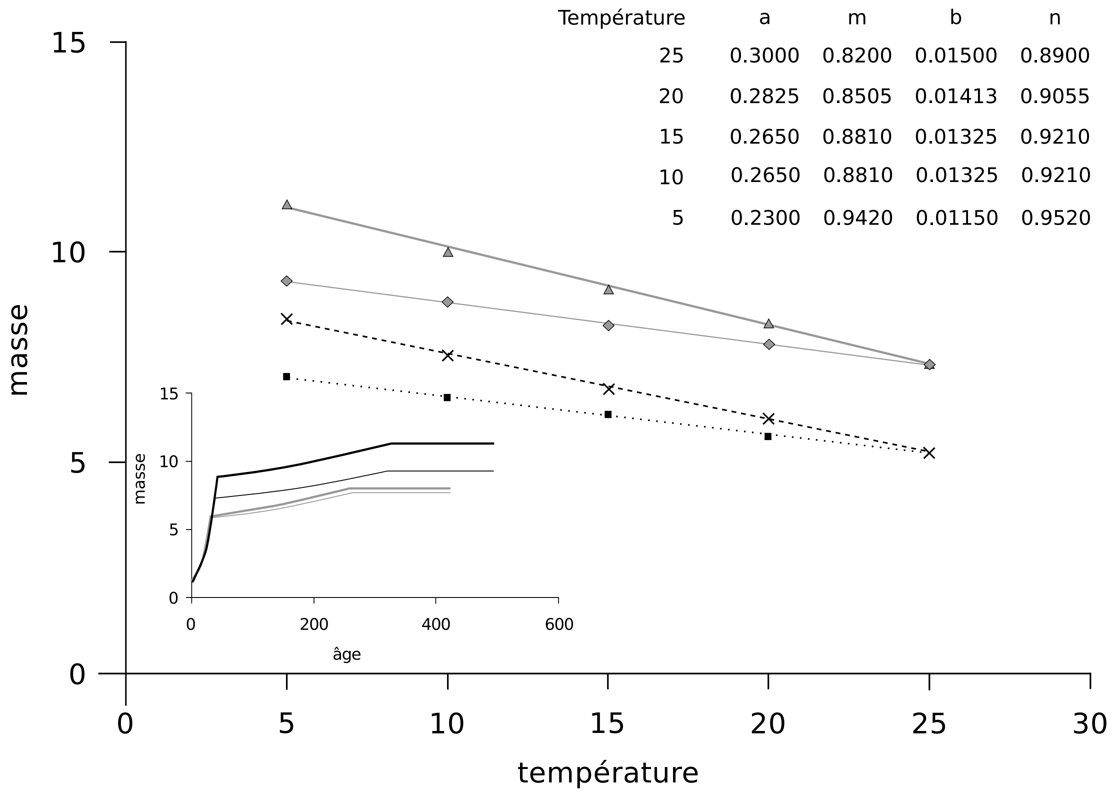
où $m(w)$ représente le taux de mortalité (Perrin & Sibly 1993; Kozlowski et al. 2004). Cette condition est nécessaire mais pas suffisante et il convient également que la fonction $\frac{P(w)}{m(w)}$ soit convexe au voisinage de w_{opt}. L'attribution de la production à la reproduction peut être exclusive (la croissance stoppe instantanément) sauf si $P(w)$ et $m(w)$ sont croissantes avec w ou si la maturation des organes reproducteurs n'est pas instantané (on a alors une période transitoire où la production est attribuée à la croissance et à la reproduction). Le modèle peut être utilisé dans un environnement où la reproduction est saisonnière ou non.

Dans son étude, Kozlowski et al. (2004) étudie les liens entre taille à maturité optimale et température en jouant (i) sur la variabilité thermique des coefficients et exposants de l'équation du taux de production (Eq. 6) et (ii) sur la variabilité thermique et en taille de la mortalité (voir l'exemple de la Fig. 39). Globalement, le modèle de Kozlowski et al. (2004) permet de montrer qu'il existe de nombreux cas où la TSR peut être observée et en particulier lorsque la mortalité est constante (c'est à dire indépendant de la masse et de la température) mais faible, ou bien qu'elle croît fortement avec la température.

Malgré son intérêt, l'approche de Kozlowski et al. (2004) souffre selon moi d'un manque de bases théoriques biologiques qui se traduit par (i) l'absence d'hypothèses claires relatives à la variabilité des différents paramètres du modèle et (ii) des lacunes dans l'appréhension des trade-offs d'allocation des ressources. Ce dernier point vient essentiellement de la non prise en compte explicite des fonctions d'entretien et de leur lien potentiel avec la mortalité. Cela est d'autant plus dommageable que le paramètre mortalité a une très grande influence sur l'émergence des patrons de TSR. Malgré ces limites, ce travail est remarquable dans la mesure où il assoit l'importance de la prise en compte des trade-offs d'allocation des ressources dans l'étude de la TSR et qu'il permet de souligner l'importance de la mortalité dans l'appréhension des ces patrons.

6.3.2 Sénescence, stress oxydant et disposable soma theory (DST)

Sénescence La sénescence est généralement définie comme étant une augmentation inévitable et irréversible de dommages avec l'âge qui conduisent à une perte de fonctions et éventuellement à la mort (Monaghan et al. 2008). La mortalité est donc le fruit de la sénescence mais celle ci peut également être appliquée à des fonctions non vitales et en particulier la fécondité. Pour la plupart des organismes, la fécondité montre une première phase d'augmentation avec l'âge après son acquisition, atteint un maximum, puis diminue jusqu'à éventuellement devenir nulle pendant un temps plus ou moins long avant la mort de l'organisme. Outre les limites que j'ai pu souligner, le modèle de Kozlowski et al. (2004) souffre également du fait qu'il ne prend pas explicitement en compte la sénescence. Dans son modèle, la fécondité dépend de la production qui n'est pas supposée décroître avec l'âge. Dans une approche conceptuellement proche de celle de Kozlowski et al. (2004), Kindlmann et al. (2001) montre que les patrons de TSR peuvent résulter d'une optimisation de l'allocation des ressources quand la production somatique et gonadique



Température	a	m	b	n
25	0.3000	0.8200	0.01500	0.8900
20	0.2825	0.8505	0.01413	0.9055
15	0.2650	0.8810	0.01325	0.9210
10	0.2650	0.8810	0.01325	0.9210
5	0.2300	0.9420	0.01150	0.9520

FIGURE 39 – Exemple de résultats du modèle de Kozlowski (d'après la Fig. 7B de Kozlowski et al. 2004). Effet de la température sur la taille à maturité (courbes en pointillées) et taille maximale (courbes en traits pleins) lorsque la reproduction est non saisonnière et qu'il existe une allocation graduelle de la production ($P(w)$) vers la reproduction du fait d'une production et d'une mortalité ($m(w)$) croissante avec la masse. Les courbe en traits fins représentent les résultats obtenus quand seul les paramètres de production sont température dépendants (voir les valeurs du tableau inclus) et les courbes en gras représentent les résultats lorsque la mortalité est température dépendante (q croît linéairement avec la température depuis 0.0085 à 5°C jusqu'à 0.01 à 25°C). T représente la longévité maximale et diminue linéairement avec la température de 496 unités à 5°C à 400 à 25°C. La figure insérée montre les courbes de croissance pour deux températures (courbes en gris pour la température chaude).

sont sujettes à la sénescence. Selon (Kindlmann et al. 2001), la production somatique (P) est allométriquement dépendante de la masse somatique ($P = nas^{\alpha}$; avec a le taux d'assimilation, s la masse somatique et n une fonction de sénescence) et cette production va être investie soit dans la croissance de la masse somatique ($s' = Pu$), soit de celle de la masse gonadique ($g' = P(1-u) - Mg$, où M est le taux de conversion de la masse gonadique en progéniture et g la masse gonadique, le produit des deux représentant la fécondité). Contrairement à Kozlowski et al. (2004), il considère donc clairement les deux types de tissus et leur sénescence. Sous certaines conditions ($n = e^{-dt^2}$; $u = 1$, $n = 1$ avant l'âge à maturité D ; $u = 0$ ensuite ; $s(0) = 1$, $g(0) = 0$, $g(D) = 1$; taux de mortalité λ constant), il calcule ensuite l'âge et la taille à maturité qui optimisent le taux de croissance r de la population. Sous ces conditions, il montre que si la température augmente simultanément et proportionnellement le taux de sénescence (d), le taux d'assimilation (a) et le taux de conversion gonadique (M), une maturité précoce est favorisée à haute température parce qu'elle permet la reproduction avant que la sénescence n'affecte trop la fécondité (Fig. 40).

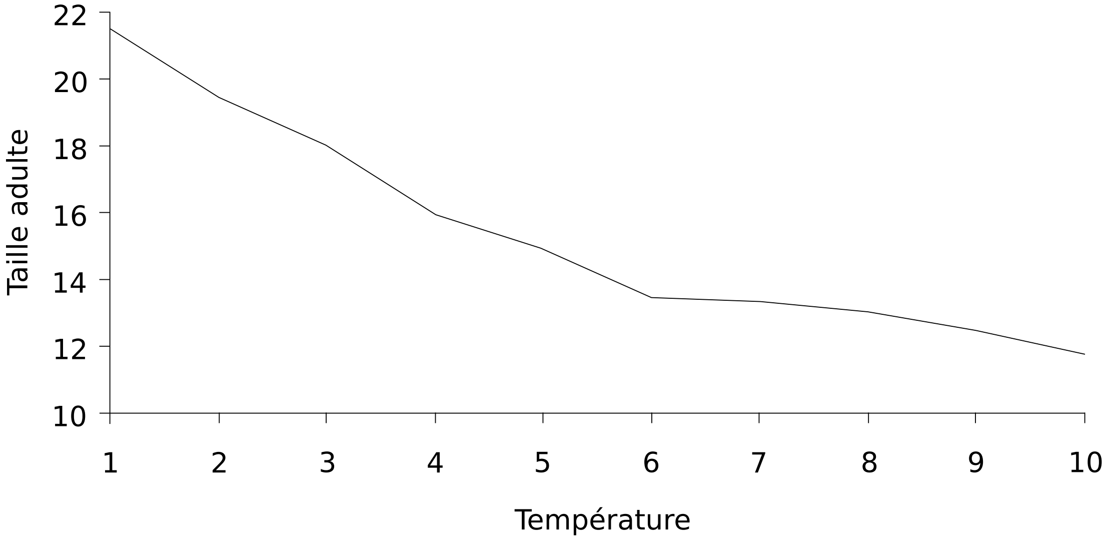


FIGURE 40 – Exemple de résultats du modèle de Kindlmann (d'après la Fig. 4B de Kindlmann et al. 2001). Taille adulte en fonction de la température, c'est à dire lorsque le taux de sénescence (d), le taux d'assimilation (a) et le taux de conversion gonadique (M) augmentent simultanément et proportionnellement ($a = M = 3d$ et d variant de 0.5 à 1).

Kindlmann et al. (2001) utilise r comme paramètre d'optimisation et la fonction de production est relativement différente de celle de Kozlowski et al. (2004) (elle est somatique et n'est pas construite comme un bilan d'énergie). Cependant, si on considère que l'hypothèse d'une augmentation des taux de sénescence, d'assimilation et de conversion gonadique sont raisonnables, le modèle permet de montrer une plus grande généralité des patrons de TSR. En particulier, aucune hypothèse relative au taux de mortalité n'est nécessaire. Malgré tout, les hypothèses exposées sont assez fortes et discutables et, comme Kozlowski et al. (2004) il y a des lacunes dans l'appréhension des trade-offs d'allocation, la fonction d'entretien étant totalement négligée alors que c'est, avec les fonctions de croissance et de reproduction, une des fonctions clés de la théorie des traits d'histoire de vie et des compromis d'allocation des ressources (Kirkwood 1987; Kirkwood & Rose 1991; Roff 1992; Stearns 1992; Reznick 2010; Monaghan et al. 2009; Selman et al. 2012). Cela est d'autant plus surprenant que le travail de Kindlmann et al. (2001) focalise explicitement sur la sénescence. Il aurait également été intéressant de lier sénescence et mortalité, ce qui n'a pas été fait dans cette étude. Enfin, aucun mécanisme n'est proposé pour expliquer le lien positif entre température et sénescence. Malgré ces limites le travail de Kindlmann et al. (2001) est, pour moi, remarquable dans la mesure où il permet de souligner l'importance (i) des trade-offs d'allocation, (ii) de la distinction soma/germen et (iii) de la sénescence. Complémentaires, les papiers de Kindlmann et al. (2001) et Kozlowski et al. (2004) offrent d'après moi des bases de réflexion très intéressantes et permettent de faire le lien entre la TSR et des théories d'écologie générales liées aux traits d'histoire de vie. Relativement peu cités et malgré leurs limites, ils sont très liés à mes activités actuelles.

DST et stress oxydant De nombreux travaux ont porté sur la nature évolutive de la sénescence, c'est à dire le pourquoi évolutif de son existence (Kirkwood 1987; Kirkwood & Rose 1991; Monaghan et al. 2008). Il existe trois grandes théories, non mutuellement exclusives, pour expliquer cette nature évolutive. La première suggère que, comme les individus ont des chances de mourir de causes extrinsèques (prédation, maladie, accident) et que cette probabilité augmente avec le vieillissement, la force de sélection de la sénescence décroît avec l'âge. Une seconde théorie a développé cette idée en supposant l'existence de gènes pleiotropes antagonistes ayant des effets délétères aux âges avancés mais qui sont néanmoins sélectionnés car ils ont des effets positifs sur la survie et la fécondité des jeunes individus (Williams 1957). La troisième et dernière hypothèse, connue sous le nom de "disposable soma theory", est basée sur les compromis d'allocation des ressources à différentes fonctions et en particulier à la croissance, la reproduction et l'entretien du soma (Kirkwood & Holliday 1979; Kirkwood 1987; Kirkwood & Rose 1991). La DST suppose qu'il n'y a pas d'intérêt à entretenir le soma au delà d'une longévité qui peut raisonnablement être atteinte dans un environnement donné (Fig. 41 ; Monaghan et al. 2008) et que plus un environnement est contraignant, moins il est utile d'investir de ressources dans la maintenance (Fig.42B). Ce surplus d'énergie peut être investi dans une croissance rapide, une reproduction précoce et abondante, au coût d'une sénescence plus marquée (longévité réduite, déclin reproductif précoce ; Kirkwood & Rose 1991; Selman et al. 2012). En d'autres termes, un investissement qui donne un avantage aux jeunes stades va raisonnablement être favorisé par la sélection naturelle. Dans ce sens la DST peut être vue comme une version phénotypique de la pleiotropie antagoniste (Monaghan et al. 2008). Quoiqu'il en soit, ces théories soulignent l'existence d'un continuum rapide-lent ("fast-slow") dans les stratégies écologiques depuis une stratégie de type "live fast, die young" à une stratégie de type "live slow, die old", la position des espèces ou des populations le long de ce continuum résultant de l'action de la sélection naturelle dans les différents environnements (Ricklefs & Wikelski 2002; Selman et al. 2012). On retrouve donc globalement le classique continuum "r-K" mais avec des bases physiologiques explicites.

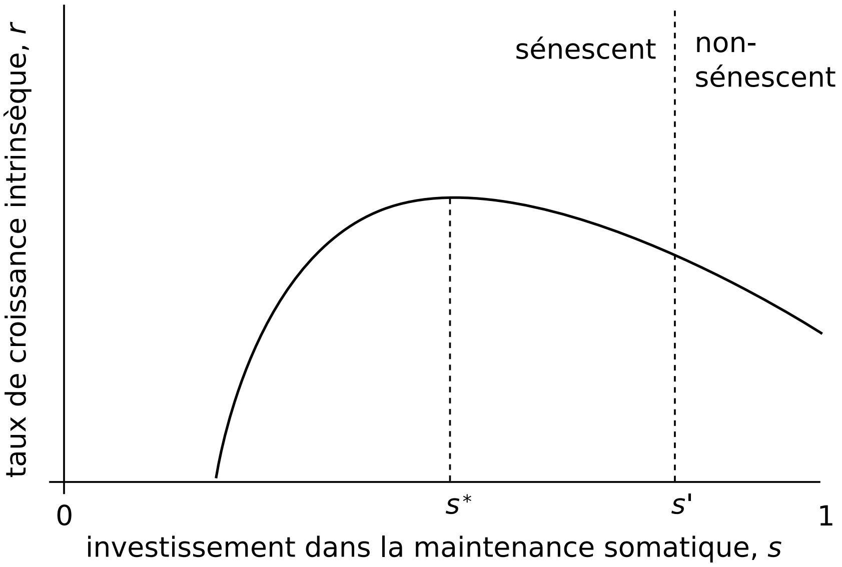
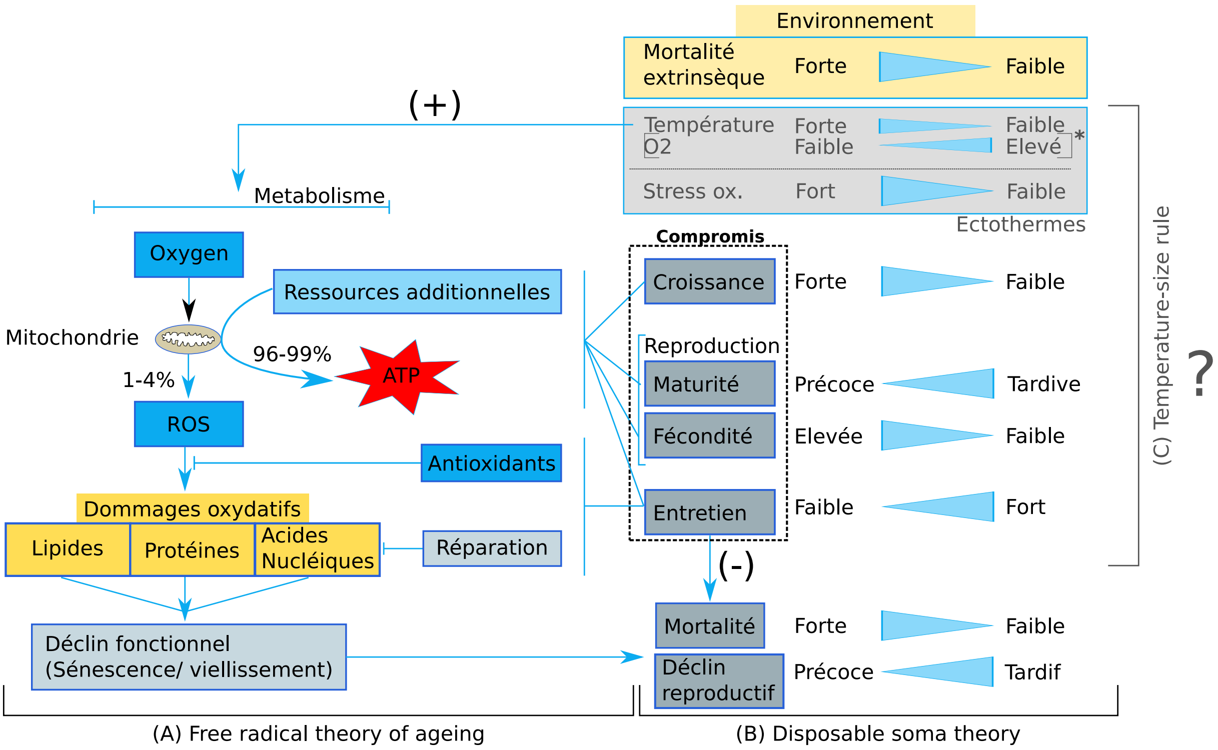


FIGURE 41 – Relation entre la fitness, mesurée comme le taux de croissance intrinsèque r, et le niveau d'investissement dans l'entretien somatique s, dont dépendent la mortalité adulte et la fécondité. Il n'y a pas de sénescence pour $s > s'$. L'optimum $s = s^*$ est prédit comme étant toujours inférieur à s'. (D'après Kirkwood & Rose 1991).

De nombreux facteurs sont susceptibles d'influencer la sénescence (Nemoto & Finkel 2004; Munch et al. 2008; Ricklefs 2008). Cependant, il est considéré depuis très longtemps que les dommages oxydatifs jouent un role majeur dans la sénescence et que ces dommages sont la conséquence de composés chimiques ayant une forte affinité pour l'oxygène : les radicaux libres, ou ROS (pour "reactive oxygen species") (Harman 1957; Monaghan et al. 2008, 2009; Selman et al. 2012). On parle de "free radical theory of ageing" (FRTA). Initialement énoncée par Harman (1957), la FRTA stipule qu'une forte production de ROS est liée à une sénescence accrue et une mortalité précoce, même si il existe des exceptions à la règle (Selman et al. 2012). Remise dans le contexte de la DST, la FRTA suggère que les ROS peuvent être des médiateurs importants des stratégies d'histoire de vie et de nombreuses études tendent à confirmer ces liens

(Fig. 42, voir par exemple Monaghan et al. 2008; Cohen et al. 2008; Monaghan et al. 2009; Selman et al. 2012; Hemmer-Brepson 2013). Le niveau d'investissement s de la DST est alors directement traduit comme un investissement dans la production d'antioxydants (défense) et la réparation des dégâts oxydatifs, et cet investissement va être mis en balance avec les fonctions de croissance et de reproduction.



*: milieux aquatiques

FIGURE 42 – Liens entre la free radical theory of ageing (FRTA, Harman 1957) et la disposable soma theory (DST, Kirkwood & Holliday 1979) et implications potentielles pour la temperature size rule (TSR, Atkinson 1994). (A) La FRTA stipule que les radicaux libres (ROS) produits lors de la synthèse d'ATP dans la mitochondrie entrainent des dégâts responsables de la sénescence et éventuellement de la mort. (B) La DST suppose qu'il existe un lien entre la mortalité extrinsèque et les compromis d'allocation des ressources vers les fonctions d'entretien du soma, de la reproduction et de la croissance. Si la mortalité extrinsèque est forte, il est inutile d'investir dans l'entretien et le surplus d'énergie est investi dans les autres fonctions au prix d'une sénescence et d'une mortalité intrinsèque forte. (C) Lien possible avec la TSR. L'augmentation de la température entraînerait une augmentation du stress oxydant qui serait à l'origine d'une sénescence accrue (mortalité élevée, déclin reproductif précoce). Cela pourrait générer des patrons de DST, c'est à dire, en plus des patrons de TSR liés à la croissance et à l'âge à maturité, une fécondité élevée et un entretien du soma faible au chaud (une stratégie de type “live fast, die young”).

Outre le fait d'offrir des piste mécanistes, l'inclusion des ROS dans cette théorie apporte de nouveaux éclairages. En particulier, un investissement fort dans les fonctions de reproduction et de croissance ont tendance à augmenter la production de ROS et les dégâts associés (Alonso-Alvarez et al. 2004, 2006, 2010; Christe et al. 2012; Rollo 2002; Salin et al. 2012). Un exemple bien connu est la “croissance compensatoire” qui survient si un stress est subi au début de l'ontogénèse (Metcalfe & Monaghan 2001). Le fort investissement vers la croissance va jouer sur la quantité de ROS produits et expliquerait une longévité réduite (Metcalfe & Monaghan 2001;

Alonso-Alvarez et al. 2007; Bize et al. 2008; Criscuolo et al. 2008, 2011). Dans ce sens, le coût d'une diminution des fonctions d'entretien (pour investir dans la reproduction ou la croissance) est probablement plus marqué qu'initialement imaginé dans la DST.

6.3.3 La TSR, un cas particulier de la DST ?

Il est légitime de poser l'hypothèse que la température pourrait entraîner une sénescence précoce chez les ectothermes du fait d'une production accrue de ROS. En effet, le métabolisme est directement lié à la masse des individus et à la température interne selon :

$$B_i = b_0 e^{-E_i/kT} M_i^{\alpha} \tag{7}$$

$e^{-E_i/kT}$ est le facteur de Boltzmann-Arrhenius où k représente la constante de Boltzmann ($8.62.10^{-5} eVK^{-1}$) et E_i est l'énergie d'activation de la voie métabolique i (Gillooly et al. 2001). Ei vaut typiquement en moyenne 0.65 eV pour la respiration hétérotrophe. La temperature interne étant directement liée à la température externe chez les ectothermes, un réchauffement environnemental se traduit par une augmentation du métabolisme. Les liens entre accroissement du métabolisme et accroissement de la production de ROS est toujours débattu (Monaghan et al. 2009; Salin et al. 2015). Cependant, quelques récentes études se sont intéressées à l'effet de la température et de la désoxygénation sur la production de ROS chez des espèces ectothermes aquatiques (Abele et al. 1998, 2002; Lushchak & Bagnyukova 2006a,b; Abele et al. 2011; Clotfelter et al. 2013; Almroth et al. 2015; Simcic et al. 2015). Ces études semblent montrer un effet positif de la température sur la production radicalaire (Abele et al. 2002) et les dégâts dûs aux ROS (Lushchak & Bagnyukova 2006a; Almroth et al. 2015) malgré une activité accrue des enzymes antioxydantes (Abele et al. 1998; Lushchak & Bagnyukova 2006b; Clotfelter et al. 2013; Almroth et al. 2015; Simcic et al. 2015). Selon la FRTA, cela fourni un mécanisme sous-jacent à l'hypothèse de Kindlmann et al. (2001) concernant l'augmentation de la sénescence avec la température.

L'accroissement de la sénescence malgré une augmentation de l'activité des enzymes antioxydantes (fonction d'entretien) permet de replacer l'effet d'un réchauffement dans le cadre de la DST. Les organismes n'investissent semble-t-il pas assez dans l'entretien pour compenser les effets délétères de la surproduction radicalaire et le surplus métabolique au chaud pourrait être investi dans les fonctions de croissance et de reproduction. La croissance rapide et la maturité précoce de la TSR pourraient donc être des patrons de DST (Fig. 42C) dont la version minimaliste (c.-à-d. sans prise en compte de l'investissement dans la fonction d'entretien) a été explorée par Kindlmann et al. (2001). Tenter d'explorer l'hypothèse selon laquelle la TSR serait un cas particulier de la DST contribuerait :

- Du point de vue de la TSR, d'apporter, en dehors d'une croissance initiale forte et d'une maturité précoce, les prédictions supplémentaires que sont (i) une sénescence accrue (mortalité, précocité d'un déclin reproductif), (ii) une fécondité élevée et (iii) un entretien faible du soma au chaud.
- Du point de vue de la DST, d'affiner la notion de sur-mortalité. En effet le modèle de Kirkwood & Rose (1991) distingue explicitement la mortalité adulte extrinsèque (ou "environnementale") de la mortalité adulte intrinsèque, conséquente de la sénescence (la mortalité juvénile est considérée constante). En réalité des facteurs environnementaux pourraient également jouer sur la mortalité intrinsèque (même si la désignation perd un peu de son sens dans ce cas !). On sait par exemple que des polluants chimiques peuvent augmenter la production de ROS et impacter la mortalité (Valavanidis et al. 2006; Bonisoli-Alquati et al. 2010; Agarwal et al. 2012; Blahova et al. 2013). Il est difficile de savoir jusqu'à quelle point il peut être intéressant d'un point de vue évolutif d'investir dans l'entretien pour pallier à une sur-mortalité intrinsèque mais il est très probable que la DST puisse s'appliquer au moins à un degré élevé de sur-mortalité. La TSR pourrait montrer un exemple de DST

où la sur-mortalité est certes due à une cause extrinsèque mais est directement lié à la sénescence.

La question du lien entre DST et TSR a occupé, occupe, et va occuper une part relativement importante de mon activité. Pour l'heure cette question est au coeur de trois thèses que j'ai pu encadrer de manière plus ou moins proche, la thèse de Claire Hemmer (2010-2013), celle d'Ayala Loisel (2015-2018) et celle de Lisandrina Mari (2015-2018).

6.3.4 Matériels et Méthodes

Trois approches distinctes ont été et vont être utilisées pour aborder la question d'un lien entre TSR et DST, l'une purement expérimentale et basée sur le médaka (*Oryzias latipes*) comme modèle biologique, une autre de terrain et basée sur l'analyse de données récoltées autour des CNPE de Bugey, Tricastin, Cattenom et de la centrale thermique d'Aramon, et une dernière à la fois expérimentale et de terrain et basée sur des données d'omble chevalier (*Salvelinus alpinus*) récoltées sur divers lacs. Je détaillerai ici principalement les travaux relatifs aux deux premières approches et n'aborderai les questions liées à l'omble que comme des perspectives dans la mesure où nous ne disposons pas de résultats préliminaires sur cette partie.

FIGURE 43 – Photos des installations expérimentales. Les poissons (*Oryzias latipes*) sont élevés à la densité d'environ un poisson/litre en circuit ouvert dans deux conditions thermiques différentes (20°C et 30°C), avec 6 aquariums (40x20x25 cm) par condition. Les saturations en O_2 sont maintenues à 100% à l'aide de bulleurs. Le pool de géniteurs utilisé pour les expériences était constitué de 76 individus, dont 46 femelles et 30 mâles. Tous ces poissons étaient de souches CAB qui, bien que présentant des colorations spécifiques, ne montrent pas de différences notables par rapport à la souche naturelle d'un point de vue de la croissance et de la reproduction (Sasado et al. 2010; Lawrence et al. 2012). La mise en place de ces installations c'est accompagnée d'un gros travail administratif et législatif en rapport avec les contraintes de l'expérimentation animale.

installations expérimentales Deux lignées de médakas sont élevées à deux températures non stressantes pour l'espèce (20°C et 30°C) depuis aujourd'hui une dizaine de générations (Fig 43). Le pool de géniteurs (F_0), élevé à 25°C, a été progressivement acclimaté aux températures d'élevage (augmentation/diminution de 1°C par 2 jours). Depuis cette génération, des mesures des traits d'histoire de vie et des statuts oxydatifs (dégâts, défenses) sont réalisées sur les individus des différentes générations (encadré 6.3.4) :

- F_0. 120-240 jours. L'effort de reproduction (taille moyenne journalière des pontes par femelle, taux d'éclosion, nombre moyen d'individus matures produit par femelle), la mortalité et les statuts oxydatifs après euthanasie (âge = 240 jours ; dégâts, défenses) ont été mesurés sur cette génération F_0 (Hemmer-Brepson et al. 2014). Lorsque les individus F_0 ont été euthanasiés (décembre 2011), les juvéniles contenus dans des "nassettes" ont été relâchés dans les aquariums parents et l'expérience sur la génération F_1 a commencé.

— F_1. 0-415 jours. Les lots initiaux ont été divisés en 2 groupes, l'un nourri *ad libitum* l'autre nourri un jour sur deux (=restriction calorique). Comme pour la génération précédente (i) l'effort et le succès de reproduction (ii) le taux de mortalité, (iii) la croissance et (iv) les statuts oxydatifs ont été mesurés (à 60 et 200 jours). Cependant, nous avons ici mesurés les statuts oxydatifs sur deux tissus distincts à 200 jours : (i) les muscles et (ii) les gonades afin de distinguer les lignées somatiques et germinales. Seule les femelles ont été utilisées.

— F_2-F_5. 0-60 à 100 jours. Les mêmes protocoles sont conservés pour les générations suivantes. Cependant, nous avons écourté les générations en euthanasiant les adultes de la génération n dès qu'il y avait assez de juvéniles en génération $n{+}1$ pour constituer un pool de géniteurs qui éviterait une trop grosse consanguinité (environ une 50aines/bac). Cela nous a permis d'avoir le plus rapidement possible un nombre important de générations. Les adultes obtenus à la fin de chaque génération ont été euthanasiés et stocké à -80 °C pour des mesures prochaines de balance oxydative.

— générations suivantes. Les autres générations ont été élevées comme la génération F_1 mais avec des mises à mort en général plus précoces et sans manipulation de la ressource (tous les individus sont nourris *ad-libitum*). De plus, un déménagement des installations expérimentales vers des chambres climatiques à été effectué durant l'été 2016.

Encadré 6.3.4 : Mesures expérimentales

Croissance Les mesures de croissance sont réalisées à 30, 45, 60, 100, 150, 200 et 300 jours suivant les premières éclosions sur 15 individus pris au hasard dans chacun des aquariums. Des modèles de croissance de von Bertalanffy sont ajustés aux données :

$$L_t = L_\infty(1 - e^{-k(t-t_0)}) \tag{8}$$

où L_t est la taille estimée au temps t, L_∞ est la taille asymptotique, k représente le taux de croissance initial et t_0 est l'âge théorique pour une taille nulle. Le package R "fishMethods" (fonction "vblrt" ; v. 1.9-0 ; Nelson 2015), basée sur une méthode de régression non linéaire avec estimation par moindre carrés non linéaires, a été utilisée pour ajuster les modèles. Des tests de rapport de vraisemblance ont ensuite été utilisés pour évaluer la significativité des différences entre les paramètres des modèles entre les traitements (Kimura 1980).

Effort de reproduction Nous avons utilisé différentes variables pour évaluer l'effort de reproduction :

— La taille des pontes par femelle (x), calculée comme le nombre d'oeufs pondu par femelle et par jour dans un aquarium donné. Cette valeur pouvait ensuite au besoin être moyennée par jour ou par condition.

— Le taux d'éclosion (H) :

$$H = \frac{Nl}{Ne} \cdot 100$$

avec Nl pour le nombre total de larves observées dans l'aquarium et Ne le nombre total d'oeufs pondus dans cet aquarium.

— Le taux de survie jusqu'à la maturité (M_F) :

$$M_F = \frac{Nm}{Ne} \cdot 100$$

où Nm correspond au nombre total de descendants arrivés à maturité dans un aquarium donné.

Survie Les individus morts sont répertoriés tous les jours, nous permettant de calculer un taux de survie (Sr) dans chaque aquarium. Les indivdius sacrifiés pour les mesures physiologiques ne sont pas pris en compte dans le calcul de ce taux.

Dégâts causés par les ROS

- Dégâts sur les membranes. La méthode utilisée pour quantifier les dégâts sur les membranes, connue sous le nom de méthode des “Tbars”, suit le protocole initialement proposé par Yagi (1976) et adapté pour des espèces ectothermes (Salin 2011; Lawniczak et al. 2013). Basée sur la détection de manolaldehyde (MDA) produit lors de la peroxydation des lipides, cette technique est souvent utilisée pour déterminer les dégâts causés par les ROS sur les membranes, notamment chez les poissons (Parihar & Dubey 1995; Mila-Kierzenkowska et al. 2005; Hemmer-Brepson et al. 2014; Almroth et al. 2015). Plus de détails sont présentés dans Hemmer-Brepson et al. (2014).
- Dégâts sur les protéines. Pour mesurer la part de protéines dégradées par les ROS, nous avons utilisé un kit de la marque ©Cayman (Protein Carbonyl Assay Kit, N° 10005020). Ce kit nous permet de définir la quantité de protéines carbonylées sur plasma, sérum, urine et homogénats de tissus. Nous nous sommes focalisés sur des homogénats de toutes les fibres musculaires (sans distinction). Le système de détection est basé sur le fait que lorsqu'un ROS oxyde une protéine il y a formation d'un radical carbonyle. Ce radical carbonyle peut réagir avec le DNPH (2,4- dinitrophenylhydrazine) du milieu réactionnel pour former de l'hydrazone. C'est cette dernière qui va être dosée avec un spectrophotomètre à 370 nm. Ces mesures n'ont pas été effectuées pour toutes les générations.

Défenses anti-oxydantes

- Activité de l'enzyme superoxyde dismutase (SOD). L'enzyme SOD permet la formation de peroxyde d'hydrogène à partir d'anion superoxyde (Martinez-Alvarez et al. 2005). Le dosage de son activité se fait le plus souvent de manière indirecte (Salin et al. 2012; Lawniczak et al. 2013). Nous avons utilisé le système xanthine / xanthine-oxydase comme producteur d'anion superoxyde ($O_2^{\circ -}$). Celui-ci va alors réagir avec le cytochrome c oxydé contenu dans le milieu réactionnel. La désoxydation de ce cytochrome c a alors été suivie pendant 3 minutes afin de définir l'activité de la SOD en $U \cdot min^{-1}$
- Activité de l'enzyme glutathion peroxydase (GPx). L'enzyme GPx permet la formation d'eau à partir de peroxyde d'hydrogène. De ce fait elle intervient en aval de la SOD pour terminer le travail de détoxification. La mesure de l'activité de la GPx a été mesurée par spectrophotométrie en suivant l'oxydation du NADPH (contenu dans le milieu réactionnel) en NADP, à 340 nm. Ces mesures n'ont pas été effectuées pour toutes les générations.
- Activité de l'enzyme catalase (Cat). L'enzyme catalase produit également de l'eau à partir de peroxyde d'hydrogène mais cette enzyme est surtout présente dans les peroxysomes (Lemieux 2007). Son activité a été déterminée en mesurant la dégradation du peroxyde d'hydrogène par spectrophotométrie à 240 nm. Ces mesures n'ont pas été effectuées pour toutes les générations.

Centres de production électrique En dehors des approches expérimentales, nous avons également appréhendé la variabilité des balances oxydatives dans des environnements naturels thermiquement contrastés au cours de la thèse de Claire Hemmer-Brepson. Des gardons et des perches ont été prélevés en amont et en aval des CNPE de Bugey et de Tricastin ainsi que de la centrale d'Aramon (Fig. 44). Des mesures de dégâts (Tbars, dégâts sur les protéines) et de défenses antioxydantes (activité de la SOD, GPx, Cat. ; encadré 6.3.4) ont été effectuées sur les individus entiers (Bugey, Tricastin, Aramon) ou sur les muscles et les gonades des individus (Cattenom). Les échauffements de Bugey et de Tricastin sont relativement constants et de l'ordre de +10°C. Aramon a un fonctionnement plus erratique et l'échauffement oscille entre +1 et +11.5°C en fonction des besoins. Des individus ont également été prélevés à Cattenom dans la retenue du Mirgenbach (Fig. 44). Cette retenue a été creusée dans les années 80 et sert de réservoir d'eau de refroidissement pour la centrale. Du fait de la prise d'eau et du rejet de l'eau échauffée à un débit de 5m3/s pour les 4 tranches équipées d'aéroréfrigérants, l'eau de cette retenue est plus chaude que celle des hydrosystèmes aux alentours. Ces caractéristiques thermiques particulières en font un bon modèle d'étude des effets simulés du réchauffement climatique (Dembski et al. 2006; Masson et al. 2008; Valente 2008).

6.3.5 Résultats préliminaires

F_0 Les résultats de la génération F_0 sont disponibles dans Hemmer-Brepson et al. (2014). Globalement, nous avons pu montrer que les individus au chaud pondaient plus précocement

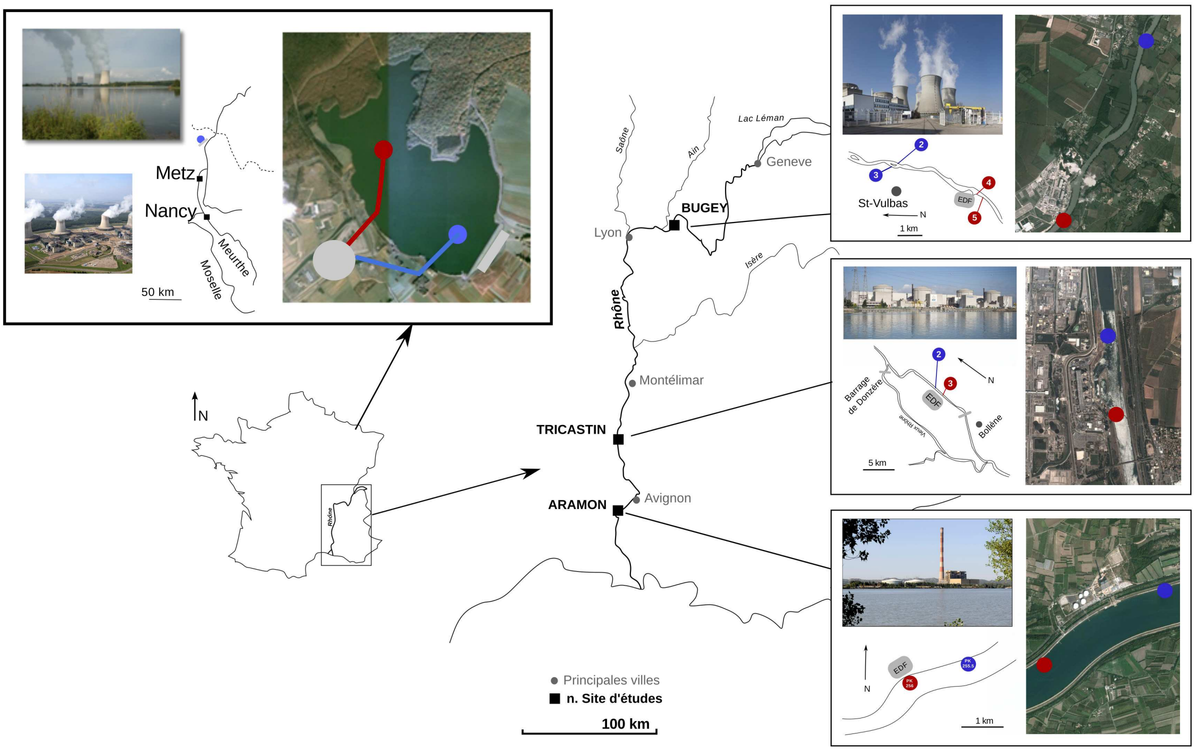


FIGURE 44 – Sites d'études de l'impact de la température sur les balances oxydatives de populations de perches et de gardons autour des centres de production électrique. Pour chaque site, les stations réchauffées (rouge) et de références (bleue) sont figurées.

et avec des pontes de plus petites tailles. Le taux d'éclosion H était aussi inférieur mais nous n'avons pas pu mettre en évidence des différences des taux de survie jusqu'à maturité M_F. Il est intéressant de noter que si l'activité de la SOD (défense) était supérieur au chaud, nous n'avons pas pu déceler de différences en terme de dégâts.

F_1 Les résultats de la génération F_1 sont plus intéressants dans la mesure où nous avons suivi cette génération et sa progéniture sur près de 400 jours. Les poissons élevés au chaud présentaient :

- Des patrons de croissance de type TSR, c'est à dire un taux de croissance initial significativement plus fort qu'au froid ($\chi^2 = 14.32$; $P < 0.001$) mais une taille asymptotique significativement plus petite ($\chi^2 = 25.96$; $P < 0.001$), de même qu'une taille à maturité plus petite (Fig. 45a).
- Un âge à maturité (Fig. 45a) et une sénescence reproductive plus précoce mais des tailles de ponte supérieures (Fig. 46a–b, Tab. 2).
- Un taux d'éclosion plus faible (Fig. 46c) et une mortalité après éclosion plus forte (Fig. 45b, Tab. 2), conduisant au final à un nombre de géniteurs produits par femelle très similaire à celui du traitement froid (Fig. 46c).

D'un point de vue de la balance oxydative, les poissons élevés au chaud présentaient :

- Des dégâts oxydatifs plus importants dans les muscles mais moins importants dans les gonades (Fig. 47, Tab. 2).
- Des niveaux de défense en opposition aux dégâts, avec des activités de SOD plus importantes dans les gonades et moins importantes dans les muscles (Fig. 47, Tab. 2).

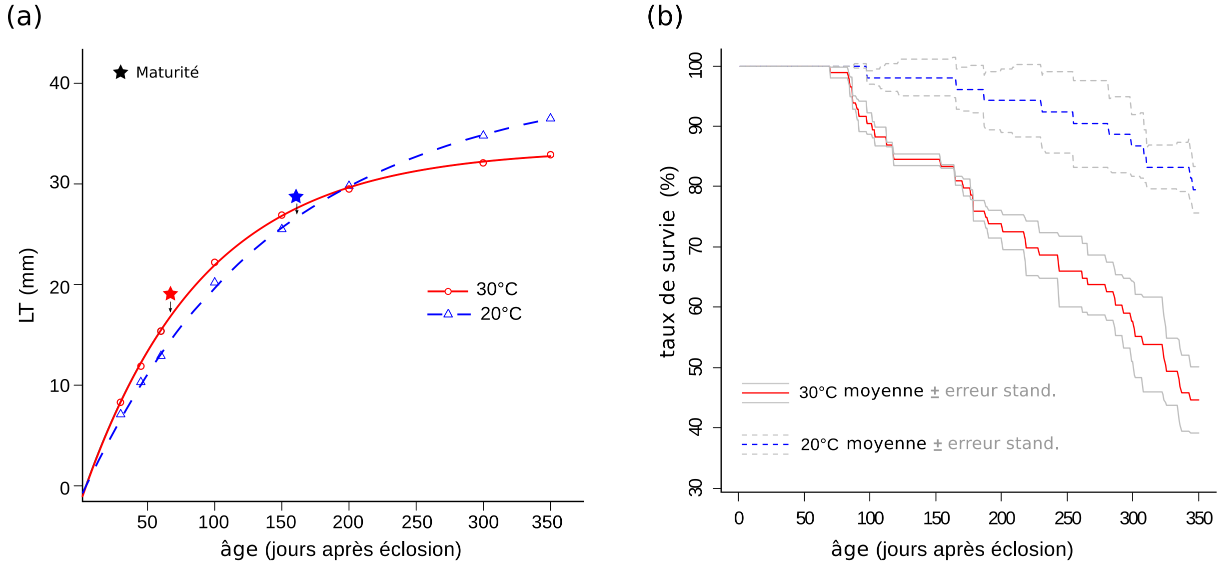


FIGURE 45 – Courbes de croissance (a) et de survie (b) des individus de la génération F_1 à 20 et 30°C. Des modéles de von Bertalanffy ont été ajustés aux données de croissance (voir encadré 6.3.4).

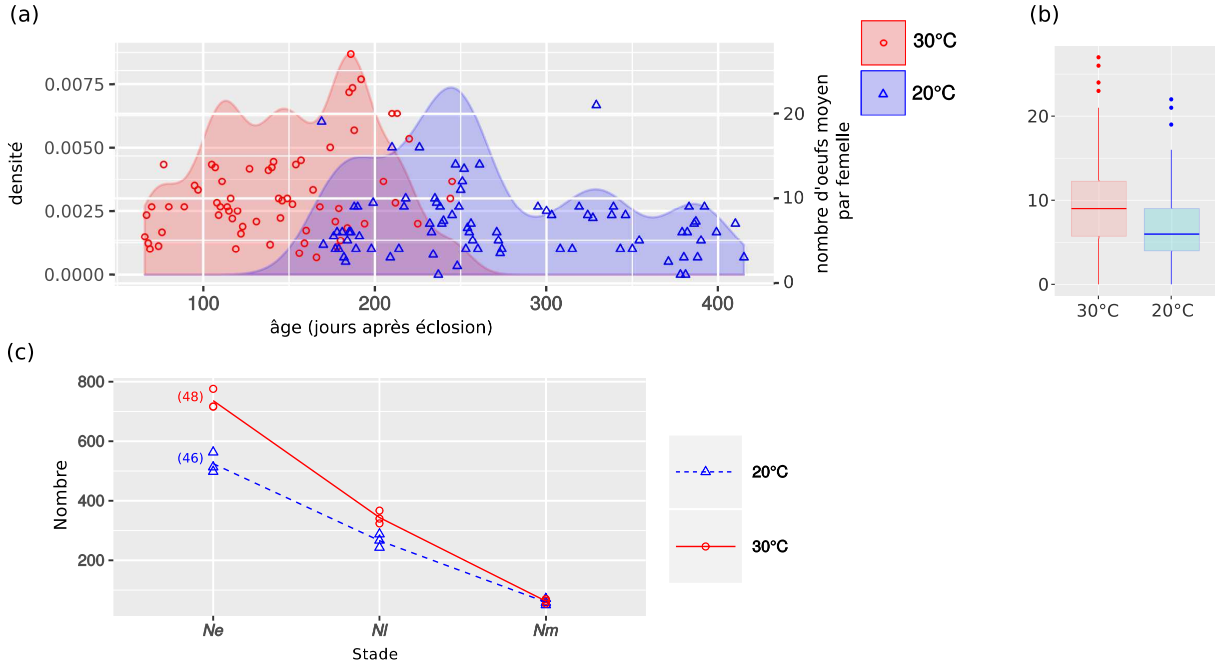


FIGURE 46 – Caractéristiques reproductives des individus de la génération F_1. (a) Nombre moyen d'oeufs pondus ($\bar{x}_{condi}$) et densité du nombre d'oeufs pondus (x) par jour et par condition. Les distributions des $\bar{x}_{condi}$ sont significativement différentes (χ^2-test, $P < 0.001$) (b) Boîtes à moustaches, par condition, du nombre d'oeuf moyen pondu par jour et par aquarium $\bar{x}_{condi \cdot aqua}$. (c) Nombres totaux d'oeufs (Ne), de larves (Nl) et d'individus matures (Nm) produits par femelles dans les deux conditions. Le nombre total de femelles est donné entre parenthèses.

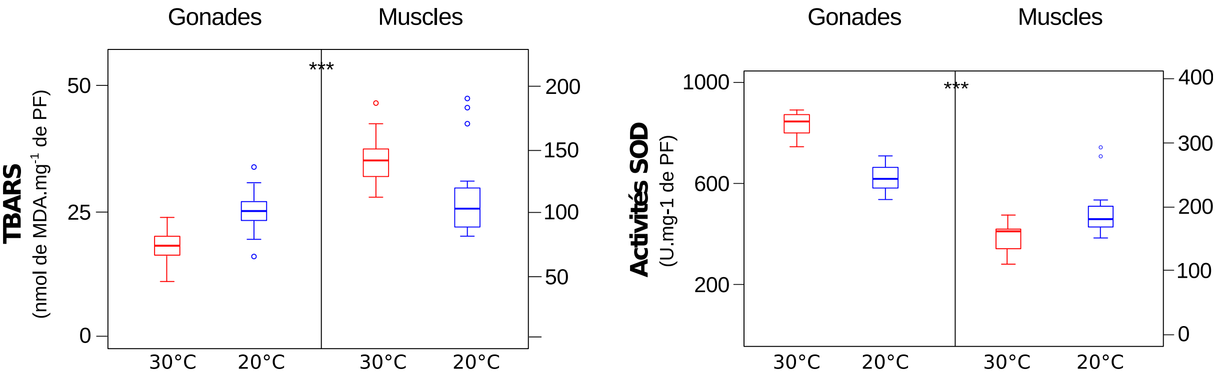


FIGURE 47 – Dégats oxydatifs sur les membranes et défenses anti-oxidantes pour les muscles et les gonades des individus de la génération F_1.

L'ensemble de ces résultats vont dans le sens de nos hypothèses. Il semble que la TSR s'accompagne de patrons typiques de la DST avec un investissement fort dans la défense du germen au dépend du soma et donc au prix d'une sénescence accrue/précoce. Au chaud, les individus grandissent plus vite, se reproduisent tôt, en investissant beaucoup dans la reproduction en terme d'oeufs produits et de défense des gonades au dépend de leur croissance post-maturation, de leur survie et de la pérénité de leurs capacités reproductives. En d'autres termes, le gradient chaud-froid s'accompagne d'un gradient "live fast, die young" - "live slow, die old" dans nos expériences.

TABLEAU 2 – Impacts de la température sur les différentes caractéristiques écologiques et biologiques des individus de la génération F_1.

Fonction	n (F ;C)	Variable dep.	Effets aléatoires		Effets fixes		P
			effets	é.t.	effets	estim.	
Reprod.	190(75 ;85)	$\bar{x}$	Réplicats	1.38	Ord. orig.	10.47	0.000 ***
			Date	0.04	T°C	-3.64	0.004 **
Survie	164(80 ;84)	Taux de survie	Réplicats	4.36	Ord. orig.	107.09	0.000 ***
					T°C	-3.25	0.296 NS
					Date	-0.17	0.000 ***
					T°C x Date	0.102	0.000 ***
Dégâts	62(31 ;31)	TBARS	Réplicats	5.96	Ord. orig.	18.48	0.005 **
					Tissu	122.99	0.000 ***
					T°C	4.88	0.577 NS
					Tissu x T°C	-32.94	0.002 **
Défenses	62(31 ;31)	SOD	Réplicats	0.06	Ord. orig.	831.86	0.000 **
					Tissu	-678.41	0.000 ***
					T°C	-214.36	0.000 ***
					Tissu x T°C	255.50	0.000 ***

n : taille de l'échantillon ; C : traitement 30°C ; F : traitement 20°C ; Ord. orig. : ordonnée à l'origine ; é.t. : écart type ; Reprod. : reproduction ; NS : non significatif ; * : $P < 0.05$; ** : $P < 0.001$; *** : $P < 0.0001$

Centres de production électrique Concernant les CNPE, il est intéressant de noter que nous trouvons une certaine cohérence avec les résultats expérimentaux, en particulier de la F_0 (Hemmer-Brepson et al. 2014), avec des dégâts supérieurs dans les environnements impactés par un réchauffement et des activités enzymatiques de la SOD également significativement supérieures (Fig. 48, Tab. 3). Les balances oxydatives sont donc clairement impactées par les échauffements en accroissant les dégâts malgré un investissement plus fort dans les défenses. Pour les sites du Rhône, nous n'avons pas pu tester si cet investissement dans les défenses

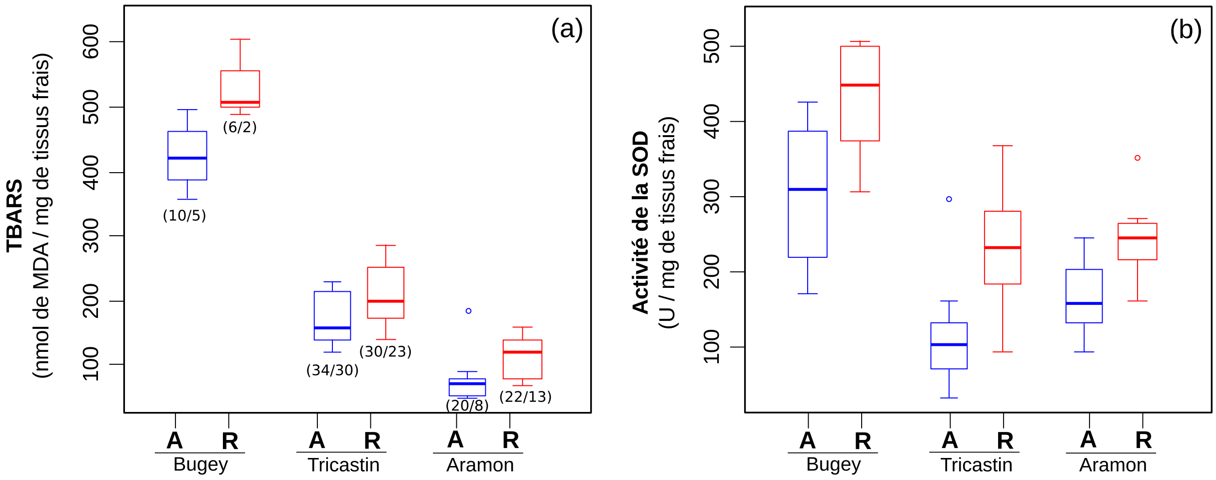


FIGURE 48 – Résultats des balances oxydatives obtenues sur les muscles de perches et gardons prélevés sur les sites lotiques. (a) Dégâts sur les membranes. Les nombres de perches/gardons sont indiqués entre parenthèses. (b) Activités de l'enzyme SOD. Les mêmes individus ont été utilisés pour mesurer les dégâts et les défenses. Les boxplots montrent les résultats combinés pour les perches et les gardons obtenus en amont A et au rejet R sur les 3 sites situés le long du Rhône : Bugey, Tricastin et Aramon.

était dû ou non à un investissement dans les gonades au dépends du soma. De ce point de vue, les échantillonnages réalisés dans la retenue du Mirgenbach sont très instructifs. Même si nous n'avons pas réalisé d'échantillonnages dans des stations témoins, il apparaît que la balance oxydative est très différente dans le soma et le germen des individus prélevés dans la retenue (Fig. 49). Les dégâts sont bien plus importants dans les muscles que dans les gonades, en rapport inverse avec le degré d'investissement dans la défense des différents tissus. On retrouve des patrons de DST très similaires à ceux observés chez les individus de médaka que nous avons élevés au chaud dans nos expériences (boîtes à moustaches rouges de la Fig. 47). Ces résultats peuvent être mis en relation avec les effets connus du réchauffement sur les espèces de cette retenue qui présentent des stratégies de type “live fast, die young”, avec des croissances rapides mais une diminution de la longévité, de l'âge à maturité et des tailles et masses maximales (Dembski et al. 2006; Masson et al. 2008). Tout laisse à penser que les liens DST-TSR observés dans nos expériences s'appliquent aussi ici. Enfin, concernant les CNPE, il est intéressant de noter que nous avons mis en évidence un effet clair des rejets, que ce soit du fait du réchauffement ou d'autres contaminants éventuellement présents dans les rejets. En effet, la plupart des tentatives de mise en évidence d'un effet de ces rejets basées sur l'étude de structures de communautés s'étaient jusque là soldées par des échecs ou par la mise en évidence d'effets très spécifiques, limités à quelques espèces (Daufresne et al. 2004; Daufresne 2004; Daufresne et al. 2005; Daufresne 2007; Daufresne et al. 2008; Daufresne 2009).

6.3.6 Développements

Approche expérimentale Le travail expérimental suit son cours et nous sommes à ce jour en train d'élever la 11ième génération de médakas à 30°C et la 10ième à 20°C. La valorisation du travail réalisé sur les différentes générations est en cours et a pris du retard du fait du changement d'orientation de Claire Hemmer-Brepson (qui a préféré quitter le monde de la recherche), du temps qu'il m'a fallu pour retrouver des financements pour une nouvelle thèse et de divers problèmes expérimentaux (comme des pics de nitrites ou le déménagement des installations vers des chambres climatiques). Outre les résultats de la F_1, nous disposons de données partielles sur

TABLEAU 3 – Résultats des modèles linéaires à effets mixtes pour les paramètres physiologiques mesurés sur les sites du Rhône.

Site	Variable dep.	Effets aléatoires		Effets fixes				
		effets	é.t.	effets	estim.	e.s.	*P*	
Bugey	TBars	Espèce	4.22	Ord. orig.	389.38	43.08	<0.001	***
		Date	57.76	Station	87.07	17.41	<0.001	***
	SOD	Espèce	<0.01	Ord. orig.	207.79	65.95	<0.01	**
		Date	145.60	Station	108.14	3.31	<0.01	**
Tricastin	TBars	Espèce	0.01	Ord. orig.	182.61	10.29	<0.001	***
		Date	15.26	Station	53.72	7.85	<0.001	***
	SOD	Espèce	78.27	Ord. orig.	214.01	79.75	<0.01	**
		Date	98.03	Station	84.67	14.42	<0.001	***
Aramon	TBars	Espèce	<0.01	Ord. orig.	103.24	55.48	<0.01	*
		Date	75.29	Station	94.77	22.14	<0.001	***
	SOD	Espèce	33.85	Ord. orig.	222.01	54.08	<0.001	***
		Date	66.80	Station	87.44	13.73	<0.001	***

Ord. orig. : ordonnée à l'origine ; é.t. : écart type ; e.s. : erreur standard

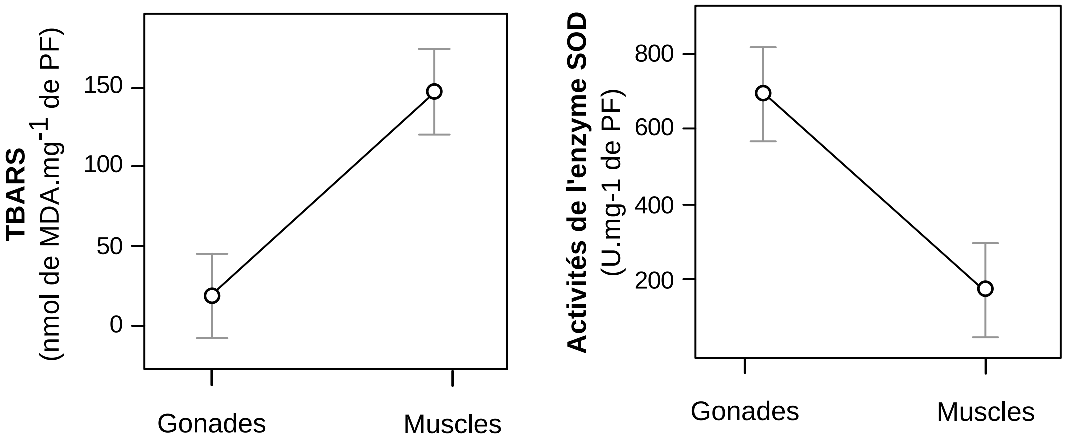


FIGURE 49 – Résultats des balances oxydatives obtenues sur les muscles et les gonades de 22 poissons (3 perches et 19 gardons) prélevés dans la retenue du Mirgenbach.

l'ensemble des générations, à la fois en terme de mesure de traits mais également d'un point de vue génétique et physiologique (tissus conservé à -80°C). Ces éléments devraient nous aider à explorer l'occurrence ou non de phénomènes de sélection naturelle au cours des générations dans nos expérimentations. En complément, un travail en common graden sera mené au cours de la thèse d'Ayala Loisel. Ce travail consistera à élever des individus provenant du traitement froid au chaud et des individus issus du traitement chaud au froid, tout en continuant également l'élevage des lignées chaude et froide. La comparaison des traits écologiques et de la physiologie des différents individus devrait nous aider à déterminer si les patrons observés sont principalement plastiques ou non. D'autres expériences sont également envisagées, en particulier pour évaluer la variabilité inter-individuelle des caractéristiques écologiques et physiologiques.

Vérité terrain Concernant les centres de production électriques, les résultats sont préliminaires et sont basés sur un nombre restreint d'individus. Concernant les CNPE, il conviendrait d'évaluer des traits écologiques pour compléter nos données. Concernant la retenue du Mirgenbach, il conviendrait d'effectuer des mesures de balance oxydative d'individus prélevés en dehors de la retenue. Par ailleurs, nous avons également entrepris d'évaluer l'impact du réchauffement sur la physiologie et l'écologie des ombles chevalier de divers plans d'eau dans le cadre des thèses d'Ayala Loisel et de Lisandrina Mari, en collaboration avec l'INRA de Thonon les Bains (Emilien Lasne).

Concernant les écosystèmes aquatiques, les augmentations de température prévues sont supposées toucher plus rapidement et avec une plus grande intensité les lacs (Adrian et al. 2009) et en particulier les lacs alpins et péri-alpins (Kajfež Bogataj 2007). Or, ces lacs et leur biodiversité fournissent de nombreux services écologiques qui sont potentiellement remis en question. L'ichthyofaune joue un rôle prépondérant dans ces services : elle occupe un poste clé dans le fonctionnement trophique des lacs et alimente la pêcherie professionnelle et amateur, participant ainsi à l'activité économique et culturelle locale. Si il n'est bien sûr pas envisageable de s'intéresser à l'ensemble des populations de poissons lacustres, il semble en revanche pertinent de se focaliser sur un modèle particulièrement sensible au réchauffement et qui pourrait ainsi être utilisé comme sentinelle du changement climatique et servir de modèle pour construire des indicateurs pour mettre en évidence les effets de ce processus. Parmi les différentes espèces de poisson des lacs alpins et péri-alpins, l'omble chevalier est singulier à plus d'un titre. C'est l'espèce d'eau douce qui possède la limite de distribution la plus septentrionale avec des populations présentes jusqu'à plus de 80° de latitude Nord. Elle possède des exigences très strictes en termes de température et d'oxygénation des eaux (Johnson 1980). En France, il n'existe que deux populations natives d'omble chevalier - celles du Léman et du lac du Bourget - qui sont considérées comme des reliques de la dernière ère glaciaire. Ces populations se situent en limite méridionale de distribution naturelle de l'espèce mais le pays abrite aussi de nombreuses populations introduites tout le long du gradient altitudinal, de quelques centaines de mètres d'altitude jusqu'à plus de 2000 m. Actuellement, par exemple dans le Léman, les conditions thermiques au niveau des frayères sont proches du seuil supérieur de fonctionnalité de 8°C (Fig. 50a ; Rubin & Buttiker 1992). Dans le contexte actuel de réchauffement climatique, il est très probable que l'augmentation des températures ou une modification des dynamiques de brassage dans les lacs (Perroud & Goyette 2010) pourraient entrainer le franchissement de ce seuil et l'extinction des populations. Ces populations semblent donc être de bons candidats à la fonction de sentinelles du changement climatique. Par ailleurs, outre sa valeur patrimoniale et socio-économique, l'omble chevalier est une espèce emblématique de la qualité des eaux des lacs alpins et périalpins et serait un excellent vecteur de sensibilisation du public et des politiques à la question de l'impact du changement climatique.

L'objectif de notre travail est (i) de confirmer les liens entre TSR et DST dans des milieux naturellement thermiquement contrastés (ii) de caractériser le niveau actuel d'adaptation à la température de différentes populations le long d'un gradient thermique par une approche

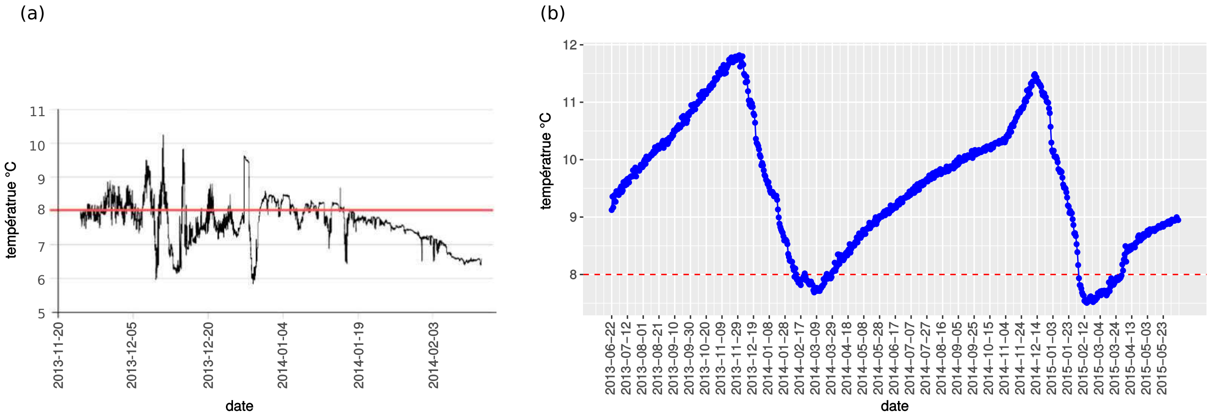


FIGURE 50 – Chroniques de température de deux plans d'eau. (a) Température moyenne horaire du Léman sur une frayère historique à 40 m de profondeur (E. Lasne, comm. pers.). (b) Température moyenne journalière du lac de Sainte Croix à 60 m de profondeur (point le plus profond de notre ligne de thermomètres sur le plan d'eau). Le seuil de 8°C est figuré en rouge.

expérimentale en 'common garden' (l'existence d'adaptation locale accréditerait l'hypothèse que la température est une force sélective majeure pour l'omble) et (ii) d'évaluer le potentiel adaptatif des populations en mesurant la variance génétique de la réponse au stress thermique, par une approche de génétique quantitative (une forte variance indiquerait un bon potentiel de réponse adaptative à l'augmentation des températures). Nous avons dors et déjà effectué quelques prélèvements dans les lacs d'Allos, de Sainte Croix et Léman (Fig. 51). La grande profondeur du Léman permet à l'omble de trouver des refuges thermiques et des zones de fraie. Cependant, des données récentes sur des frayères historiques montrent que la température est proche, et parfois dépasse, le seuil de fonctionnalité de développement embryonnaire. Le lac de Sainte-Croix est situé à relativement basse altitude en région méditerranéenne et les ombles y sont exposés à des conditions thermiques plus contraignantes que dans le lac Léman. L'introduction de l'espèce est relativement récente (années 1990), mais la température y est élevée pour l'espèce (>8°C) constituant probablement une pression sélective assez forte pour qu'on puisse s'attendre à une divergence adaptative rapide (Crozier & Hutchings 2014). Enfin, le lac d'Allos est le plus grand lac d'altitude d'Europe, situé à 2220m d'altitude, où les ombles sont exposés à des conditions plus froides (2-4°C) que dans les deux autres lacs. Cette population a été introduite en 1920 (Machino & Rivier 2002). Par ailleurs, les populations d'Allos et de Sainte-Croix présentent la particularité d'avoir été fondées à partir d'individus issus du Léman et sans rempoissonnement ultérieurs. Cela implique qu'ils ont à la base le même patrimoine génétique et permet de s'affranchir de facteurs confondants (des analyses génétiques menées en parallèles permettront de quantifier la distance génétique entre ces populations et celles du Léman et de mettre à jour les études anciennes (Potvin & Bernatchez 2003)). D'autres prélèvements pourront être envisagés dans d'autres lacs et en particulier dans les lacs de Constance (Allemagne) et Pavin.

Une première partie du projet consistera à comparer les balances oxydatives dans le soma et le germen de femelles des différents plans d'eau (thèse d'Ayala Loisel). Une deuxième partie consistera à caractériser les conditions actuelles de reproduction de l'omble chevalier (approche in situ, thèse de Lisandrina Mari). Il s'agira d'analyser, sur le terrain, les conditions naturelles de reproduction des ombles dans des contextes thermiques contrastés. Dans ces différents lacs, des sondes de température seront installées au niveau des frayères à l'interface eau-sédiment et resteront en place pendant une période suffisamment longue (avant, pendant et après la reproduction) pour appréhender les variations temporelles des conditions thermiques. Une troisième partie, la plus conséquente, visera à évaluer l'effet de la température sur l'embryon d'omble chevalier (approche ex situ). Des fécondations artificielles seront réalisées avec des géniteurs issus

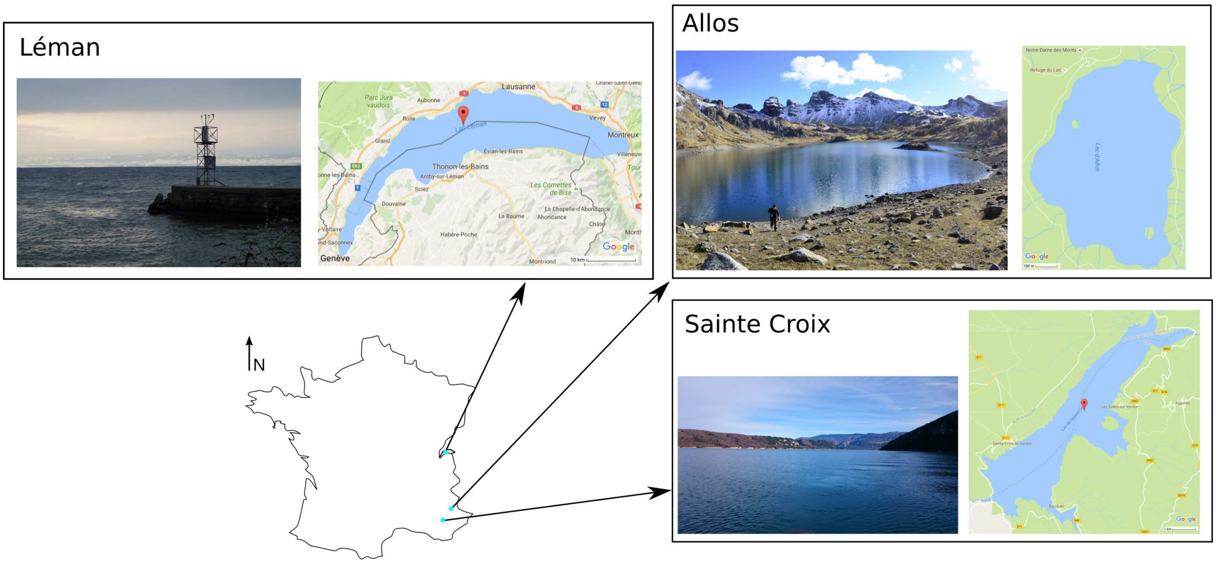


FIGURE 51 – Plans d'eau sélectionnés pour l'étude.

des différentes populations selon un schéma de croisement factoriel permettant de mesurer les différentes composantes de la variance phénotypique, dont la variance génétique additive. Les embryons issus des différentes origines seront soumis à diverses conditions réalistes de température (selon la seconde partie). Afin d'obtenir des proxy de la fitness, différents traits seront mesurés sur les embryons : la survie, la durée de développement et la taille aux différents stades (éclosion, résorption) (collaboration G Evanno, INRA). Enfin, des mesures de balances oxydatives seront réalisées. A ce jour, les premiers résultats, encourageants, sont en cours d'analyse et de nouveaux prélèvements seront effectués fin 2016 et début 2017.

6.4 Axe 3 – Allométries, "Metabolic Scaling Theory" et écologie thermique

6.4.1 Considérations métaboliques

Comme nous l'avons vu prédédemment, de nombreuses variables ecologiques et biologiques (Y) sont dépendantes de la masse M suivant des équations allométriques (Eq. 3 ; Peters 1983; Calder 1984; Brown et al. 2004). C'est en particulier le cas pour le métabolisme individuel, qui dépend de plus explicitement de la température (T) comme le montre l'équation 7. Pour rappel,

$$Y = Y_0 M^b \tag{3}$$

$$B_i = b_0 e^{-E_i/kT} M_i^{\alpha} \tag{7}$$

Comme je l'ai signalé plus haut, Ei vaut typiquement en moyenne 0.65 eV pour la respiration hétérotrophe mais on sait également qu'elle vaut 0.85 eV pour la méthanogénèse et 0.32 eV pour la photosynthèse "effective" (parce que la dépendance thermique de la photosynthèse en C3 est en réalité hyperbolique) (Yvon-Durocher et al. 2010a). Le métabolisme individuel est donc très bien défini par la masse d'un organisme et sa température corporelle.

Par ailleurs, il apparaît que l'exposant b des relations allométriques est souvent proche d'un multiple de $\frac{2}{3}$ ou $\frac{3}{4}$. C'est le cas pour l'exposant allométrique α et certains auteurs ont rapidement considéré qu'il existait une dépendance entre les allométries. Plus précisément, la " Metabolic Theory of Ecology" (MTE) (Brown et al. 2004) suppose que toutes les relations allométriques découlent de la relation métabolique. Cela implique que b peut explicitement être évalué à partir de α et que Y_0 dépend de b_0, du facteur de Boltzmann-Arrhenius et, éventuellement, de la ressource (Brown et al. 2004). En d'autres termes :

$$Y = f_Y(b_0 e^{-E_i/kT}) M^{g_y(\alpha)} \tag{9}$$

où f_y et g_y sont des fonctions dépendentes de la variable Y considérée (il est dans ce sens plus juste de nommer la MTE “Metabolic Scaling Theory” (MST ;Atkinson, comm. pers.), ce que je ferais dans cet ouvrage). Il convient cependant de noter (i) qu'il existe des déviations non négligeables du modèle général, (ii) que la valeur de α ($\frac{2}{3}$, $\frac{3}{4}$, ou une valeur comprise entre 0 et 1) et ses causes sont fortement discutées (Brown et al. 2004; Glazier 2005) et (iii) que les bases théoriques de la MST font également débat (voir par exemple Kozlowski & Konarzewski 2004, 2005). Quoiqu'il en soit, cette théorie a été validée de manière empirique sur bon nombre de jeux de données, et présente l'intérêt d'évaluer de manière quantitative des processus écologiques dans des milieux dont on connaît la température et la structure en taille des organismes qui les occupent.

Mes travaux ont récemment beaucoup portés sur la taille et sa dépendance à la température. Celle ci n'est pas explicitement considérée dans la MST mais, suivant la TSR, on peut réécrire l'équation 9, au moins pour les ectothermes, comme :

$$Y = f_Y(b_0 e^{-E_i/kT}) M(T)^{g_y(\alpha)} \tag{10}$$

où $M(T)$ est par exemple à l'échelle individuelle défini au temps t par une fonction de type von Bertalanffy dont les coefficients et exposants dépendent de la température (voir les sections précédentes).

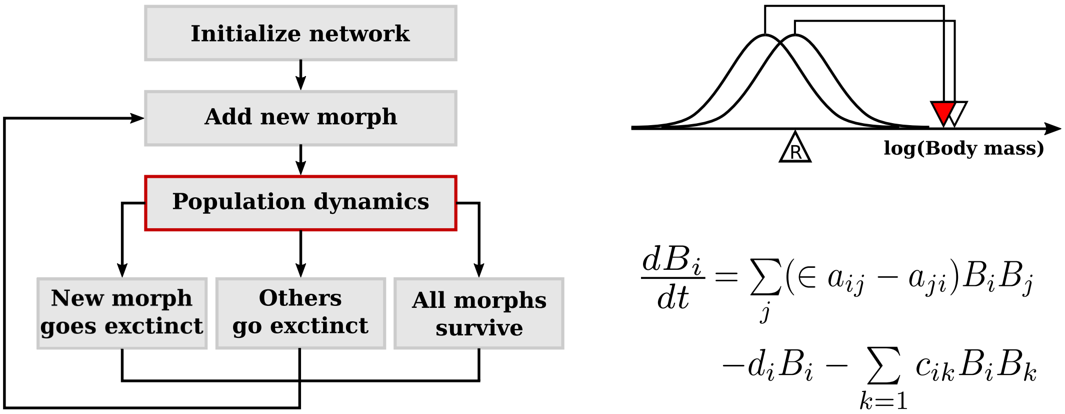
Des impacts écologiques du réchauffement climatique Mon travail actuel vise, en plus des axes 1 et 2, à explorer les impacts écologiques du réchauffement climatique dans ce cadre théorique. Comme pour l'axe 1, et probablement encore plus, cet axe n'a pas encore été très développé par manque de temps et de financements. Il était également important de travailler sur la question de la taille avant de s'attaquer aux conséquences des modifications des structures en taille à proprement parler. Plusieurs projets ont cependant récemment reçu des financements et ce travail est en train de débuter en focalisant pour l'instant sur l'impact de la température sur (i) la structure des reseaux trophiques et (ii) le métabolisme écosystèmique (c.-à-d. les flux de CO_2, de CH_4 et d'O_2) aux interfaces air-eau et eau-sédiment.

6.4.2 Température et réseaux trophiques

L'étude des liens température - structure de réseaux avait été proposée dans ClimSize mais a finalement été partiellement financée dans le cadre du projet ANR Arsenic, porté par Nicolas Loeuille et François Massol. Les liens température - structure et stabilité des réseaux sont explorés dans ce projet par Korinna Allhoff, en utilisant un modèle d'évolution de réseau basé sur les modèles de Loeuille & Loreau (2005) et Allhoff et al. (2015). L'idée est qu'un réseau est composé de différents morphes, chaque morphe consommant des proies dans une gamme de taille donnée, toutes plus petites que sa propre taille (Fig. 52). Des morphes ayant des tailles et des centres de prédation dans l'espace des tailles différents des morphes présents sont introduits dans le modèle que l'on laisse évoluer. Les nouveaux morphes se développent ou disparaissent aux dépends, ou à cause, des morphes présents et de leurs taux de mortalité propres (Fig. 52).

K. Allhoff a ensuite considéré une dépendance à la température des taux de mortalité des morphes ($d_i(T)$), et du taux de croissance ($R(T)$) et de la capacité d'accueil ($K(T)$) de la ressource au travers d'équations d'Arrhenius :

$$d_i(T) \propto e^{\frac{E_{act} \cdot (T-T_0)}{kTT_0}} \tag{11}$$



$$\frac{dB_i}{dt} = \sum_j (\in a_{ij} - a_{ji}) B_i B_j - d_i B_i - \sum_{k=1} c_{ik} B_i B_k$$

FIGURE 52 – Modèle d'évolution de réseau utilisé par Korinna Allhoff dans le projet Arsenic (figure reproduite d'après une présentation orale avec l'autorisation de l'auteur). Chaque morphe consomme des proies dans une gamme de taille donnée. La structure du réseau dépend de la dynamique des populations des morphes. La dynamique d'un morphe dépend de sa consommation de morphes plus petits et de la prédation exercés par les morphes plus gros (qui dépendent des taux d'assimilation a_{ij} et a_{ji}), de son taux de mortalité (d_i), et des interaction compétitives (c_{ij}).

$$R(T) \propto e^{\frac{E_{act}\cdot(T-T_0)}{kTT_0}} \tag{12}$$

$$K(T) \propto e^{-\frac{E_{act}\cdot(T-T_0)}{kTT_0}} \tag{13}$$

Sous ces conditions, il apparaît que les réseaux ont tendance à devenir moins diversifiés, avec une perte importante du nombre de morphes, principalement ceux des plus bas niveaux trophiques (Fig. 53). La biomasse totale à en revanche tendance à augmenter, principalement pour les bas niveaux trophiques, caractérisés par des morphes de petite taille (Fig. 53). Ces résultats sur les tailles sont intéressants puisqu'ils vont dans le sens des règles taille-température à l'échelle inter-spécifiques mais qu'ils mettent le point sur des contraintes principalement trophiques. Ces travaux débutent et des développements sont en cours (prise en compte des liens entre le taux d'attaque et la manipulation des proies, impact de changements brusques de température ou spatialisation du modèle). Je n'ai pas encore formellement collaboré sur ce projet mais devrait le faire de manière plus intense avant sa fin, par exemple en explorant l'effet d'une prise en compte explicite des liens taille-température à l'échelle spécifique.

6.4.3 Du métabolisme individuel au métabolisme écosystémique

Comme je l'ai souligné plus tôt, une question majeure de la MST est la valeur de l'exposant α de l'équation 7. De nombreuses études ont porté sur la détermination de sa valeur et de sa variabilité (voir Glazier 2009a,b; Killen et al. 2010; Glazier 2010, 2005). Si sa dépendance thermique a été signalée (Glazier 2005; Killen et al. 2010), sa généralité n'a été finalement que peut étudiée alors que cette variabilité me semble d'une importance majeure. En effet, considérer une dépendance thermique $\alpha = h(T)$ permet de réécrire l'équation 10 selon :

$$Y = f_Y(b_0 e^{-E_i/kT}) M(T)^{g_y \circ h(T)} \tag{14}$$

Cette équation développe la dépendance des variables écologiques à la température. Évidemment, elle souffre des même maux que la MST, c'est à dire qu'elle sous entend que les variables écologiques sont reliées d'une manière ou d'une autre à l'équation métabolique. Je ne vais pas

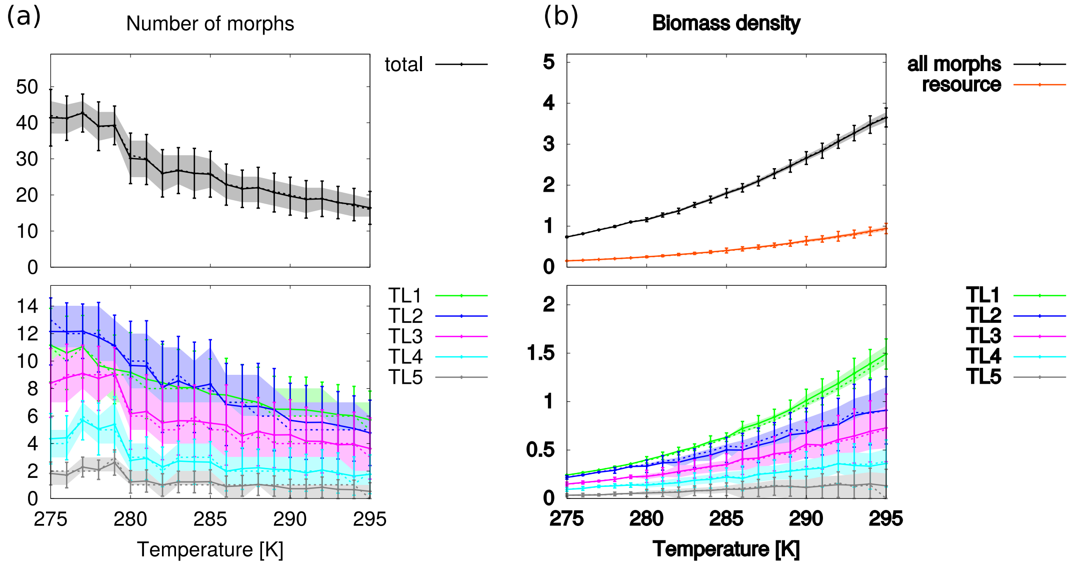


FIGURE 53 – Exemples de résultats des analyses préliminaire de K. Allhoff sur l'impact de la température sur la structure des réseaux. (a) Nombre de morphes total et par niveau trophique (TL) en fonction de la température. (b) Biomasse totale et par niveau trophique en fonction de la température.

discuter de cette assertion ici tant elle est débattu dans la littérature. Il est vrai cependant que la MST a probablement souvent usé de conclusions hâtives. Il est en effet toujours possible de construire des dépendances entre des fonctions allométriques de même nature (c'est à dire d'expliciter f_y et g_y dans le cas de la MST). Ces dépendances peuvent être des pistes pour comprendre les liens entre métabolisme et variables écologiques. En trouver ne démontre en revanche pas formellement des liens de causes à effets. Cependant, je dirais simplement que je suis persuadé de l'importance du métabolisme individuel en écologie et qu'il est très probable que certaines dépendances explicitées dans la MST sont justes, en particulier celles étudiées à l'échelle individuelle. De plus, il est important de noter que l'exposant métabolique est ici considéré comme variable, ce qui constitue un avantage certain par rapport à la MST telle qu'expliciter par exemple par Brown, West, Savage, Enquist et leurs collaborateurs qui ont eu tendance à défendre l'existence d'une valeur de $\frac{3}{4}$ et d'une explication pour cela (West et al. 2003; Brown et al. 2004) et que ce point est probablement le plus discuté dans leur approche (Kozlowski et al. 2004; Kozlowski & Konarzewski 2005).

Si cette équation me parait prometteuse sur le fond, il reste de nombreuses interrogations sur la forme des différentes fonctions. Dans ce sens, j'ai commencé, en collaboration avec Alejandro Isla, à étudier $h(T)$ sur différents jeux de données. Ce travail nécessite d'être poussé plus en avant, en particulier pour clarifier les mécanismes sous-jacents, mais il semble qu'il existe une relation linéaire négative entre α et la température (Fig. 54).

Il est intéressant de noter que les ordonnées à l'origine des différents modèles sont proches, en particulier si on considère les données restreintes de Fishbase et de la biobliographie ($\in [2.10, 2.40]$; Fig. 54). Les pentes ont également des valeurs proches ($\in [-0.0057, -0.0048]$ si on considère les données restreintes de Fishbase et de la bibliographie). La valeur moyenne de α sur les données restreintes vaut 0.75. Cependant, un autre point remarquable est que la plupart des données sont récoltées autour de 290°K, soit environ 17°C et que la valeur de α prédite par le modèle à cette température est de 0.73, soit très proche de $\frac{3}{4}$. Pour une température de 20°C, cette valeur est de 0.71. Il n'est pas étonnant d'observer autant de données à ces températures puisqu'elles représentent des températures confortables pour travailler, autant in situ qu'ex situ et qu'elles sont fréquentes en milieu tempéré. Dans ce sens, nous ne pouvons

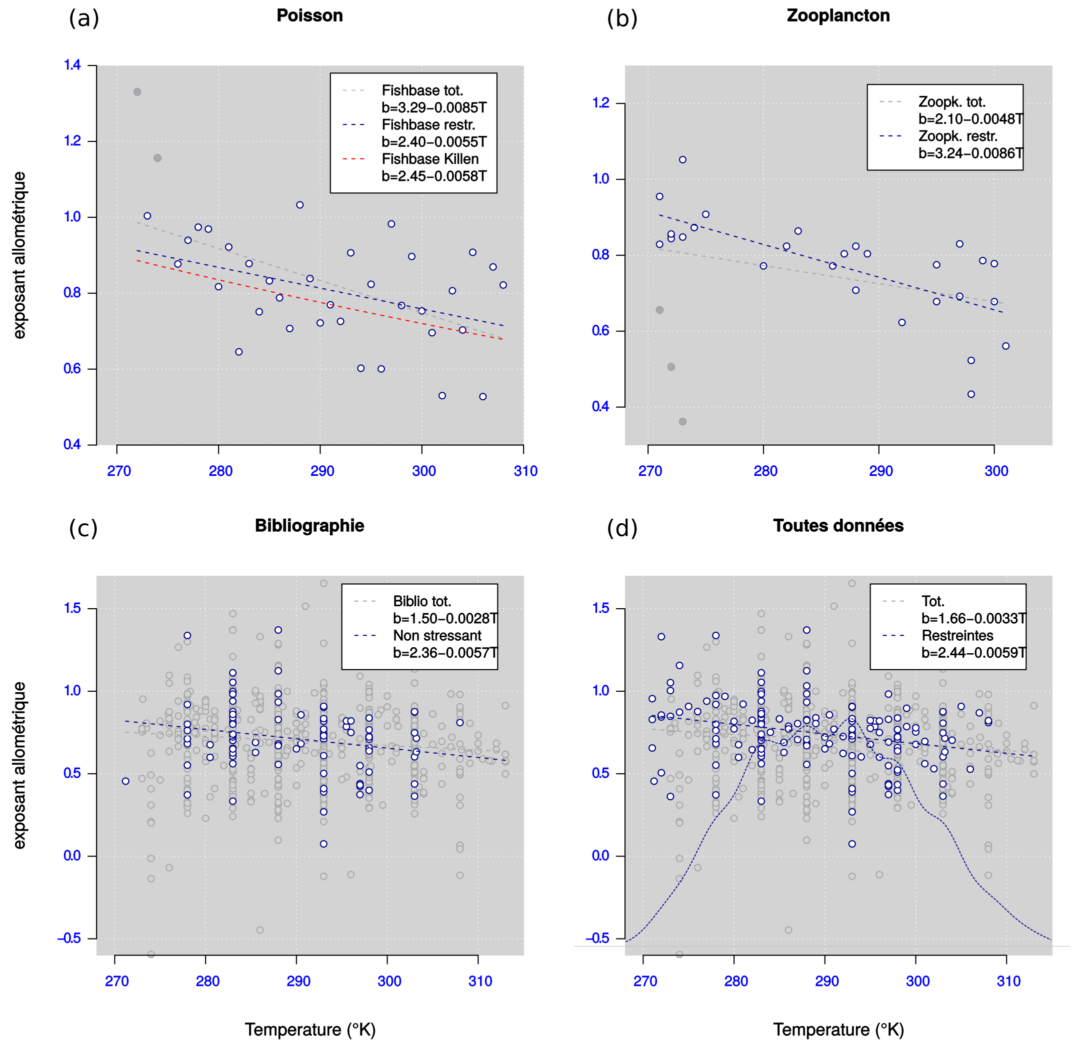


FIGURE 54 – Exemples de résultats préliminaires de l'étude des liens entre l'exposant allométrique du métabolisme et la température. (a) Données issues de la base Fishbase de 2013. Une comparaison des résultats avec ceux de Killen et al. (2010) est donnée. (b) Données zooplacton de T. Ikeda disponible en ligne. (c) Données issues de la bibliographie. La courbe de densité des données restreintes est représentés. Dans tous les cas, Les points grisés représentent les points non utilisés dans les analyses sur données restreintes. Pour le panneau (c), les données correspondant à des études pour lesquelles les durées d'acclimatation étaient supérieures à la semaine et où la température d'expérimentation était proche de la température du milieu ont été considérées comme "non stressantes".

pas exclure que la valeur de $\frac{3}{4}$ souvent défendu comme valeur universelle ne puisse pas être un artefact expérimentale.

Quoiqu'il en soit, dans l'esprit de la MST, et comme débuté dans le cadre d'Arsenic, il est intéressant d'étudier les dépendances thermiques des variables biologiques ou écologiques et leurs conséquences au travers de l'équation 14. Une des dépendances les plus directement liées à cette équation est le métabolisme écosystémique (Yvon-Durocher et al. 2010b,a). A ce jour, je suis impliqué dans deux projets qui devraient permettent d'appréhender cette question. Le premier projet est une fiche action Onema qui vise à construire des bioindicateurs du fonctionnement des écosystèmes lacustres (programmation 2016-2018 du pôle ; action 9bis "Biodiversité et Fonctionnement des Ecosystèmes Lentiques : Indicateurs de fonctionnement des plans d'eau"). Dans le cadre de cette action un ingénieur de recherche est en charge du développement de chambres de mesure des flux d'O_2, de CO_2 et de CH_4 aux interfaces eau-atmosphère et eau-sédiment et des campagnes de mesures devraient, à termes, être planifiées sur le territoire national dans des environnements environnementalement contrastés, en particulier d'un point de vue thermique et en termes de niveau d'eutrophisation. En parallèle, le projet DHEMISA, financé dans le cadre d'un appel d'offre du Labex OTMED, vise à étudier la dynamique des micro-organismes aquatiques, depuis les procaryotes hétérotrophes jusqu'au microzooplancton, à l'aide d'un cytomètre de flux automatique et de terrain développé spécialement pour cela dans le projet. Le projet est développé en collabraotion avec l'institut méditerranéen d'océanographie (MIO ; Michel Denis, Gérald Grégori). Ce projet devrait nous permettre de coupler les flux de gaz à des structures en taille et en fonction du type de métabolisme (photosynthèse, respiration, méthanogénèse) des micro-organismes.

7 Conclusion

– Torrent du ravin de l'Auriac, Allos, Août 2016. Le brassage de l'eau emprisonne l'air qui va oxygéner le milieu aquatique. –

La majeure partie de mon travail a porté sur l'étude de l'impact du réchauffement climatique, à une époque où les conséquences de ce réchauffement étaient encore très hypothétiques, en particulier dans les milieux aquatiques où la variabilité hydrologique apparaissait comme le moteur principal des dynamiques biologiques. Dans ce cadre, mon travail a suivi une construction pas à pas, en se basant d'abord sur la richesse des données récoltées sur le long terme autour des CNPE. Ce travail exploratoire m'a permis de mettre en évidence un tel impact. Les patrons décrits dans mon travail comme dans celui de beaucoup d'écologistes restent cependant assez grossiers. Les modifications des aires de distribution et de la phénologie des espèces sont des phénomènes importants dans l'appréhension des modifications de notre environnement mais je pense que l'on ne peut pas s'arrêter là et je suis surpris que les appels d'offres comme celui du Gicc portent déjà plus sur des processus d'atténuation que sur la recherche de mécanismes d'influence de la température sur les systèmes écologiques (APR GICC 2016 ≪ L'adaptation au changement climatique dans la transition écologique ≫). Je pense que cela traduit la tension actuelle qui existe entre recherche dite “fondamentale” et recherche dite “finalisée”. Il me semble que la finalité de la recherche devrait être plus une propriété émergente qu'un but affiché. L'histoire des sciences à en effet tendance à confirmer ce type de fonctionnement. Il existe probablement aujourd'hui un trop grand pilotage de la recherche tant dans son financement que dans son évaluation. Cela peut paraître surprenant de la part d'un membre d'un institut de recherche tel qu'Irstea. Je ne remets pas en cause l'importance de la transférabilité de la recherche mais il me semble qu'il serait plus clair et plus efficace d'avoir des organes spécifique de transferts, proches mais indépendants des structures de recherche. Concernant le changement climatique, si je ne remets pas en cause l'urgence de la situation et la nécessité de trouver des solutions, il me semble en revanche qu'il est primordial de continuer à développer des connaissances sur les impacts du réchauffement climatiques et sur les mécanismes sous-jacents. Mon travail tend dans ce sens, en accordant une attention particulière à la prise en compte de règles d'écologie générale aussi bien qu'au développement d'approches spécifiques, relevant de ce que l'on pourrait appeler l'écologie thermique. Si il a put être assez vivement critiqué, je pense que le travail lié à la MST est intéressant dans ce cadre. Pourtant, la dépendance thermique du métabolisme et ses conséquences ont finalement été peu exploitées dans ce sens, la plupart des assertions de la MST étant faites en contrôlant la température plutôt qu'en étudiant son effet.

Plus généralement, mon travail m'a intimement (mais subjectivement j'en conviens) persuadé que la température était une variable centrale en biologie et en écologie. La cinétique de toute réaction chimique est soumise aux lois de la thermodynamique et c'est une base de la biologie terrestre (au sens planétaire ici et pas en opposition au milieu aquatique). Les liens température-organismes sont donc une évidence et les conséquences écologiques qui en découlent le sont tout autant. Elles en restent néanmoins pour la plupart énigmatiques et c'est la raison pour laquelle je pense que la dimension thermique a de beaux jours devant elle en écologie. Je m'interroge dans ce cadre sur les liens qu'il serait intéressant de tisser entre écologie et physique en général et thermodynamique en particulier.

Les écosystèmes peuvent être considérés comme des systèmes ouverts et dissipatifs au sens de Prigogine (1968). Dans ce sens il existe un cadre conceptuel dans lequel écologique et thermodynamique sont liées (Kleidon 2010). Cela explique l'engouement que certains physiciens mais également chimistes, biologistes ou écologues ont portés aux bases thermodynamiques du fonctionnement de la vie et des écosystèmes (Schrödinger 1944; Margalef 1963; Prigogine 1968; Tonnelat 1977; Margalef 1996). Beaucoup de ces études ont porté une attention particulière à la seconde loi de la thermodynamique. En effet, l'equilibre thermodynamique correspond au maximum d'entropie (l'entropie étant l'énergie thermique non utilisable pour générer un travail). Dans les systèmes écologiques, loin des conditions d'équilibre, on va donc avoir des processus qui ont tendance à produire de l'entropie. Ainsi, une des pistes d'étude des contraintes thermodynamiques pesant sur les écosystèmes et de leurs conséquences porte sur l'optimisation de cette production d'entropie (connu sous l'appellation de "principle of maximum entropy production", ou MEP ; Kleidon 2010). Naturellement, un lien s'est par ailleurs établi avec la théorie de l'information de Shannon (voir Margalef 1996), même si certains considèrent que l'entropie de Boltzmann et de Shannon sont conceptuellement différentes bien que mathématiquement semblables au signe près. En dehors de l'entropie des systèmes, un intérêt particulier a également récemment été porté sur leur exergie (Jørgensen 1992; Jørgensen & Fath 2004; Jørgensen 2007), qui correspond à la quantité de travail que peut fournir un système à l'extérieur lorsqu'il se met en équilibre thermodynamique avec son milieu. Pour un système biologique Jørgensen (2002) définie l'eco-exergie pour laquelle le travail est représenté par l'énergie chimique de la biomasse et des composés biochimiques en général contenus dans celui ci. L'eco-exergie est calculée comme la somme des concentrations des différents composés organiques pondérée par l'information contenue dans leur gènes (Jørgensen & Fath 2004; Jørgensen 2006; Jørgensen & Nielsen 2007; Xu et al. 1999).

Plus généralement, les utilisations de la thermodynamique sur les systèmes écologiques peuvent être regroupées sous la notion de "thermodynamic extremization principles" (Yen et al. 2014). En effet, toutes ces utilisations reprosent sur le principe de maximisation ou de minimisation de variables energétiques (liées à des questions de stockage ou de dissipation d'énergie) dans des systèmes qui ne sont pas à l'equilibre. Une liste de ces applications est donnée par Yen et al. (2014) et en particulier le principe de maximisation de la production d'entropie, l'exergie, la MST, ou encore la dynamic energy budget (DEB ; Kooijman 2010). Si l'utilisation de la thermodynamique en écologie est une réalité de longue date, elle peine pourtant encore à se développer. Outre le fait qu'elle offre certainement des bases mécanistes fortes des patrons écologiques, je pense qu'elle pourrait être particulièrement utile dans l'étude des impacts écologiques du réchauffement climatique, un champ dans lequel elles n'ont encore, à ma connaissance, que peu été utilisées.

Pour finir, je ne pense pas avoir à développer ici les enjeux que représentent le changement climatique. D'autres s'en chargent bien mieux que moi et il suffit de lire les derniers rapports du Giec pour se faire une bonne idée de l'ampleur du phénomène et de ses conséquences (voir par exemple IPCC 2012, 2013, 2014). Je me permettrais simplement de dire que ces changements sont non seulement inquiétants pour l'humanité mais que je trouve également désolant d'imaginer que la nature de mon enfance est passée, et que mes enfants ne pourront plus la contempler à l'identique. Il me semble de ce fait primordiale de trouver des solutions d'atténuation des effets des changements globaux. Pour ce faire, je suis convaincu de l'importance de l'identification des mécanismes sous-jacents, même si ces travaux peuvent paraître très fondamentaux. Mon travail sur les contraintes oxydatives va dans ce sens et j'aimerai dans un future proche travailler par exemple avec des pisciculteurs pour connaître l'ampleur des effets de réduction en taille et leur conséquences économiques. L'identification de l'influence du stress oxydant comme moteur de ces changements pourrait offrir des pistes pour trouver des solutions d'atténuations. Des travaux portant sur la thermodynamique pourraient également être d'une grande utilité. Dans la mesure où elle traduit le fonctionnement des systèmes, l'exergie à par exemple récemment été utilisée comme bio-indicateur de leur état (Xu et al. 1999, 2011). Il est clair que l'homme dispose donc encore aujourd'hui de forces, en particulier intellectuelles, pour réagir face à ces changements. Puisse notre sagesse nous aider à les mettre efficacement, et rapidement, à l'oeuvre... Un défit d'autant plus grand qu'il nécessite certainement de gros changements de paradigmes dans le fonctionnement de nos sociétés.

Annexes

– Un rocher vole sur le cours de l'Huveaune. Roquevaire, Février 2016. –

Vous trouverez dans cette partie 5 articles en lien direct avec la thématique des impacts écologiques du changement climatique. J'y ai ajouté 4 articles portant sur des thématiques en marge des impacts climatiques mais sur lesquels j'ai eu une très forte implication

Quelques articles en lien avec la thématique du changement climatique

[20] Mouthon J. and **M. Daufresne** (2015). Resilience of mollusc communities of the River Saone (eastern France) and its two main tributaries after the 2003 heatwave. *Freshwater Biology.* **early view**.

[18] Hemmer-Brepson C., L. Replumaz, C. Romestaing, Y. Voituron and **M. Daufresne** (2014). Non-stressful temperature effect on oxidative balance and life history traits in adult fish (Oryzias latipes). *Journal of Experimental Biology.* **217**, 274–282.

[10] **Daufresne M.**, K. Lengfellner and U. Sommer (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences of the United States of America.* **106**, 12788–12793.

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[1] **Daufresne M.**, M.C. Roger, H. Capra and N. Lamouroux (2004). Long-term changes within the invertebrate and fish communities of the Upper Rhône River : Effects of climatic factors. *Global Change Biology.* **10**, 124–140. (106)

[20] Mouthon J. and **M. Daufresne** (2015). Resilience of mollusc communities of the River Saone (eastern France) and its two main tributaries after the 2003 heatwave. *Freshwater Biology*. **early view**.

Freshwater Biology

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Resilience of mollusc communities of the River Saone (eastern France) and its two main tributaries after the 2003 heatwave

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SUMMARY

1. Global climate change can increase the mean and variability of temperature and may increase the occurrence of extremes such as heatwaves. Both gradual and abrupt warming may perturb freshwater communities, but understanding of ecological resilience to such events is limited.
2. We report the response of mollusc communities in the River Saone and its two main tributaries to the European heatwave of 2003 and determine the extent of community recovery over 8 years following this rare extreme event, during a period of gradual warming.
3. The 2003 heatwave had a major impact on the density and species richness of mollusc communities across the study area. After the heatwave, abiotic conditions favoured recolonisation by molluscs, yet full recovery of community states (defined as the combination of structure × density × species richness) was not observed at any site.
4. Given the profound changes observed in the mollusc community structure in the Saone River and in its two main tributaries and the observed slow pace of recovery relative to that observed elsewhere for pulse disturbances (typically < 3 years), we suggest that global change, including warming, may preclude community recovery from pulse disturbances, such as extreme events, and instead yield communities comprising new combinations of species.

Keywords: climate change, community shift, disturbance, extreme climatic event, recovery

Introduction

Global climate change can lead to gradual shifts in ecological communities. For instance, environmental warming can alter species distributions, local community composition and phenology over decades (Parmesan & Yohe, 2003; Root *et al*., 2003). However, where key thresholds are crossed, gradual environmental change may also cause abrupt shifts in community structure (Van Nes & Scheffer, 2004). Slight erratic change in the environment, combined with gradual change, may be sufficient to invoke such shifts. However, extreme climatic events may have more pronounced physical and ecological impacts, as evidenced by research on heatwaves (Mouthon & Daufresne, 2006) and floods (Daufresne, Bady & Fruget, 2007). Extreme events can be defined as statistically rare or unusual weather or climatic occurrences, such as extremes of precipitation or temperature, which can have severe natural impacts on the environment (IPCC, 2012). Future shifts in the occurrence of extreme events could challenge the resilience of freshwater ecosystems, either by themselves or in combination with underlying gradual environmental change.

Resilience can be defined as the amount of disturbance a community can undergo and still remain within the same state (Holling, 1973) or as the ability of a community to recover and return to its original state (Klein, Nicholls & Thomalla, 2003). Although freshwater ecosystems contain species adapted to environmental stressors, climate change could produce novel future regimes or combinations of stressors that exceed the capacity of communities to recover. Thus, from a management perspective, it is important to lessen anthropogenic

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pressures that could impair resilience to anthropogenic or natural disturbances, including extreme climatic events such as heatwaves.

One of the most notable recent extreme climatic events in Europe was the heatwave of summer 2003 (Schar *et al*., 2004; Stott, Stone & Allen, 2004; Trigo *et al*., 2005; Twardosz & Kossowska-Cezak, 2013). Heatwaves of such magnitude are exceptionally rare, occurring only once every several thousand or million years (Schar *et al*., 2004). Although research capturing the ecological consequences of the 2003 event for freshwater ecosystems is scarce (but see Jankowski *et al*., 2006; Mouthon & Daufresne, 2006; Daufresne *et al*., 2007; Wilhelm & Adrian, 2007; Jöhnk *et al*., 2008; Wegner *et al*., 2008), we know that the very hot summer of 2003 caused intense thermal stratification in European eutrophic, temperate lakes, depleted hypolimnetic oxygen and stimulated blooms of harmful cyanobacteria. These harsh conditions caused the mass mortality of fish and birds in the Netherlands (Jankowski *et al*., 2006; Jöhnk *et al*., 2008) and suppressed the abundance of *Dreissena polymorpha* larvae in Müggelsee, a shallow eutrophic lake in Germany (Wilhelm & Adrian, 2007). In large lowland rivers in France, the heatwave caused a rapid, substantial change in the structure of mollusc communities, as well as drastic declines in species richness and density (Mouthon & Daufresne, 2006). Coupled with major droughts and floods, it led to the development of assemblages of tolerant and invasive taxa (Daufresne *et al*., 2007). The heatwave also increased parasitism and mortality of fish in an experimental study in the lake Grosser Plöner See in Germany (Wegner *et al*., 2008).

The occurrence of hot extremes is expected to increase in the future (IPCC, 2012, 2013), and thus, understanding how aquatic communities recover from such disturbance is a priority for research. According to Stott *et al.* (2004), the heatwave of 2003 was a manifestation of global warming. Freshwater communities were exposed to a short-term pulse disturbance (heatwave) overlying a ramp disturbance (environmental warming) intensifying steadily over time (see Lake, 2000). How the impact of these two disturbances combined to influence the resilience of the freshwater communities remains uncertain. For instance, the recovery time of most aquatic invertebrates to a pulse disturbance is <3 years (Resh *et al*., 1988; Niemi *et al*., 1990; Yount & Niemi, 1990; Lake, 2000; Watanabe, Yoshimura & Omura, 2005). However, Mouthon & Daufresne (2006) and Daufresne *et al.* (2007) did not observe clear signs of recovery of macroinvertebrate communities in the 3–4 years after the heatwave, and since then, more research has been undertaken to track the extent of recovery. In this paper, we analyse the change in structure of the mollusc communities in the Saone River and its two main tributaries, the Doubs and Ognon, over 8 years following the heatwave. The aim of the study was to evaluate the resilience of the different river systems to the heatwave within the context of global warming.

Methods

Study area

The River Saone (catchment area 29 900 km^2, length 473.4 km) rises in the Vosges mountains at an altitude of 405 m and falls 245 m along its course, joining the Rhone at Lyon (Fig. 1). For 300 km of its lowest reaches, the river is channelised for navigation, with a low gradient (0.08 m km^{-1}). As the river is prone to heavy flooding, surrounding land is only sparsely urbanised, with industrial zones being located near the main towns. Land use in the catchment is predominantly agricultural (58%), mixed with forestry and other semi-natural environments (36%). Livestock farming is common in the upper river valley, with cereal farming, market gardening and wine growing widespread in the lower valley. River water quality has been degraded by navigation, agricultural inputs and urban and industrial discharges, so concentrations of nitrates, phosphates and various toxic pollutants increase from upstream to downstream (Agence RMC, 2005). At all sites, the bed of the river is dominated by carbonate-rich sediments, mainly fine sand and silt. River water is alkaline (mean pH ± SD ranging from 7.88 ± 0.14 to 8.16 ± 0.07 in the different sampling sites, see Table S1), well oxygenated (mean ranging from 9.62 ± 0.36 to 10.27 ± 0.38 mg L^{-1}) and nutrient-rich (e.g. mean total phosphorus concentration ranging from 0.05 ± 0.02 to 0.17 ± 0.09 mg L^{-1}). Water quality is poorest at Lyon where high concentrations of chloride (40.55 ± 11.07 mg L^{-1}) and sodium (23.90 ± 6.96 mg L^{-1}) reflect industrial activity.

The Saone receives *c.* 20 tributaries of which the Ognon and the Doubs are the largest. The river is linked to the basins of the Moselle, Seine and Loire by canals that have probably accelerated colonisation by exotic species (Bij de Vaate *et al*., 2002).

Environmental variables

Mean daily discharge (1977–2011) for three sites on the Saone River (Ray/Saône, Pagny-la-Ville, Mâcon) and two sites on the Saone's main tributaries at Pesmes

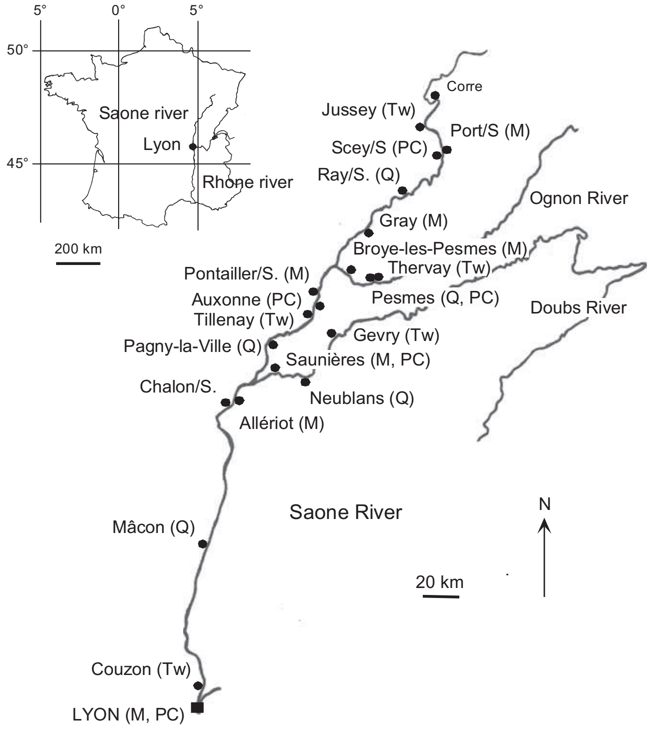

Fig. 1 Study area and location of the mollusc sampling sites (M) and discharge (Q), water temperature (*T*w) and water physico-chemical (PC) recording sites.

(River Ognon) and Neublans (River Doubs) (Fig. 1) was obtained from the Banque Hydro (data available at http://www.hydro.eaufrance.fr). Mean daily water temperature data (1977–2011) recorded automatically at the Saone at lock of Couzon (*c.* 10 km from the confluence with the Rhone) were supplied by EDF (Electricité de France). Water temperature data collected by Office National de l'Eau et des Milieux Aquatiques (ONEMA) were available for the Saone at Jussey and Tillenay, in the Ognon at Thervay and in the Doubs at Gevry (Table S2). We used mean daily air temperature at these sites (SAFRAN model; Vidal *et al.*, 2010) to estimate missing values from 1 January 1977 to 31 December 2011. Water temperature at date t was regressed on the mean air temperature over the $t-6$ to t period (1-week period, R^2 ranging from 0.91 to 0.97).

Mollusc sampling sites

Mollusc communities were sampled monthly (September 1996–December 2011) from the River Saone at Lyon and annually (in September or October, various years, see Table S3) at a further six sites across the river network (Fig. 1). The Saone at Lyon is *c.* 150 m wide with two-thirds of this width consisting of a navigable channel. The sampling site was *c.* 150 m long, and more than half of its surface area was covered with macrophytes (*Nuphar*, *Ceratophyllum*, *Potamogeton* and filamentous algae) from May to October. The sampling sites at Port/Saône, Gray and Pontailler/Saône were located in bypassed river sections (non-navigable old channels). The site at Allériot was downstream of the confluence of the Doubs. Broye-les-Pesmes and Saunières were in the lower reaches of the Ognon and Doubs, respectively, immediately upstream of their confluence with the Saone (Fig. 1).

At each site, one 0.25 m^2 sample was taken at each of four stations, at a depth between 0.50 m (depth at which wake effects lessen) and 1.5 m, using a rectangular hand-net (25 × 18 cm, 315 μm mesh). Samples were kept separately (Lyon) or combined into a single sample (annual sampling sites), fixed on-site in 12% neutralised formaldehyde, and molluscs later separated from sediment, identified to species and counted.

Statistical analysis

Temporal variation in mollusc community structure was evaluated for univariate (species richness and total density) and multivariate (species × months, species × years matrix of mollusc density) data sets. For each site, a principal components analysis (PCA) was performed on $\ln(x + 1)$-transformed mollusc density data. Species with an occurrence <10 in the site-specific data set were excluded from the analysis because PCA can be sensitive to rare taxa.

To detect trends in environmental (discharge, temperature) and biological time series at the Lyon site, we used a modified Mann–Kendall trend test developed by Hamed & Rao (1998). This nonparametric analysis (based on ranks) tested for temporal trends once autocorrelation effects were removed. For the Lyon site data, analysis of variance (ANOVA) was used to test for the effect of the heatwave on (i) factorial site scores from the PCA, (ii) species richness and (iii) density of gastropods and bivalves. Data were allocated to one of two groups depending on their collection date before (September 1996–July 2003) or after (August 2003–December 2011) the heatwave. Autocorrelation consistent (HAC) estimators were used to assess the covariance of the ANOVA model parameters and, in turn, the statistical significance of differences between the two groups (Zeileis, 2004). Temporal change at the other sampling sites was described only qualitatively because of limited data before 2003.

To determine the return of the biological variables to an initial state, we used a method based on the maximisation of between-group sample variance. This analysis was performed only on time series which showed a clear post-heatwave shift in n values, that is when the first samples following the heatwave were clearly outside the range of the pre-heatwave values. With the heatwave occurring at time t, values could be divided into groups of $t-1$ pre-heatwave values and $n-t+1$ post-heatwave values. Under the hypothesis of a return to the initial state, we evaluated the extent to which the x most recent samples (n, $n-1$ … $n-x+1$; $x \in [0, n-t]$) were closer to the pre-heatwave values than to the $(n-t+1)-x$ post-heatwave values. The procedure consisted of finding the value x which maximised the variance between the two groups: (i) a group of samples where the $t-1$ pre-heatwave samples were pooled together with the x most recent samples and (ii) the rest of the data. Note that if the variance was maximal for $x = 0$, we considered there was no return to the initial state. All analyses were performed using Statistica package library (version 9.0), R (R Development Core Team, 2013) and sandwich packages (Zeileis, 2004).

Results

Discharge and temperature data

Mean annual discharge varied among wet and dry years and declined significantly at all the sites from 1977 to 2011 (Mann–Kendall trend test, $P < 0.0001$ at Ray/S. and Pagny-la-Ville, $P < 0.01$ at Mâcon and Pesmes, $P < 0.05$ at Neublans) (Fig. 2). The post-heatwave years, especially 2005, 2009 and 2011, caused this negative trend, as no obvious tendency was observed between 1977 and 2003 (Mann–Kendall trend test, $P > 0.05$ at all the sites). The lowest mean daily discharge in summer (July–September) was in 2003 (for Neublans, Pagny-la-Ville, Pesmes and Ray/S.), with flows 3.1–4.4 $m^3 s^{-1}$ lower than the mean summer discharge for the period 1977–2011 (Fig. 2f). Conversely, the 2003 summer discharge at Mâcon did not deviate markedly from conditions in 1977–2011 (Fig. 2f).

Mean annual water temperature at Couzon (*c.* 10 km from Lyon) increased significantly ($P = 0.0003$) by 0.05 °C $year^{-1}$ from 1977 to 2011. However, this warming mainly occurred before the heatwave and no significant trend could be distinguished from 2003 to 2011 ($P = 1$). We observed the same pattern for water temperature at Jussey and Tillenay (Saone River), Thervay (Ognon) and Gevry (Doubs River) for the period 1977–2003 ($P < 0.05$ at all sites) and 2003–11 ($P > 0.6$ at all sites). The mean summer (July–September) water temperature was lowest in 1977 at Gevry and Couzon and in 1996 at the other sites. The mean daily temperature was highest in 2003 for all the sites. For instance, at Couzon in 2003, the maximum temperature was 29.5 °C

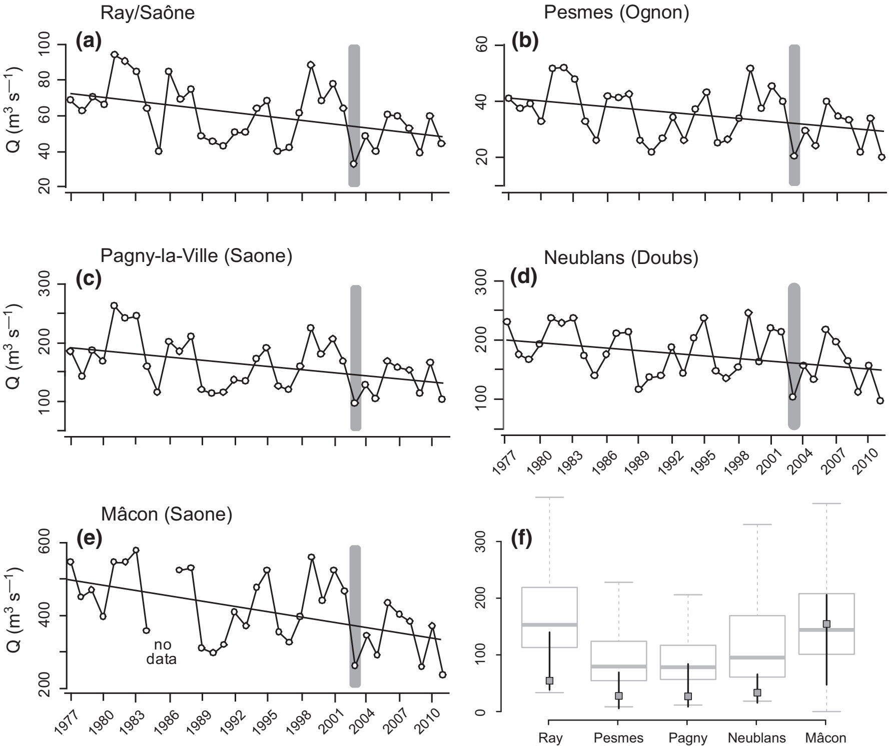

Fig. 2 Mean annual discharge in the Saone, Ognon and Doubs rivers (a–e), with linear trends shown as solid lines. *Y* axes are scaled to data. The 2003 heatwave is highlighted in grey. Boxplot of the summer (July–September) mean daily discharge at the different sites between 1977 and 2012 (f). Lower and upper limits of the boxes represent the first and third quartiles of the data. The thick horizontal grey segments represent the median values. For each boxplot, the whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. Range of the 2003 values (thick vertical black line) and the 2003 median (large grey squares) is highlighted. To increase readability, some values have been multiplied by a factor *f* (indicated in the figure as ×*f*).

(versus 24.3–26.4 °C from 1996 to 2002) and exceeded 25 °C for 75 days (versus 0–17 days from 1996 to 2002). The median summer water temperature of 2003 was particularly high at all sites (21.5 °C at Jussey, 23.8 °C at Thervay, 24.3 °C at Tillenay and 25.5 °C at Couzon) and deviated strongly from the distributions of the summer data 1977–2011 (Fig. 3f).

Mollusc data

A total of 156 673 molluscs were collected at Lyon between September 1996 and December 2011, but of these, only 38 613 (24.6%) were found after the heatwave. Thirty-two species (18 gastropods, 14 bivalves) were collected at this site over the study period, of which eight (*Ancylus fluviatilis*, *Stagnicola* sp., *Galba truncatula*, *Gyraulus laevis*, three unionid species and *Pisidium milium*) were rare (<10 individuals in the full data set). After the heatwave, the formerly dominant species *Valvata piscinalis* was replaced by the exotic *Corbicula fluminea*. Between 1997–2003 and 2004–11, the mean relative abundance of *Valvata* at Lyon declined from 28.6 to 5.4% whereas that of *Corbicula* increased from 13.4 to 39.8%. A total of 90 775 individuals were collected from the other six sites, and the number of mollusc species observed at individual locations was ranged from 27 (Gray) to 32 (Saunières). Twenty species were common to all the sites.

Temporal change in mollusc community at Lyon

The 2003 heatwave had a major impact on the richness (43.1% reduction) and abundance (74.2% reduction) of molluscs (ANOVA d.f. = 163, all P-values < 0.001). Richness was more strongly reduced for gastropods (52.3%) than bivalves (34.8%). Abundances of both groups were strongly suppressed (gastropods by 85.5% and bivalves by 64.0%). After the heatwave, both species richness and density of molluscs continued to decline until 2007 and then increased progressively until 2011 (Fig. 4). Mollusc

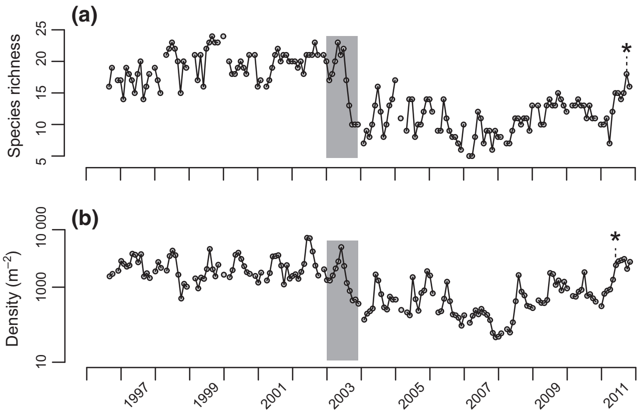

Fig. 4 Monthly variation of species richness (a) and density (b) of molluscs in the Saone River at Lyon. The 2003 heatwave is highlighted in grey. Dates of recovery are highlighted by stars.

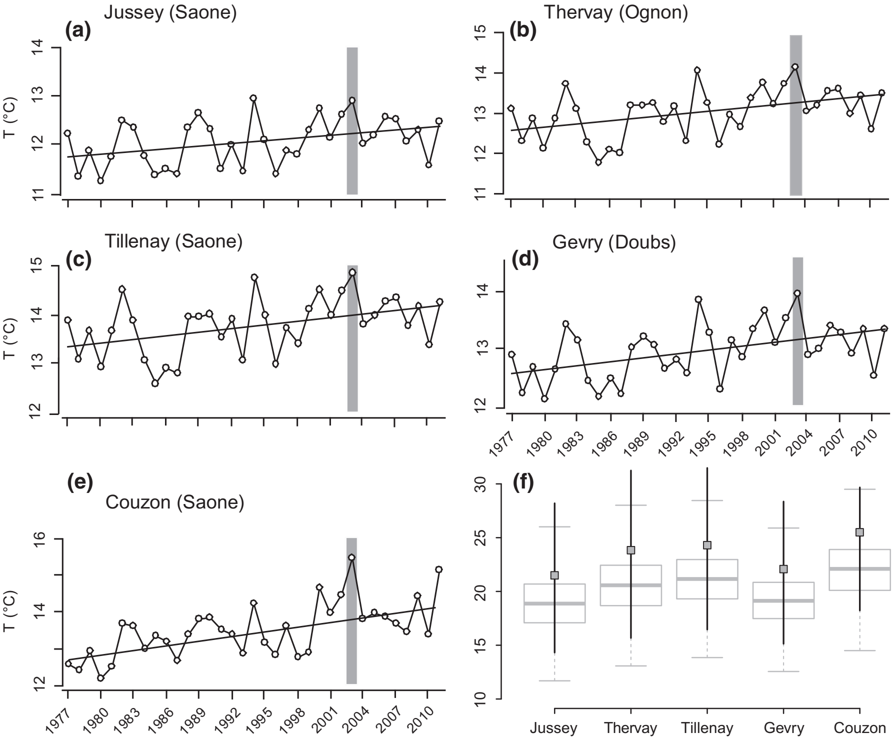

Fig. 3 Mean annual water temperature in the Saone River at Jussey, Tillenay and Couzon, in the Ognon at Thervay and in the Doubs at Gevry (a–e), with linear trends shown as solid lines. *Y* axes are scaled to data. The 2003 heatwave is highlighted in grey. Boxplot of the summer (July–September) mean water temperature at the different sites between 1977 and 2011 (f). Lower and upper limits of the boxes represent the first and third quartiles of the data. The thick horizontal grey segments represent the median values. For each boxplot, the whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. Range of the 2003 values (thick vertical black line) and the 2003 median (large grey squares) is highlighted.

Table 1 Time (year or year and month according to sampling frequency) at which we observed a return to initial conditions for mollusc density, species richness and community structure. Community structure is summarised by scores of the samples on the two-first axes (F1 and F2) of principal components analysis (PCAs) performed on ln(x + 1)-transformed densities of species

			Community structure	
Site (river)	Density	Species richness	F1	F2
Port/Saône	2008	No return	2008	No shift/gradual change
Gray (Saone)	2008	2009	2008	No return
Pontailler/Saône	2009	No return	2008	No return
Allériot (Saone)	2011*	2010	2006*	No return
Lyon (Saone)	June 2011	October 2011	June 2011	No shift/gradual change
Broye-les-Pesmes (Ognon)	2008	2008	2008	No return
Saunières (Doubs)	2005	2004	2004	No shift/gradual change

*No clear shift (the first post-heatwave samples correspond to extreme values of the pre-heatwave period).

density and richness returned to pre-disturbance levels in June and October 2011, respectively (Fig. 4, Table 1).

The first two axes of the PCA accounted for 57.4% of the inertia in the mollusc data (F1 = 41.5%, F2 = 15.9%, respectively). The sudden change in the structure of the mollusc communities caused by the 2003 heatwave (see Mouthon & Daufresne, 2006) is shown by the clear separation of scores from 1997–2003 and 2004–11 from left to right along axis 1 of the PCA (Fig. 5a). Axis 2 shows the temporal evolution of community structure in the years before (from the bottom of the axis upwards) and after (from the top of the axis downwards) the heatwave. The test performed on the monthly factorial scores on F1 revealed a negative trend before the heatwave (Mann–Kendall trend test, $P < 0.001$) and no trend after the heatwave (Fig. 5b, c). There was a significant difference in the mean of the monthly factorial scores before and after 2003 on F1 (ANOVA, d.f. = 163, $P < 0.0001$), but not on F2 ($P = 0.58$). The heatwave effect explained 76.6% of the variance on F1 and only 2.3% on F2. In fact, the monthly factorial scores on F2 did not show a shift in their values but a positive trend (Mann–Kendall trend test, $P < 0.001$) versus negative trend (Mann–Kendall trend test, $P < 0.001$) before versus after the heatwave (Fig. 5b, c). We observed a recovery in F1 values in June 2011. The situation is more complex for F2, where the 2011 scores are closer those of 1996 than 2003.

Species with low scores on F1 and F2 (e.g. *Pisidium moitessierianum*, *Pisidium henslowanum*, *Pisidium subtruncatum*, Fig. 6) declined in abundance with time, especially after the heatwave. However, some species showed signs of recovery, especially those with higher scores on F1 (e.g. *Potamopyrgus antipodarum*, Fig. 7). Species with high scores on F2 and low scores on F1 (e.g. *Musculium*

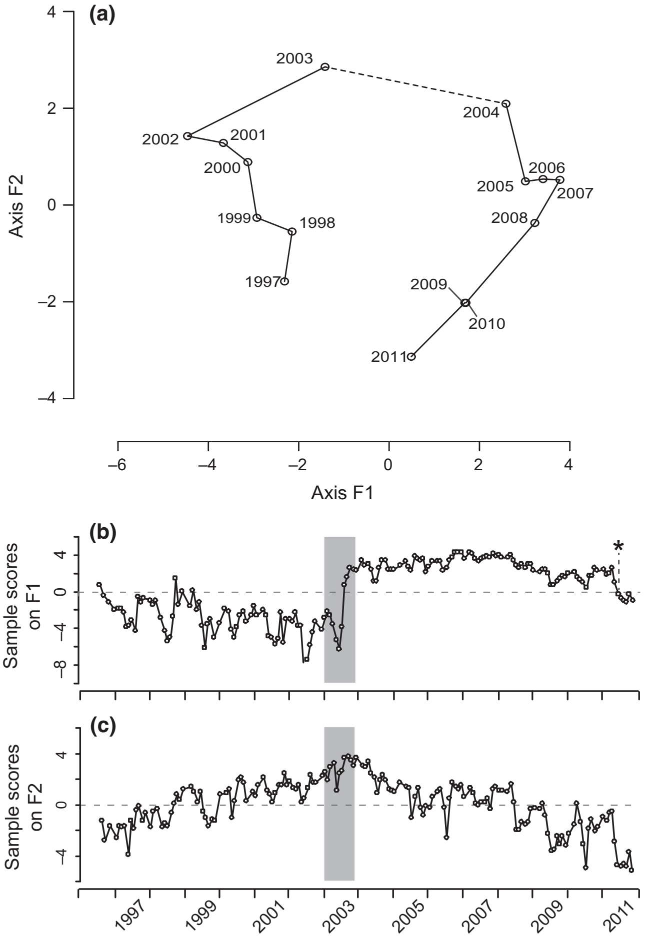

Fig. 5 Results of the principal component analysis performed on mollusc quantitative data in the Saone River at Lyon. (a) Mean yearly (black line) factorial scores of the monthly samples on the two-first axes of the principal components analysis (PCA) (F1, F2). (b, c) Times series of scores of the monthly samples on F1 and F2. Horizontal dashed lines represent overall means. The 2003 heatwave is highlighted in grey. Dates of recovery are highlighted by stars.

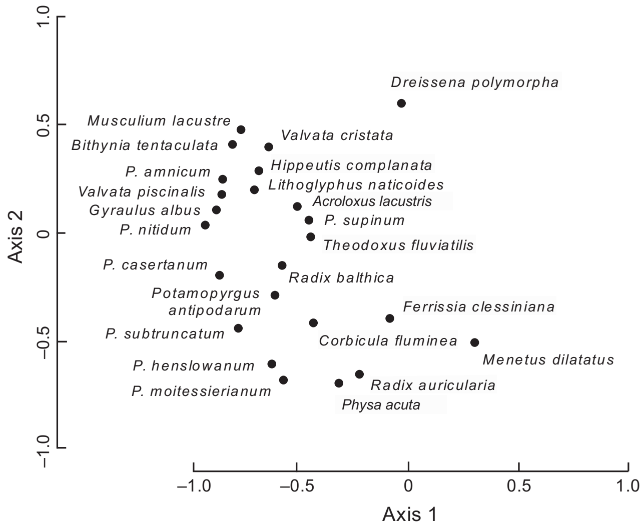

Fig. 6 Results of the principal component analysis performed on mollusc quantitative data in the Saone River at Lyon, scores of the species on the two-first axes (*P* = *Pisidium*).

lacustre, *Bithynia tentaculata*, *Pisidium amnicum*, *V. piscinalis*, Fig. 6) increased up to the heatwave. Densities then rapidly declined and remained very low (e.g. *M. lacustre* and *V. piscinalis*, Fig. 7). Species with high scores on F2 and F1 showed similar patterns up to the heatwave, but this temporarily boosted, rather than suppressed, their densities. For these species, the subsequent decrease in density occurred later in the time series (e.g. *D. polymorpha*, Fig. 7). Finally, species with low F2 scores and intermediate F1 scores showed an initial decrease in density and an increase after the heatwave (e.g. *Physa acuta*, Fig. 7). Species with intermediate scores on F1 and F2 did not show marked trends in their densities (e.g. *C. fluminea*). Note that the mean annual density of the invasive *D. polymorpha*, which prefers stony habitats, remained low over the whole study period (maximum 10.8 individuals m^{-2} in 2003).

At Lyon, persistent signs of community recovery from the heatwave were not observed until 2009, when several gastropod species reappeared (*V. piscinalis*, *Valvata cristata* and *B. tentaculata*). Further recovery was evident by 2011 when *Gyraulus albus* and *M. lacustre* returned and the density of sphaeriid bivalves increased. Nonetheless, post-heatwave mollusc communities remained different in structure from pre-heatwave communities (F1 × F2 score combination remains different), with several species common pre-2003 (*Lithoglyphus naticoides*, *Theodoxus fluviatilis*, *Radix balthica* and *P. amnicum*) still absent in 2011.

Temporal change across the Saone catchment area

We observed a decline in species richness and mollusc density after the heatwave at the six sites (Fig. 8, see

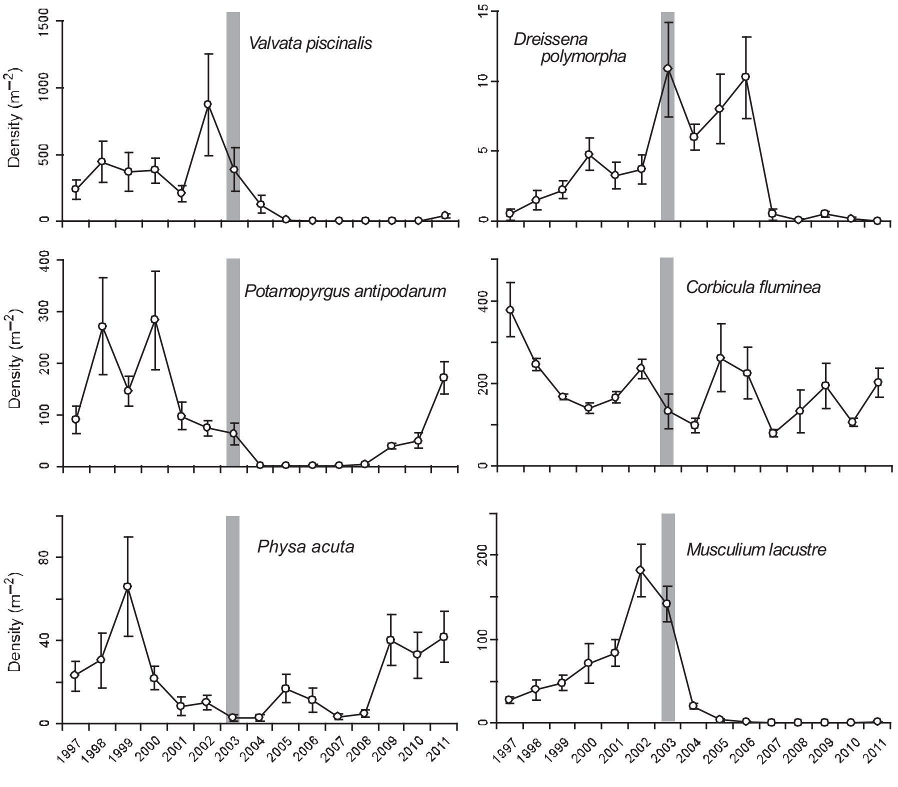

Fig. 7 Time series of mean annual density (±SD) of six key species: *Valvata piscinalis*, *Potamopyrgus antipodarum*, *Physa acuta*, *Dreissena polymorpha*, *Corbicula fluminea* and *Musculium lacustre* in the Saone River at Lyon. *Y* axes are scaled to data. The 2003 heatwave is highlighted in grey.

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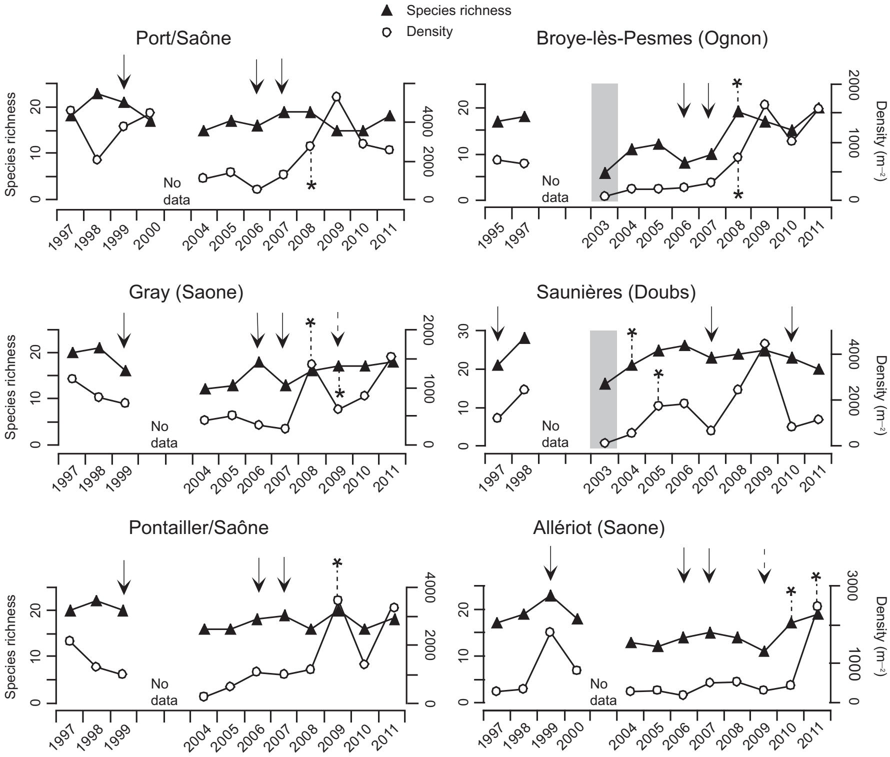

Fig. 8 Annual variation of species richness and density of mollusc communities at the annual sampling sites in the Saone, Ognon and Doubs rivers. Sampling dates (solid arrow) preceded by summer floods and dredging of river bed (dotted arrow) are shown. *Y* axes are scaled to data. The 2003 heatwave is highlighted in grey. Dates of recovery are highlighted by stars.

also Mouthon & Daufresne, 2006), although the change in density at Allériot was less clear than in the other sites (the first post-heatwave values are close to those of 1997–98). The rate of recolonisation varied among sites (Fig. 8, Table 1). Mollusc densities increased rapidly (from 2005) at Saunières, but recovery was slower elsewhere. Complete recovery of mollusc densities (to pre-heatwave levels) was observed at Port/S., Gray and Broye in 2008 and at Pontailler/S. in 2009 (Fig. 8, Table 1). In contrast, sphaeriid bivalves never returned to pre-heatwave densities at Broye and Saunières. At Allériot, a site degraded by chemical discharges, recovery time is difficult to identify due to marked variation before the heatwave. At this site, low densities recorded in 1997 and 1998 were attained in 2004, whereas higher densities observed in 1999 (after the high water levels of the Doubs in June and July), were not exceeded until 2011 (Fig. 8). Gastropods (mainly the numerically dominant *P. antipodarum*, 55–83% total numbers) were the main contributors of community recovery at all sites except Gray, where sphaeriid bivalves were more common (53.3% total numbers). Trends in species richness were similar to those observed in density, except at Port/S. and Pontailler/S. where the number of species present did not recover (Fig. 8, Table 1).

The PCA revealed how the heatwave of 2003 modified the structure of mollusc communities at all the sites (Fig. 9). For four sites (Port/S, Gray, Broye and Saunières), marked shifts in community structure along the first axis suggest pronounced impacts of the heatwave. For two sites (Pontailler/S and Allériot), change along the second axis (accounting for less variation) was more prominent, revealing less severe heatwave effects. Mollusc communities were also affected by low temperature during summer (all sites in 2007), floods in summer (all sites in 2007, Saunières in 1997, 2010 and 2011) and autumn (Saone River in 1998 and in August 2006), and dredging (Gray and Allériot in 2009). The timing of recovery from the heatwave differed among the sites, ranging from 2004 (Saunières) to 2008 (Pontailler/S. and Broye, Table 1). Recovery of mollusc populations was faster in the Doubs than in the Saone and the Ognon, and overall, the years 2008–11 proved to be a favourable period for recolonisation. Nonetheless, scores on the second axis (F2) remained very different from the initial state at all the sites, and no complete returns to the pre-heatwave community structures were observed.

The rate of recovery accelerated late in the time series, with the cold and wet year of 2007 being apparently particularly favourable for recolonisation (the mean discharge in summer in 2007 was 2.0–2.2 times higher than the mean summer value of the period 1977–2011 for each of these sites, and summer 2007 was the second coldest in terms of mean values at Tillenay, Thervay and

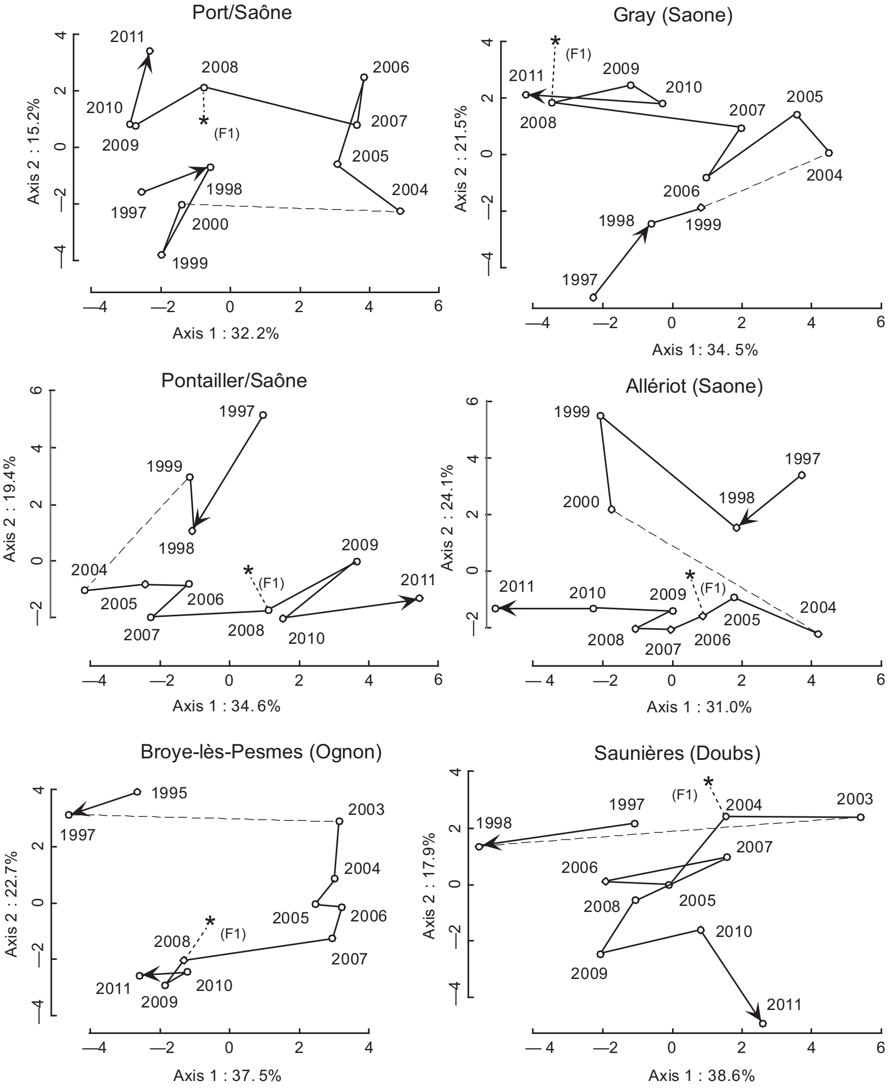

Fig. 9 Factorial scores of the yearly samples on two-first axes (F1, F2) of the principal component analysis performed on mollusc quantitative data at the annual sampling sites in the Saone, Ognon and Doubs rivers. Dates of recovery are highlighted by stars.

Couzon and the fourth coldest at Gevry and Jussey). Eight biological variables among the 28 studied showed signs of recovery in 2008 (Table 1).

Discussion

Long-term monitoring in the River Saone and its tributaries provided evidence for slow and incomplete recovery of mollusc community structure from the severe impacts of the 2003 heatwave (see Mouthon & Daufresne, 2006). Nevertheless, recovery of specific community descriptors (PCA scores, species richness, density) was observed and progressed at different rates across the river network. Typically, the recovery time (e.g. of total density and species richness) of aquatic invertebrates to a pulse disturbance is <3 years (Resh *et al.*, 1988; Niemi *et al.*, 1990; Yount & Niemi, 1990; Lake, 2000; Watanabe *et al.*, 2005). In this study, complete recovery to pre-heatwave densities required 2–8 years, whereas recovery of species richness was incomplete at some sites but occurred rapidly (1 year) at one site (Saunières). A complete return to the initial community structure was not observed at any site, although there were clear signs of partial recovery after 1 (at Saunières) to 8 years (at Lyon).

The magnitude and duration of responses to major pulse disturbances, such as a heatwaves, can depend on the presence or absence of an underlying press (Collier & Quinn, 2003) or ramp disturbance. Both the magnitude of change and the recovery time were the lowest at Saunières on the Doubs (Table 1), the site least impacted by non-climatic anthropogenic pressures. The species-rich assemblages at Saunières suggest that the lower reaches of the Doubs are more suitable for molluscs than the middle (bypassed sections) and lower reaches of the Saone and Ognon rivers. Rapid recovery at Saunières may be explained by the presence of donor sites like backwaters permanently connected with the main stream, and tributaries with good water quality, that favour mollusc recolonisation by drift (Niemi *et al.*, 1990; Yount & Niemi, 1990; Mackay, 1992; Collier & Quinn, 2003; Watanabe *et al.*, 2005). This capacity of mollusc populations for rapid recovery suggests that the resilience of the Doubs is greater than that of the Saone and the Ognon. Nevertheless, there is a clear underlying trend in the structure of the mollusc community of the Doubs which is a likely consequence of gradual environmental change, probably global warming. Such changes in community structure may modify resilience to disturbance, and the question remains as to whether the present community will recover as quickly following future heatwaves. Surprisingly, in the lower Saone where the impact of these press and ramp disturbances was greatest, the time to complete return of mollusc density to pre-heatwave levels was highly variable. Recovery was especially rapid at Allériot (<1 year; Table 1, Fig. 9), a degraded site where mollusc density is generally low. Again, this site located right downstream of the confluence with the Doubs probably benefited from high-quality donor sites upstream. At the other sites, recovery from the heatwave may have been delayed and/or diverted by gradual warming and/or navigation and pollution of the river over the sampling period.

A progressive increase in water temperature from 1977 to 2011 affected the ecology of the Saone, Doubs and Ognon rivers (Mouthon & Daufresne, 2006, 2010; Fig. 3), while the decade 2001–11 was the warmest recorded in France since 1900 (http://climat.meteofrance.com). At Lyon, between the September 1996–July 2003 and August 2003–04 periods, the decline in mean density was >50% for 19 species (12 gastropods and seven bivalves) and <50% for three species (the gastropod *Ferrissia clessiniana* and two bivalves *M. lacustre* and *Pisidium supinum*). The species colonising both the upper and lower part of rivers or reaching their maximal density in the lower part are eurythermic (Meier-Brook, 1975; Mouthon, 1999). Nevertheless, most of them were strongly affected by the heatwave. Besides potential direct thermal effects, a fall in dissolved oxygen concentrations at the end of the night may also have affected molluscs during the heatwave. A better knowledge of the thermal niches of the species and their relative ability to face anoxia would further understanding of underlying mechanisms. However, the increase in the density of the exotic *Menetus dilatatus* (gastropod) and *D. polymorpha* (bivalve) tends to show that the most eurytolerant species were favoured, although their populations remained very low (Mouthon & Daufresne, 2006). Regarding climatic drivers, moderate floods in summer 2007 appeared to have initiated (Saone and Ognon) or reactivated (Doubs) the recolonisation process and favoured the return of density to pre-heatwave levels, possibly by increasing drift. In addition, the summer water temperatures after the heatwave of 2003 were generally close to mean values for 1996–2011, while the low discharges after the heatwave undoubtedly promoted the recovery of mollusc densities. Lower variation in discharge (absence of strong floods) and temperature favouring good oxygenation of the water during the summer period ensured conditions beneficial to the development of these quiet-water organisms (Boycott, 1936; Dillon, 2000).

Boat-generated wakes and resuspension of bed sediment by boat propellers negatively affect the growth and distribution of aquatic macrophytes (Murphy & Eaton, 1983; Vermaat & De Bruyne, 1993) which constitute an important habitat for molluscs (especially gastropods). More than 5000 boats (3/4 carrying goods and 1/4 river cruises or sailing) transit the Couzon lock each year, so navigation activities may have slowed recovery in the most downstream sites. Chemical pollution has also been identified as a problem in the river and could potentially prevent the recolonisation of sensitive recolonists. However, while toxic substances (e.g. pesticides, metals, PAHs) were particularly prevalent in the downstream part of the Saone (Agence RMC, 2005), there has been no significant reduction in water quality since the heatwave (SEQ-Eau, available at http://sierm.eaurmc.fr/eaux-superficielles/fichiers-telechargeables/grilles-seq-eau-v2.pdf), and hence, influences on recovery are uncertain. Nevertheless, unusually low chl-*a* concentrations in the Saone downstream of the Doubs confluence since at least 1987, the cause of which remains unknown but possibly involved pollution (Fruget & Persat, 2000; Agence RMC, 2005), may have affected the recovery of molluscs in these reaches. In fact, molluscs that feed on phytoplankton (*C. fluminea*, *D. polymorpha* and the Unionidae) or periphyton (mainly gastropods) may be food-limited (Mouthon, 2001; Mouthon & Daufresne, 2008). However, pedal feeding on organic matter by *C. fluminea* (Way *et al.*, 1990; Hakenkamp & Palmer, 1999) could explain the persistence of this species (Fig. 7), although its growth is generally slower in such instances (Mouthon, 2001). Finally, recent introductions of invasive species may have influenced recovery. New mollusc species such as the bivalve *C. fluminea* and other seston and deposit feeders, such as *Hypania invalida*, *Chelicorophium curvispinum* and mysids, may compete for food with native molluscs. Invasive non-mollusc predators such as *Dikerogammarus villosus* (Van der Velde *et al*., 2000; Dick, Platvoet & Kelly, 2002; Devin *et al*., 2005) may also modify the structure of mollusc communities (Strayer, 2010).

In conclusion, mollusc densities did recover across the river network. However, we did not observe complete recovery of mollusc community state (defined as the combination of structure × density × species richness) at any site, even though 8 years have elapsed since the heatwave. The arrival of exotic species (Strayer, 2010), and an increase in the frequency of extreme events possibly linked to global warming in future years, could impede complete recovery of the mollusc community in the Saone and its major tributaries (Beniston *et al*., 2007; IPCC, 2007; Planton *et al*., 2008). It could be that the abrupt shift of mollusc community structure caused by the heatwave and underlying warming trend represents a new stable state, as outlined by theory (Scheffer *et al*., 2001; Scheffer & Carpenter, 2003; Van Nes & Scheffer, 2004). Our study suggests that global warming could shape how biotic communities respond to future pulse disturbances, and may lead to the formation of communities consisting of species that are extant today, but in new combinations not found at present (Williams & Jackson, 2007).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean ± SD values of physico-chemical variables at five sites (see Fig. 1 for their location) over the 1997–2011 period.

Table S2. Periods during which mean daily water temperatures were available in the different recording sites (see Fig. 1 for their location).

Table S3. Distance from the confluence of the Rhone River, sample frequency, and sampling periods for five sites on the Saone River and two of its tributaries Ognon and Doubs.

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RESEARCH ARTICLE

Non-stressful temperature effect on oxidative balance and life history traits in adult fish (*Oryzias latipes*)

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ABSTRACT

Temperature is well known to affect many biological and ecological traits, especially in ectotherms. From a physiological point of view, temperature is also positively correlated to metabolism and is often associated with an increase in reactive oxygen species (ROS) production. It has recently been suggested that ROS play a role in lifespan and resource allocation. However, only a few authors have attempted to explore the relationships between temperature, resource allocation and oxidative balance in ectotherms. Here, we measured the effect of temperature on growth, reproductive effort, offspring quantity and quality, hatching and survival rates, and the associated proximal costs, which were evaluated through the quantification of oxidative balance elements. We reared adult fish (*Oryzias latipes*) at two non-stressful temperatures (20 and 30°C) during a relatively long period (4 months, approximately the entire adult life). The results show a trade-off between reproduction and maintenance because investment toward growth could be neglected at the adult stage (confirmed by our results). Intriguingly, ROS-dependent damages did not differ between the two groups, probably because of the higher rate of activation of the antioxidant enzyme superoxide dismutase for warm-acclimated fish. The allocation toward antioxidant defences is associated with an earlier reproduction and a lower quality of offspring. These interesting results bring new perspectives in terms of the prediction of the impact of global warming on biota through the use of ecological theories based on oxidative balance and metabolism.

KEY WORDS: Maintenance, Reproduction, Oxidative metabolism, Ectotherms

INTRODUCTION

Global warming is now considered a major threat for terrestrial and aquatic ecosystems. The two most well-known ecological impacts of climate change on biota are: (1) shifts in the distribution areas of species towards higher latitudes and altitudes, and (2) shifts in phenology, with spring events occurring earlier under warmer conditions (Wood and McDonald, 1997; Parmesan and Yohe, 2003; Root et al., 2003). Recently, Daufresne et al. (Daufresne et al., 2009) highlighted that the size of aquatic ectotherms also tends to decrease with global warming. Changes in phenology and organism size are necessarily related to physiological changes, but such physiological changes are not yet understood. Of course, the thermal sensitivity of the physiology of ectothermic organisms has been widely studied, but most of these studies have investigated thermal stress and not a slow and slight deviation from thermal optima [i.e. above the 80% performance breadth (‘B80’) reviewed by Angilletta et al. (Angilletta et al., 2002)].

Temperature has a direct influence on metabolism (Gillooly et al., 2001; Pörtner et al., 2006), playing a role in enzymatic kinetics (Rao and Bullock, 1954) and, in turn, increasing oxygen demand (Fry and Hart, 1946) and respiratory activity (Clarke, 2004; Gillooly et al., 2006). The core of metabolism is oxidative phosphorylation [i.e. consumption of oxygen in order to generate energy as ATP (=ATP)], which mainly occurs in mitochondria. These organelles are involved in a range of other processes [e.g. cellular differentiation, cell death and cell growth (Lane, 2006)] and probably play a role in ageing (Kirkwood and Austad, 2000). Rubner (Rubner, 1906) was the first to suggest that higher mass-specific metabolism could lead to a shorter lifespan, and later Metchnikoff (Metchnikoff, 1908) proposed that ageing and death could be the consequences of toxic by-products of metabolism. Harman (Harman, 1956) developed the idea and stated that these toxic by-products are the reactive oxygen species (ROS), which derive from oxygen affecting lipids, proteins and DNA integrity. According to Harman’s ‘free radical theory of ageing’ (FRTA), the increase in ROS production or change in oxidative balance (e.g. increased production or fewer antioxidant defences) leads to a decrease in lifespan (Finkel and Holbrook, 2000). Recently, the idea of oxidative balance led some authors to integrate the FRTA into the concepts of life history trade-offs and resource allocation, especially regarding growth and reproduction (Alonso-Alvarez et al., 2004; Alonso-Alvarez et al., 2006; Alonso-Alvarez et al., 2007; Dowling and Simmons, 2009; Monaghan et al., 2009; Metcalfe and Alonso-Alvarez, 2010). The main idea is that enzymatic defences require energy that may not be allocated to other functions [‘physiological cost’ described by Zera and Harshman (Zera and Harshman, 2001)]. Most of these studies have focused on endotherms and few have dealt with ectotherms (Salin et al., 2012). Regarding ectotherms, increasing environmental temperature leads to an increase in oxidative metabolism and thus to an increase in ROS production [1–4% of oxygen is transformed into ROS (Abele et al., 2002; Speakman, 2005)], especially because mitochondria exposed to high temperatures are less efficient [the ADP/O ratio is lower in warmer conditions (Abele et al., 2002)].

Here, we predict that the thermal stimulation of aerobic metabolism in ectotherms will lead to an increase in ROS production, which may, in turn, induce damage to macromolecules. This damage may influence the trade-off in resource allocation between growth, reproduction and maintenance (mechanisms against the ROS damage). Considering that, at the adult stage, allocation of energy toward growth could be neglected (von Bertalanffy, 1957), the trade-off should be between the two other important traits: reproduction and maintenance. From this, there are two non-mutually exclusive ways that could be used by individuals to deal with this extra ROS production. First, fish living in warm conditions may upregulate their antioxidant activities in order to

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List of symbols and abbreviations

FRTA	free radical theory of aging
GPx	glutathione peroxidase
H	hatching rate
H_2O_2	hydrogen peroxide
j_0	first day that a female laid
j_f	last day that a female laid
L_{egg}	egg size
L_f	final body size
L_{female}	female size
L_i	initial body size
MDA	malondialdehyde
M_{F1}	survival rate until maturity of the offspring
NADPH	nicotinamide adenine dinucleotide phosphate
N_e	total number of eggs from a given tank
N_{eji}	number of eggs laid at a given date
N_{fji}	number of females that laid eggs at a given date
N_j	number of days where females laid eggs
N_l	total number of larvae from a given tank
N_m	total number of individuals that achieved maturity from a given tank
$O_2^{\cdot -}$	superoxide anion
$OH^{\cdot -}$	radical hydroxyl
Q_{10}	thermal coefficient
ROS	reactive oxygen species
SOD	superoxide dismutase
TBARs	thiobarbituric acid reactive substances
$\bar{x}$	mean number of eggs laid per female per day

limit oxidative damage. However, because of the amount of energy invested in defences, reproductive effort could be reduced. The second scenario may result from a no-energy allocation to antioxidant defences in order to sustain the ATP pool necessary for reproduction. As a consequence, an increase in oxidative damage and, in turn, mortality rate, may result. In this paper, we used an experimental approach to test these hypotheses. Adult fish were reared at two non-stressful temperatures. We used *Oryzias latipes* Temminck & Schlegel 1846 because this species can live in a wide range of temperatures without exhibiting any stress. We observed responses throughout the duration of the adult stage (from 4 to 8 months old). This duration is relatively long for this species [mean lifespan in controlled conditions at 27°C is 1 yr (Shima and Mitani, 2004), confirmed in Leaf et al. (Leaf et al., 2011)] and corresponds to almost the entire reproductive period (Hirshfield, 1980). This study was conduced over this duration because the physiological responses observed after short-term acclimation (<1 month) could be different than the responses seen after a long-term exposure [>1 month (Sidell et al., 1973; Greaney et al., 1980; Sellner and Hazel, 1982)]. To our knowledge, no study has focused on such a long-term temperature exposure (relative to lifespan) in controlled conditions for fish species at the adult stage. The results of our study provide evidence that increasing temperature leads to changes in oxidative balance and life history traits in fish.

RESULTS

Initial versus final size after 4 months of acclimation

In the warm treatment (30°C), the mean initial size of fish (L_i) was 34.9±2.3 mm and the mean final size (L_f) was 35.0±2.3 mm. In the cold treatment (20°C), the mean initial size was 35.4±2.0 mm and the mean final size was 35.5±2.6 mm. We observed that all individuals grew during the experiment because the initial size was significantly smaller than the final size for the two thermal groups (Table 1). However, no interaction between final size and temperature was found ($P>0.05$; we removed this effect from the model presented in Table 1), suggesting that individuals from the warm group grew similarly to individuals from the cold group. The gain in size was extremely limited (*ca.* 1 mm), presumably because individuals were already mature at the beginning of the experiment. Finally, there was also no significant effect of sex on growth of adult *O. latipes*.

Reproduction

Clutches: quantity and quality

There was a significant difference in the mean clutch sizes (=number of eggs) per day and per female ($\bar{x}$) between the two temperature

Table 1. Details of the different linear mixed models

			Random effects		Fixed effects		
Property	N (cold; warm)	Dependent variable	Effect	s.d.	Effect	Estimate	P
Growth	76 (38; 38)	L_f	Replicate	0.031	Intercept	0.755	$0.437^{n.s.}$
					L_i	1.022	0.004**
					Temperature	–0.023	$0.401^{n.s.}$
					Sex	0.007	$0.947^{n.s.}$
Reproduction	131 (67; 64)	$\bar{x}$	Replicate	0.279	Intercept	10.382	0.000***
			Date	1.249	Temperature	–3.105	0.000***
	279 (142; 137)	L_{egg}	Replicate	0.029	Intercept	0.706	0.003**
			FemaleID	0.028	L_{female}	0.030	0.000***
					Temperature	–0.112	0.000***
Damage	43 (23; 20)	TBARs	Replicate	0.844	Intercept	18.208	0.000***
					Temperature	1.833	$0.195^{n.s.}$
Defence	43 (23; 20)	SOD	Replicate	0.410	Intercept	386.541	0.000***
					Temperature	258.170	0.008**
	42 (23; 19)	GPx	Replicate	0.168	Intercept	374.300	0.000***
					Temperature	14.500	$0.823^{n.s.}$
	42 (22; 20)	Catalase	Replicate	0.660	Intercept	2.792	0.000***
					Temperature	–0.136	$0.842^{n.s.}$
Oxidative status	42 (23; 19)	TBARs	Replicate	0.256	Intercept	13.956	0.000***
					SOD/GPx	1.168	0.000***
					Temperature	–0.829	0.006**
					SOD/GPx × Temperature	0.725	0.001**

GPx, glutathione peroxidase; L_{egg}, egg size; L_f, final body size; L_{female}, female size; L_i, initial body size; SOD, superoxide dismutase; TBARs, thiobarbituric acid reactive substances; $\bar{x}$, mean clutch size.
**$P<0.01$; ***$P<0.001$; n.s., not significant.

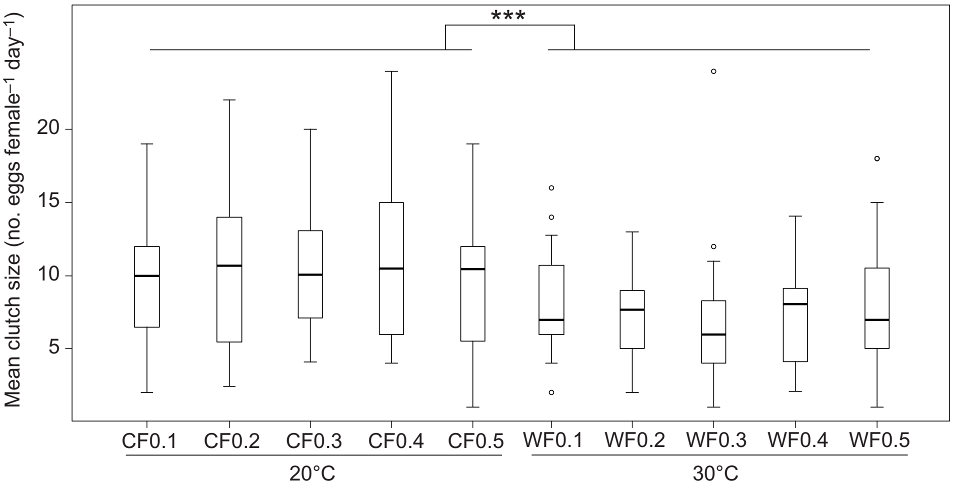

Fig. 1. Eggs laid by female *Oryzias latipes* at 20°C (cold group) and 30°C (warm group). Boxplots represent the first and the third quantiles and the medians of the mean size of clutch per day observed in each tank (WF0.1 to WF0.5 correspond to the tanks from the warm group and CF0.1 to CF0.5 correspond to the tanks from the cold group). The whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range from the box. Open circles (outliers) are defined as data points that are located outside the whiskers of the boxplot. Asterisks indicate a significant difference between thermal treatments (***P<0.001).

treatments (Table 1), with fewer eggs laid by the individuals of the warm group (Fig. 1). The hatching rate (H) was significantly lower in the warm group (Z=3.90, P<0.001; Table 2), but survival until maturity (M_{F1}) seemed to be the same in both groups (Z=1.77, P=0.083; Table 2).

Phenology

Temporal distributions of the egg production over the experiment were significantly different between the two temperature treatments (χ^2=1544.63, d.f.=226, P<0.001). Indeed, spawning started immediately in the warm group whereas the first clutch was observed 35 days after the beginning of the experiment in the cold group.

Egg size

Egg sizes were positively correlated to female size (Fig. 2). The linear model showed a similar slope for the two temperature treatments, the interaction term being non-significant. Temperature decreased the intercept of the egg size–female size relationship and eggs were, for a given female size, on average 0.11 mm bigger in the cold group compared with the warm group (Table 1).

Table 2. Reproductive effort parameters determined for both temperature treatments

Temperature	Replicate	N_c	N_e	N_l	N_m	H	M_{F1}
20°C	CF0.1	30	309	154	67	49.8	21.7
	CF0.2	31	327	179	53	54.7	16.2
	CF0.3	28	299	176	50	58.9	16.7
	CF0.4	33	320	164	55	51.3	17.2
	CF0.5	32	350	201	65	57.4	18.6
	Total	154	1605	874	290	54.5***	18.1$^{n.s.}$
30°C	WF0.1	30	273	140	52	51.3	19.0
	WF0.2	31	294	136	50	56.3	17.0
	WF0.3	38	315	133	39	42.2	12.4
	WF0.4	34	306	148	44	48.4	14.4
	WF0.5	40	341	169	55	49.6	16.1
	Total	173	1529	726	240	47.5***	15.7$^{n.s.}$

Results are detailed by tank and means per treatment are determined. The differences were determined only between the rate of hatching (H) and the rate of survival until maturity (M_{F1}) because these parameters take into account the numbers of eggs, larvae and adults.

N_c, number of clutches observed; N_e, number of eggs; N_l, number of larva at the end of the experiment; N_m, number of offspring that achieve maturity.

***P<0.001; n.s., not significant.

Oxidative balance

Quantification of oxidative damage

The mean values concerning the membrane damage were 16.36±1.75 nmol MDA mg^{-1} fresh tissue for the cold group and 16.43±1.20 nmol MDA mg^{-1} fresh tissue for the warm group. No significant differences between the two groups were observed. Similarly, no significant thermal effect was observed for carbonylated proteins despite a mean that was two times lower for fish from the cold group compared with fish from the warm group (4.6±2.6 vs 8.3±3.6 nmol mg^{-1} fresh tissue for 20 vs 30°C, respectively; W=16, P=0.19).

Quantification of antioxidant defences

Our linear model highlighted a significant difference between the two temperature treatments regarding the activity of superoxide dismutase (SOD) (Table 1), with a higher activity in the warm group (Fig. 3). Conversely, glutathione peroxidase (GPx) activity did not differ between the two groups (Table 1, Fig. 4). In the same way, we showed no significant differences for catalase (Table 1, Fig. 4). Finally, there was more membrane damages when the SOD/GPx ratio was low (Fig. 4, Table 1). The interaction between temperature treatment and SOD/GPx ratio was significant, indicating that the increase in damage on membranes is more efficiently controlled by the increase in SOD/GPX ratio for fish from the cold group than for fish from the warm group.

DISCUSSION

Throughout this 4-month experiment, very little energy was allocated to growth for adult *O. latipes* for both temperatures. This result contrasts with that observed by Leaf et al. (Leaf et al., 2011), probably because of their acknowledged overestimation of adult growth and the differences in experimental design. Because no differences in growth were observed, we have especially focused on

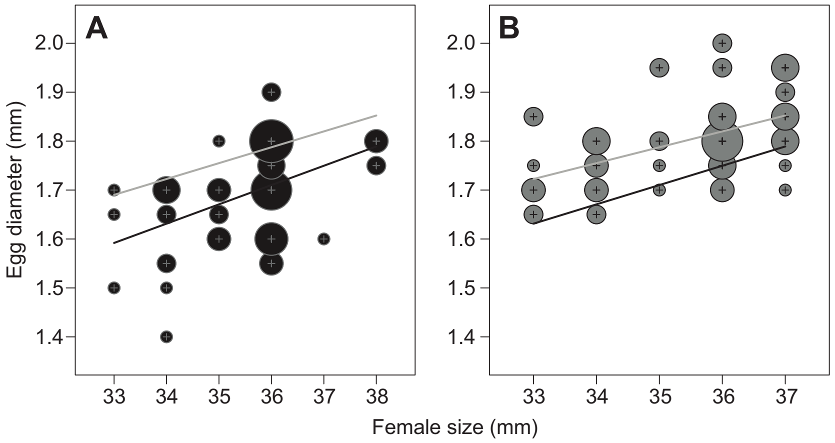

Fig. 2. Relationship between female size and egg size of *Oryzias latipes* at 30°C (A; warm group) and 20°C (B; cold group). Black and grey linear regression lines correspond to the warm and cold groups, respectively. The size of the circles is proportional to the number of females that present the same body size and the same egg size. The centre of each circle is indicated by a ‘+’ symbol to increase readability.

the trade-off between reproduction and maintenance (here assessed through antioxidant processes). Concerning reproduction, we observed: (1) differences in phenology and in reproductive effort between females exposed to 20 and 30°C, and that females from the cold group laid (2) more eggs per day and (3) larger eggs than fish from the warm group, and (4) their hatching rates were higher, suggesting a better quality of offspring.

From a physiological point of view, adults seemed to be similarly impacted by ROS despite the higher aerobic metabolism theoretically observed under higher temperature (Rao and Bullock, 1954). The present study demonstrates that long-term (relative to lifespan) exposure to high (but non-stressful) temperature has an impact on oxidative metabolism without affecting oxidative damage. However, the lack of significant differences in oxidative damage between temperature treatments raises some questions regarding oxidative markers as sensors of trade-offs.

Concerning the antioxidant defence (SOD activity), we observed higher activity in individuals in the warm group. More surprisingly, temperature seemed to have a potential effect on the antioxidant capacity (evaluated through the SOD/GPx ratio). This effect could thus become a key element regarding the sensitivity of individuals to temperature.

Our study show no direct negative correlation between reproduction and maintenance [as previously observed (Monaghan et al., 2009; McGraw et al., 2010; Metcalfe and Alonso-Alvarez, 2010)], but the increase in SOD activity associated with the small clutch size observed under warm treatment suggests a possible trade-off between maintenance and reproduction.

Thermal effect on oxidative damage and antioxidant defences

The thermal sensitivity of the metabolism of ectothermic species is well known and well documented (Rao and Bullock, 1954). Thus, we expected a higher metabolism for fish exposed to the warm treatment and higher associated ROS damage. However, the results of our study showed no significant differences concerning damages to membranes or proteins.

First of all, it is important to note that the lack of a significant effect of temperature on oxidative damage could be due to the limited number of samples used to measure damage, especially to proteins (i.e. the type II statistical error is high). In addition, the absence of observed differences could be related to the age of the fish. Indeed, the individuals tested were 8 months old, which does not exactly correspond to the end of life for *O. latipes* [mean lifespan at 27°C: 1 yr (Shima and Mitani, 2004)]. According to the FRTA, older individuals exhibit greater damage. In this context, we cannot exclude that older fish could exhibit higher MDA contents in the warm group towards the end of their life. Thus, complementary analysis on damage to proteins or DNA over a much longer duration could help to achieve a more accurate picture of the relationship between temperature and oxidative damage.

In addition, with increasing temperature, the degree of unsaturation of the fatty acids of the membranes is known to change (Hazel and Williams, 1990; Cossins, 1994). This change, commonly referred to as ‘homeoviscous adaptation’ (Hazel and Williams, 1990), has been interpreted as a mechanism for compensation of membrane fluidity and/or order, a physical property (Cossins, 1994). This also makes the membranes of highly acclimated fish probably less sensitive to oxidative stress (Bielski et al., 1983). We cannot exclude that such a phenomenon occurred in our experiment, and this could explain a stronger between-temperature difference in damage to proteins than to membranes. Overall, even if our results for damage have to be analysed cautiously, our experiment reveals that if a sharp increase in temperature induces an oxidative stress (Lushchak and Bagnyukova, 2006), this effect is subtler when organisms are submitted to slight changes in temperature over the long term.

Regarding antioxidant defence, we confirmed higher antioxidant activities for fish from the warm group than from the cold group. Indeed, SOD tested at the same temperature (25°C) for the two groups showed higher activities for the individuals that were exposed to 30°C. The higher SOD activity for fish from warm conditions has been shown previously in a shrimp species (*Macrobrachium nipponense*) (Wang et al., 2006). Surprisingly, the other enzymes involved in oxidative defence (GPx and catalase) presented the same activity in both thermal groups. The higher activity for SOD under warm conditions may thus have induced an increase in H_2O_2 concentration in the cell. We indeed observed that the positive correlation between the SOD/GPx ratio and the membrane damage indicated that the more incomplete the antioxidant defence chain, the more individuals are affected by ROS. This correlation is thermally dependent and the different slopes suggest once again higher ROS production at 30°C. In addition, de Haan et al. (de Haan et al., 1995) proposed the existence of a biological optimum for the ratio between SOD and GPx + catalase activities, which might be more relevant than the absolute activities of the enzymes themselves. The accumulation of H_2O_2 could also induce another deleterious effect via the Fenton reaction (Mao et al., 1993), which leads to the production of the radical hydroxyl ($OH^{\cdot -}$), which is the most dangerous oxidative molecule. In other words, accumulation of H_2O_2 because of higher SOD

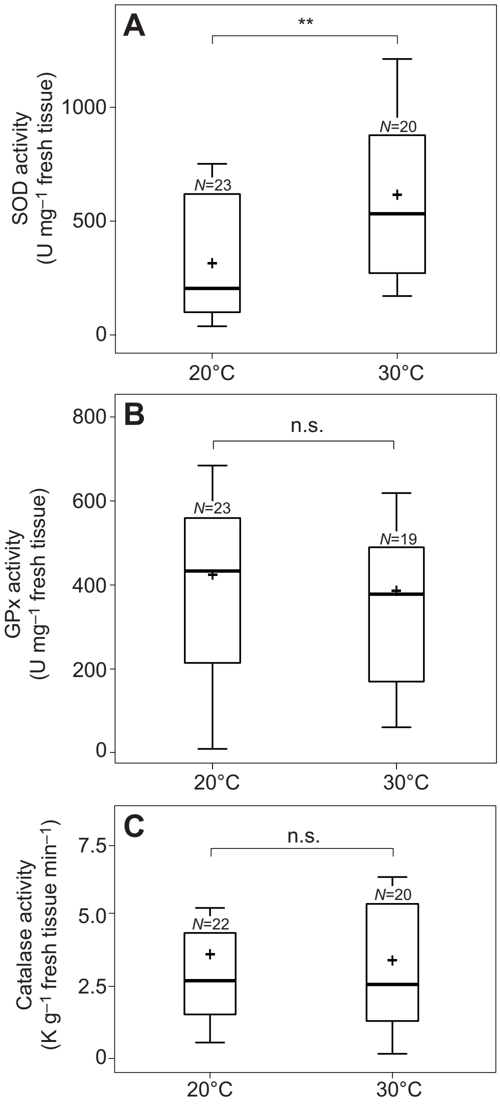

Fig. 3. Antioxidant enzyme activities measured in *Oryzias latipes* at 20°C (cold group) and 30°C (warm group). Boxplots represents the mean (cross) the median (middle line) and the first and third quantiles of the results obtained for (A) superoxide dismutase (SOD), (B) glutathione peroxidase (GPx) and (C) catalase. The whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range from the box. Asterisks indicate significant differences between thermal treatments (***P*<0.01; n.s., *P*>0.05). The sample sizes (*N*) are given.

activity under warm conditions could have induced an increase in OH$^{-\cdot}$ concentration with harmful effects, even if we failed to detect them here. Further studies focusing on other cell components (e.g. genetic material) and including more individuals would help generate a more complete picture of oxidative damage to cells under warm conditions. Nevertheless, the higher antioxidant activities for fish from the warm group is a crucial point within our resource allocation context.

Mortality and reproductive effort

Quality versus quantity of offspring is one of the trade-offs often studied by evolutionary biologists (Charnov and Gillooly, 2004). According to Hirshfield (Hirshfield, 1980), offspring mortality increases with temperature for *O. latipes* and our results partially validate this relationship. Indeed, the hatching rate was significantly higher for eggs from the cold group. However, no significant differences in survival until maturity were observed.

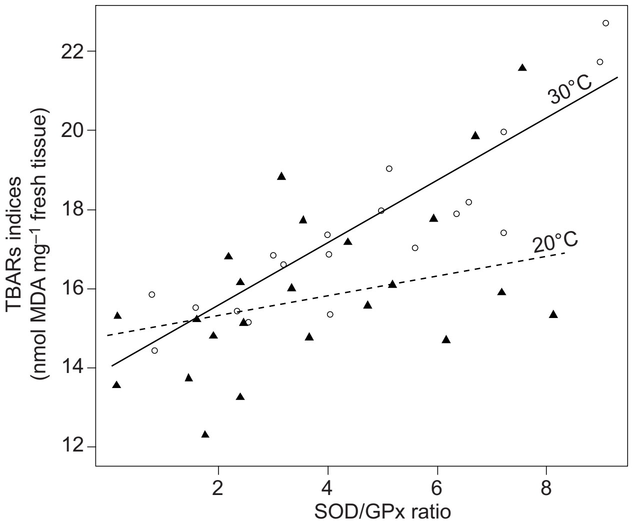

Fig. 4. Log–log relationship between SOD/GPx ratio and oxidative membrane damage of *Oryzias latipes* individuals at 20°C (cold group) and 30°C (warm group). The solid linear regression line and open circles correspond to results obtained for the warm group, and the dashed linear regression line and filled triangles are for fish from the cold group. TBARs, thiobarbituric acid reactive substances.

Besides the mortality rate of eggs and offspring, we observed a delay in the occurrence of the reproductive pattern in the cold group. This delay can probably be explained by the time necessary to produce sexual cells at a low temperature (Robinson and Rugh, 1943). In addition to this temperature effect on the timing of the reproduction pattern, we observed a change in the clutch size. Indeed, clutches from the warm group were significantly smaller than those from the cold group. Regarding medakas, this is dissimilar to what has been suggested by Hirshfield (Hirshfield, 1980), but is in accordance with results from Leaf et al. (Leaf et al., 2011). Interestingly, because females from the warm group laid eggs more often than the females from the cold group, there was almost the same total number of eggs laid in both temperature treatments. Thus, despite a change in the phenology, one could consider that the investment in reproduction was similar between the two thermal groups. It is important to note, however, that for a given length, a warm-acclimated female produced eggs that were significantly smaller in diameter than those from cold-acclimated females, as previously shown by Hirshfield (Hirshfield, 1980) and Leaf et al. (Leaf et al., 2011). These authors stated that it is not the size of the egg that changes with thermal treatment, but its quality. We cannot exclude that size and quality of the eggs were related in our experiment. Actually, the hatching rate was lower under warmer conditions (and thus when eggs were smaller). Considering the absence of growth during the 4 months of the experiment and the higher investment in antioxidant defences, we can also hypothesize that eggs were smaller under higher temperatures because of a lower investment in reproduction per clutch event.

The clutch size ($\bar{x}$) given by Eqn 1 (see Materials and methods) did not take into account the sex ratio, which was different between the two groups and changed during the experiment. However, it is commonly observed that the number of males is not limiting for reproduction, notably in fish species (Andersson, 1994).

These results have several ecological implications. It is generally assumed that warmer temperatures could allow the reproductive period to increase for species that are not submitted to thermal stress. In addition, an earlier reproduction is an advantage in stressful environments (Stearns, 1992). From this viewpoint, the strategy of the fish from the warm group, which actually invested relatively steadily and quickly in reproduction, could be advantageous. However, the lower quality of the eggs (observed here with the lower hatching rate) could balance the benefit of the increase in the length of the reproductive period. Indeed, it is interesting to note that, at the end of the experiment, the total number of mature individuals of the generation F1 (a proxy of the fitness of the F0 individuals) was quite similar for the two thermal groups (240 versus 290 for the warm and the cold groups, respectively). Thus, the changes in oxidative metabolism and life history traits could help to maintain similar population dynamics in different thermal environments. Further studies focusing on the whole ontogeny and over several generations could validate such hypothesis.

MATERIALS AND METHODS

Biological material and rearing conditions

The Japanese medaka (*Oryzias latipes*) is a small (20–40 mm, 250–500 mg) and iteroparous (Robinson and Rugh, 1943; Hirshfield, 1980) freshwater fish native to East Asia. This eurythermic fish [living in 5–35°C environments, optimal temperature: 25°C (Dhillon, 2007)] requires only 10–12 weeks to reach sexual maturity at 25°C. The experimental design is detailed in Fig. 5. Here, we used 76 individuals belonging to the CAB strain from Carolina Biological Supply Company (Burlington, NC, USA; from AMAGEN, Gif-sur-Yvette, France) and WatchFrog (Evry, France). Fish of this standard strain present differences in the colour of eyes and other tissues (Loosli et al., 2000; Furutani-Seiki and Wittbrodt, 2004; Furutani-Seiki et al., 2004; Sasado et al., 2010; Kinoshita et al., 2009), but can be used in ecophysiological studies because their development characteristics are similar to those of the wildtype (Sasado et al., 2010; Lawrence et al., 2012). They were studied from 4 months old (mature) to 8 months old [almost the end of life at 27°C (Shima and Mitani, 2004; Leaf et al., 2011)]. These individuals were reared under two contrasted temperatures: 20 and 30°C. These temperatures were chosen because of the wide range of thermal conditions supported by *O. latipes* (Shima and Mitani, 2004). Because of the possible effect of food resources, fish from each thermal group were fed *ad libitum* every day at 18:00 h with TetraMin, as in Hirshfield (Hirshfield, 1980). Individuals were kept for 5 days at the same rearing temperature as the supplier (25°C). After these 5 days, the temperature was increased or decreased by 1°C every 2 days, reaching 20°C for the 'cold' group and 30°C for the 'warm' group within 10 days. During this 15 day period, the photoperiod was 12 h:12 h light:dark.

Thirty-eight individuals (generation F0) were randomly placed in the cold group and 38 in the warm group at a ratio of 1.33 to 1.66 females for one male to maximize the reproductive effort and to avoid overtaxing females [this density, far below 2–3 fish l^{-1}, causes no stress and no agonistic behaviour (Denny et al., 1991)]. In order to avoid the loss of all fish because of a common disease (e.g. *Ichthyophthirius multifilis*), fish were placed in groups of seven or eight in five 20 l tanks (see details in Fig. 5; WF0.1 to WF0.5 corresponding to the tanks of the warm group and CF0.1 to CF0.5 corresponding to the tanks of the cold group). After the 'settling' phase (5 days of animal housing and 10 days for the change in temperature), the photoperiod changed to 16 h:8 h light:dark to trigger reproduction (Hirshfield, 1980).

Eggs were collected every day and were placed in a small nursery (2.5 l) in the same tank as their parents (maximum density reported: 1 larva per

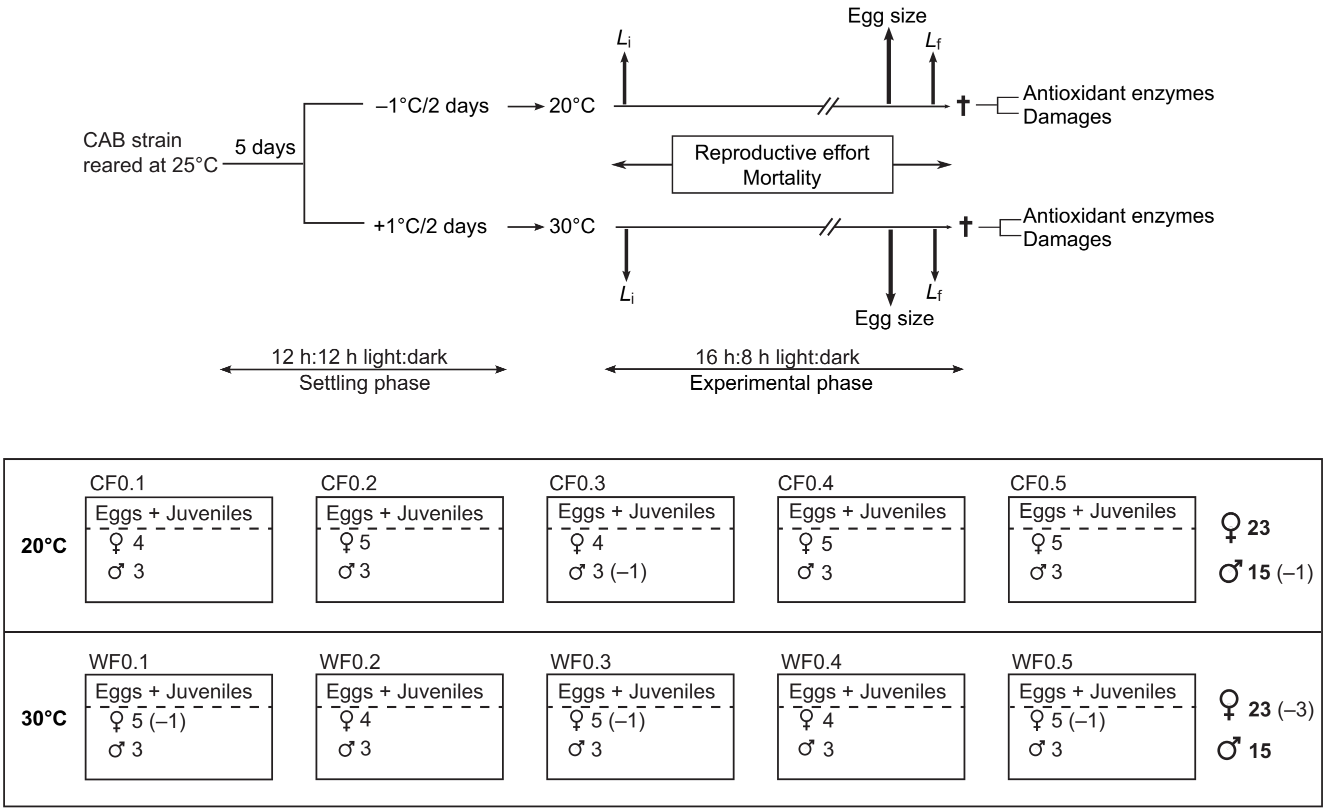

Fig. 5. Experimental design. WF0.1 to WF0.5 correspond to the tanks from the warm group (30°C) and CF0.1 to CF0.5 correspond to the tanks from the cold group (20°C). Daggers correspond to the end of the experiment, when individuals were euthanised prior to measurement of oxidative elements (TBARs and SOD/GPx). Sample sizes are indicated next to the male and female symbols. Numbers in parentheses indicate the number of dead individuals during the experiment (i.e. before euthanasia). Eggs and juveniles were placed in a nursery in the same tank as their parents.

50 ml of water). Larvae were fed *ad libitum* every day at the same time as the parents. According to legislation, our laboratory obtained an agreement for running experiments on *O. latipes* for this study (Direction départementale de la protection des populations, no. A 13-510).

Measurement of growth

In order to confirm that adult fish did not grow during the experiment, we measured the initial and the final total lengths (in mm) of each individual. At the beginning of the experiment, non-anaesthetised fish were placed in a small Petri dish with water. The sex was determined before individuals were assigned to each tank [females present a smaller anal fin than males (Kinoshita et al., 2009)] and the initial sizes (L_i) were measured with millimetre graph paper with a precision of ±0.5 mm. Four months later (at the end of experiment), the final sizes (L_f) were determined following the same protocol.

Reproductive effort

Reproductive effort (phenology, quantity of offspring, egg size and quality of offspring) was only determined in females.

Phenology

Clutches were collected every day directly from the female [females keep eggs after fertilisation (Shima and Mitani, 2004)] with a brush early in the morning (08:00 h). The 10 groups (five tanks per temperature treatment) were followed during the entire experiment in order to determine the phenology of the reproduction processes. The timing of the first clutches was noted in each tank to determine if there was a delay between the two thermal groups.

Quantity of offspring

The clutch sizes (number of eggs) were measured every day. Because the number of females was not exactly the same at the beginning of the experiment (see Fig. 5) and because it changed slightly during the 4 months (due to the loss of three females in the warm group), we determined the mean number of eggs produced per female that laid and per day ($\bar{x}$) as:

$$\bar{x} = \frac{1}{N_{\mathrm{j}}}\sum_{j_0}^{j_{\mathrm{f}}}\left(\frac{N_{\mathrm{e}ji}}{N_{\mathrm{f}ji}}\right), \qquad (1)$$

where j_0 corresponds to the first day where females from a tank laid eggs and j_f is the last day, N_j corresponds to the number of days where females from a given tank laid eggs (not every day), N_{eji} corresponds to the number of eggs laid at a given date (i) and N_{fji} is the number of females that laid eggs on the same date (i).

Egg size

A clutch was taken from each of 15 females (with no significant differences in mean female size between the two groups: t-test: d.f.=67.5, P=0.16) on a single day (27 November 2011, 101 days from the beginning of the experiment). The diameters of 137 and 142 eggs collected from the warm and cold groups, respectively, were measured with a microscope system (Leitz DIALUX 20 EB, Midland, ON, Canada) with ±0.01 mm precision.

Quality of offspring

In order to determine the quality of offspring (i.e. the individuals of the F1 generation), the number of larva were observed at the end of the experiment, which allowed us to determine the hatching rate (H) following the equation:

$$H = \frac{N_{\mathrm{l}}}{N_{\mathrm{e}}} \times 100\,, \qquad (2)$$

where N_l is the total number of larva observed in a given tank and N_e is the total number of eggs laid in this tank.

We also determined the rate of survival until maturity of the offspring generation (M_{F1}) with the equation:

$$M_{\mathrm{F1}} = \frac{N_{\mathrm{m}}}{N_{\mathrm{e}}} \times 100\,, \qquad (3)$$

where N_m corresponds to the total number of offspring (corresponding to the F1 generation) that lived until maturity in a given tank.

Oxidative stress

To avoid any bias in the results due to the possible sex effect in resource allocation associated with oxidative balance, we measured physiological properties only on females, as suggested by Heiss and Schoech (Heiss and Schoech, 2012).

After the 4 months of the experiment, fish were euthanised (by demedulation) and placed in an Eppendorf tube (2 ml). They were then placed in liquid nitrogen in order to fix enzymatic reactions and for later tissue analysis. The 23 females from the cold group and the 20 (less three individuals that died during the experiment) from the warm group were used for physiological measurements of oxidative balance. After removing the head and the fins, fish were first homogenised twice with ball milling for 4 min at 50 Hz in a monopotassium phosphate solution (KH_2PO_4 100 mmol l^{-1}). These homogenates were then divided into two equal parts, one used for damage determinations and the other for quantification of antioxidant activities.

Quantification of ROS damage

Under oxidative stress, membrane lipids are peroxidised. The product of this reaction is malondialdehyde (MDA). The thiobarbituric acid reactive substances (TBARs) method is based on the measurement of the presence of MDA in the sample following the protocol described by Yagi (Yagi, 1976). The homogenates obtained above were homogenised again with a buffer solution (100 mmol l^{-1} KH_2 PO_4, with the addition of 0.05% bovine serum albumin, 10 mmol l^{-1} EDTA, 0.13 mmol l^{-1} butylated hydroxytoluene and 0.13 mmol l^{-1} desferoxamine). These new homogenates were then centrifuged at 1100 ***g*** (4°C 10 min^{-1}) and supernatants were collected. These extracts were conserved at −80°C and analysed later. Two hundred microlitres of supernatant was added to 30 µl of 8.1% SDS, 225 µl of 20% acetic solution and 225 µl of 0.8% thiobarbituric acid. These samples were incubated and shaken at 95°C for 1 h. Then, after addition of 600 µl of butanol/pyridine (15/1), the samples were shaken again for 10 min and centrifuged once more for 10 min at 1600 ***g*** (ambient temperature). The upper phases were removed and dosed at 532 nm at 25°C and compared with a standard range with TEP (1,1,3,3-tetraethoxypropane). The results were expressed as nanomoles of MDA per milligramme of fresh tissue.

Even if the TBARs method is commonly used to assess ROS damage [examples in ectotherm species (Wilhelm-Filho et al., 2001; Sukhotin et al., 2002; Mila-Kierzenkowska et al., 2005)], its use is criticised (Almroth et al., 2005). Therefore, in order to better evaluate damages caused by ROS, we also measured carbonylated proteins on a subsample of individuals already tested for TBARs (N=5 for the cold group and N=4 for the warm group). The remains of homogenates were centrifuged at 10,000 ***g*** for 15 min at 4°C. The supernatants collected were then tested with the Cayman Protein Carbonyl Assay Kit using DNPH (2,4-dinitrophenylhydrazine) as indicative of carbonylated proteins (item N.10005020, Cayman Chemical, Ann Arbor. MI, USA). The results were expressed as nanomoles of carbonylated proteins per milligramme of total protein content (compared with a BSAe standard at 280 nm and 25°C).

Quantification of antioxidant defences: enzyme activities of SOD, GPx and catalase

The extraction method was the same for the three enzymes and quantification of enzyme activities followed the protocols described in Voituron et al. (Voituron et al., 2006). We homogenised the samples in a buffer solution (1 mmol l^{-1} dithiothreitol, 2 mmol l^{-1} EDTA, pH adjusted to 7.4 at 4°C) during 4 min at 50 Hz with ball milling. Homogenates were then centrifuged for 5 min at 4°C and 2380 ***g*** and supernatants were used for measurements.

The enzyme superoxide dismutase (SOD) catalysed the dismutation of $O_2^{\cdot\,-}$ (superoxide anion) to produce H_2O_2 (hydrogen peroxide). This enzyme is very unstable; thus, the samples were measured directly after extraction. Homogenates were frozen (−80°C) and analysed later for glutathione peroxidase (GPx) and catalase activities. Measurements of SOD activities were indirect. We used the xanthine/xanthine-oxidase system as a producer of $O_2^{\cdot\,-}$ and the oxidation of cytochrome *c* was followed to determine the activity at 550 nm and 25°C. The results were expressed as units per milligramme of fresh tissue. Regarding GPx, its function consists of the conversion of free H_2O_2 produced by SOD in water. To evaluate GPx activities, measurements were based on tracking, using spectrophotometry, and the NADPH disappearance at 340 nm and 37°C. We also measured the catalase activities. This enzyme also allows the decomposition of H_2O_2 to water and oxygen (like the GPx enzyme but not located in the same cellular compartment). The decrease in H_2O_2 was measured in the sample by spectrophotometry at 240 nm at 25°C in quartz cuvettes in order to quantify catalase activities.

Statistical analysis

Values are given as the means ± s.e.m. For all tests, the level of significance was fixed at 5%. Growth, reproductive effort and physiological properties were analysed by linear mixed models because residuals followed a Gaussian law. We took into account the pseudoreplication effect in these linear mixed models considering the tank as a random effect (‘replicates’). All tests were performed using R version 2.14.2 (R Development Core Team, 2012). The linear mixed models were performed with the package ‘lme4’ (Bates et al., 2012). The significance of the fixed effects was evaluated by Markov chain Monte Carlo tests (Baayen, 2011).

Growth

Growth was analysed by a linear mixed model with the final length (L_f) as a dependent variable and initial size (L_i), temperature (°C) and the interaction between L_i and temperature as fixed variables. To check for a potential difference in growth of male versus female individuals, sex was also considered as a fixed effect. The replicates were considered as random effects. The interaction term was removed from the model if it was not significant.

Reproduction

Differences in mean clutch size ($\bar{x}$) between the two thermal groups were estimated by a linear mixed model with temperature as a fixed effect and date and replicates as random effects. Then, in order to study the differences in the phenology of reproductive events, a χ^2 test of homogeneity was carried out. This test allowed us to observe differences regarding the distribution of clutch events over time between the two thermal groups.

To estimate differences regarding egg size (L_{egg}), a linear mixed model was used with female size (L_{female}), temperature and their interaction as fixed effects, and replicate and female identity (‘FemaleID’) as random effects.

Finally, the hatching rate (H) and survival rate until maturity for the offspring (M_{F1}) were compared between the two thermal groups using homogeneity tests to compare two proportions.

Oxidative balance

All females from a given temperature were pooled together at the end of experiment in order to have more statistical power when analysing the differences in the physiological parameters (damage and defence). SOD, GPx and catalase activities and MDA contents were analysed by linear mixed models with temperature as a fixed effect and replicate as a random effect. Because of the small sample sizes, the differences between the rate of proteins carbonylated in the two thermal groups were analysed by a non-parametric test (Wilcoxon Mann–Whitney).

In order to determine the efficiency of antioxidant enzymes, we also calculated the mean ratio between SOD and GPx activities (Sánchez-Rodríguez et al., 2007). This ratio allowed us to observe, using a linear mixed model, the relationship between damage to membranes (TBARs) and antioxidant capacities (SOD/GPx ratio), temperature and their interaction. Again, the replicates were considered as a random effect. The interaction term was removed from the model if it was non-significant.

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Competing interests

The authors declare no competing financial interests.

Author contributions

C.H.-B. and M.D. contributed to the conception of the study. C.H.-B., M.D. and Y.V. designed the experiment. C.H.-B., L.R. and C.R. performed the experiments. C.H.-B. performed the statistical analyses. C.H.-B. and M.D. wrote the paper. All authors contributed to the interpretation of the results and critically reviewed the early versions of the manuscript.

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[**10**] **Daufresne M.**, K. Lengfellner and U. Sommer (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences of the United States of America.* **106**, 12788–12793.

Global warming benefits the small in aquatic ecosystems

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Understanding the ecological impacts of climate change is a crucial challenge of the twenty-first century. There is a clear lack of general rules regarding the impacts of global warming on biota. Here, we present a metaanalysis of the effect of climate change on body size of ectothermic aquatic organisms (bacteria, phyto- and zooplankton, and fish) from the community to the individual level. Using long-term surveys, experimental data and published results, we show a significant increase in the proportion of small-sized species and young age classes and a decrease in size-at-age. These results are in accordance with the ecological rules dealing with the temperature–size relationships (i.e., Bergmann's rule, James' rule and Temperature–Size Rule). Our study provides evidence that reduced body size is the third universal ecological response to global warming in aquatic systems besides the shift of species ranges toward higher altitudes and latitudes and the seasonal shifts in life cycle events.

biological scale | body size | climate change | ectotherms | metaanalysis

At the biogeographical scale, the most noticeable ecological impact of global warming is a shift of species' ranges toward higher altitudes and latitudes in accordance with their thermal preferences (1–3). This observation has been used extensively to forecast the effect of climate change on biota by modeling future species distributions according to climate-change scenarios (4). However, such patterns and pattern-related predictions do not elaborate specific underlying ecological mechanisms. As a consequence, our understanding and, in turn, our ability to forecast the impacts of climate change on biota remains limited (e.g., it seems possible to forecast species' ranges, but it remains difficult to predict the relative abundances of species within a community). Besides the shifts in species' ranges, the second well-known ecological response to global warming is a change in phenology (3). Such patterns could lead to a decoupling of the dynamics of predators and prey (5). This mechanism-oriented hypothesis, generally referred to as the match–mismatch hypothesis (6), offers perspectives in forecasting the ecological impacts of climate change (5, 7, 8). Nevertheless, all of the components of the food web can be affected by dissimilar changes in phenology, leading to complex dynamics that are difficult to predict (5). The match–mismatch hypothesis and the shifts of species' ranges are key tools when evaluating the ecological consequences of global warming, but they are thus far insufficient to provide clear views on future ecological changes. Further general rules dealing with the impacts of a global rise in temperature on biota are needed.

Body size is a fundamental biological characteristic that scales with many ecological properties (e.g., fecundity, population growth rate, competitive interactions) (9, 10). Surprisingly, few studies have dealt with changes in body size with global warming (10), especially for ectotherms, although they represent $\approx$99.9% of species on Earth (11). Furthermore, the biological scales (individual, population, community) at which global warming should act on body size have not been studied. Three rules concerning ecogeographical and ecothermal gradients (10) are relevant in this context. First, Bergmann's rule (12), states that warm regions tend to be inhabited by small-sized species. Second, James' rule (13) states that, within a species, populations with smaller body size are generally found in warmer environments. Third, the temperature–size rule (TSR) states that the individual body size of ectotherms tends to decrease with increasing temperature (14). Combining these rules, we can build a set of 5 hierarchical and nonmutually exclusive hypotheses concerning the potential effect of climate change on size structures from the individual to the community scales (Fig. 1). The first hypothesis predicts a decrease in mean body size at the community scale under warming whatever the underlying mechanisms (community body size shift hypothesis). If there is a decrease in the mean body size at the community scale under warming, there are 4 subsequent hypotheses that could explain this decrease. According to Bergmann's rule, the first mechanism acts at the community scale is an increase in the proportion of small size species (species shift hypothesis) in terms of abundances of individuals and/or number of species. Second, according to James' rule, the decrease in size at the community scale could also be due to a decrease in mean body size at the population scale (population body size shift hypothesis). In turn, such a size decrease at the population scale could be due to 2 mechanisms. First, according to the TSR, the size-at-age (or size-at-stage; individual scale) should decrease with increasing temperature (size-at-age shift hypothesis). Note, however, that this decrease should not be observed for early ages or stages because the TSR predict a higher growth rate but a lower final size at higher temperature. In addition to this decrease in size-at-age/stage, an increase in the proportion of juveniles (population age-structure shift hypothesis) could also be expected at the population scale. The latter hypothesis does not correspond to the above-cited ecogeographical or ecothermal rules, but it is the default explanation if the population body size shift hypothesis applies whereas the size-at-age shift hypothesis does not apply. Note that, due to compensatory effects, the invalidation of a hypothesis does not imply that both subsequent hypotheses do not apply. For instance, no changes in mean size at the community scale can be due to a decrease in mean body size at the population scale and an increase in proportion of large species.

In this article, we studied changes in body size from individuals to communities under climate warming by testing the 5 hypotheses described above. The tests of the hypotheses were based on (*i*) the analysis of the effects of increasing temperature on long-term fish data sampled in French rivers and in the Baltic Sea, (*ii*) the analysis of experimental plankton data (bacteria, phyto- and zooplankton) collected in light- and temperature-controlled mesocosms (15), and (*iii*) on a review of related published work based on data collected in mesocosms and in the

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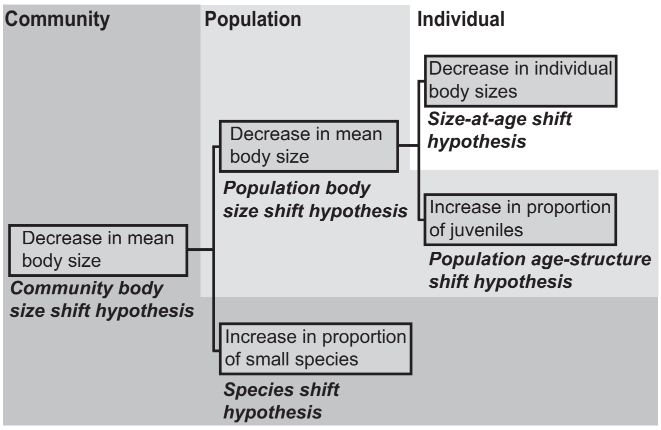

Fig. 1. The tested hypotheses regarding the impact of warming on body size at different biological scales.

North Sea (Fig. 2). Impacts of potential confounding factors, and especially of fisheries, were considered. Our results support the hypothesis that reduced body size is a third universal or very general ecological response to global warming among ectotherms in aquatic systems, besides the shift of species ranges toward higher altitudes and latitudes and the seasonal shifts in life-cycle events.

Results

We found that increased temperature acts on communities, populations and individuals through changes in species composition, growth and reproduction.

Community Body Size Shift. A metaanalysis revealed that the mean temporal trend (*S*) of mean body size of fish in large French rivers was significantly negative during the last 2–3 decades under gradual warming (Fig. 3). A decrease in mean body size with increasing temperature was also observed for bacteria in temperature-controlled mesocosms [extended linear mixed-effect (LME) model, coefficient estimate = -1.06×10^{-3}, *t* value = -5.51, number of observations = 68, $P = 3.1 \times 10^{-2}$; Fig. 4*A*]. The mean cell size of phytoplankton also tended to decrease with increasing temperature in the same mesocosms (16) (Fig. 4*B*).

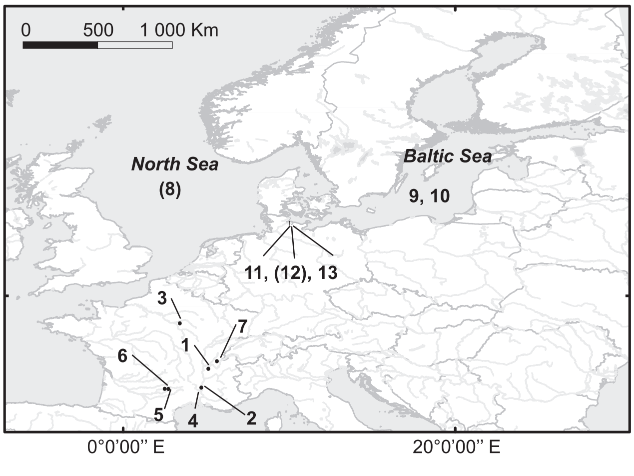

Fig. 2. Location of the study areas. 1–4: Long-term survey of freshwater fish communities in large rivers. 5–7: Long-term survey of brown trout populations. 8: Long term survey of North Sea fish community. 9–10: Long term survey of herring and sprat populations in the Baltic Sea. 11–13: Sampling of bacteria and phytoplankton communities and of *Pseudocalanus* sp. (zooplankton) in temperature-controlled mesocosms. Numbers in brackets refer to published climate–size relationships reviewed in this article (16, 17).

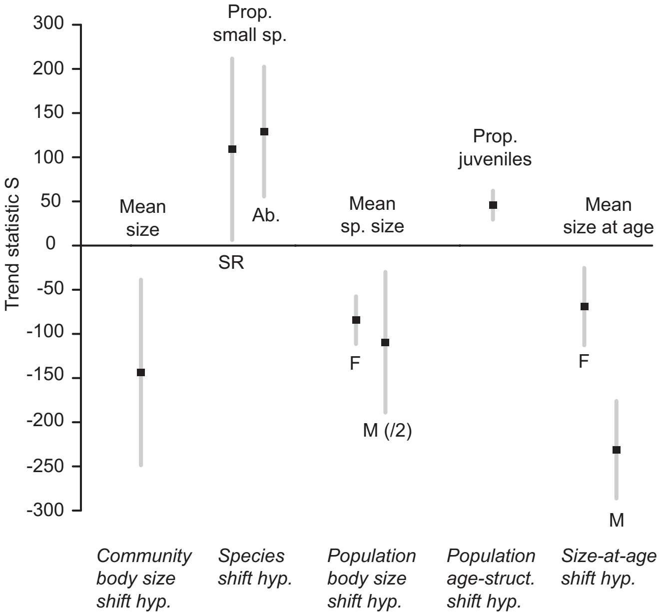

Fig. 3. Mean effect sizes (i.e., mean weighted temporal trend statistic *S*; ±95% confidence intervals). Negative or positive trend values indicate temporal decrease or increase, respectively. Mean temporal trends are significant if their 95% confidence intervals did not contain 0. Community body size shift and species shift hypotheses were tested by using 4 freshwater fish communities. To test the species shift hypothesis, small species were defined as species with a maximum size below the first quartile of the maximum size of all of the species in the community. Proportions of small species are calculated in terms of species richness (SR) and abundances (Ab.). Population body size shift and population age-structure shift hypotheses were tested by using 28 and 18 fish populations, respectively. Size-at-age shift hypothesis was tested by using 28 age classes. Significantly different means for marine (M) vs. freshwater (F) populations are represented. To increase readability some effect sizes are divided by a factor *x* (indicated in the figure as /*x*).

Species Shift. Supporting the species-shift hypothesis, the proportion of small-sized species significantly increased in communities of large French rivers (Fig. 3) both in terms of species richness and abundance. Similar patterns were also observed for the fish community of the North Sea where the geographical ranges of small species expanded, whereas those of large species shrank due to warming (17) (Fig. 4*C*). In this way, the more even distribution of small species and the patchier distribution of large species should result locally in an average temporal increase in the number of small species and an average decrease in the number of large species. Finally, because the same size s_i was attributed to all individuals from a given phytoplankton taxon *i* in ref. 16, the observed decrease in mean size described above (community body size shift; Fig. 4*B*) is entirely due to an increase in proportion of abundances of small-sized taxa.

Population Body Size Shift. Besides interspecific patterns, our metaanalysis revealed a negative temporal trend in the mean body size of individual fish populations under global warming (Fig. 3). Herring and sprat populations in the Baltic Sea showed merely significant stronger decrease in mean size than freshwater species populations (coefficient $Qb = 2.67$, $P = 0.10$), underlining the potential additive effect of fisheries.

Population Age-Structure Shift. The decrease in fish mean body size at the population scale was partially due to a significant

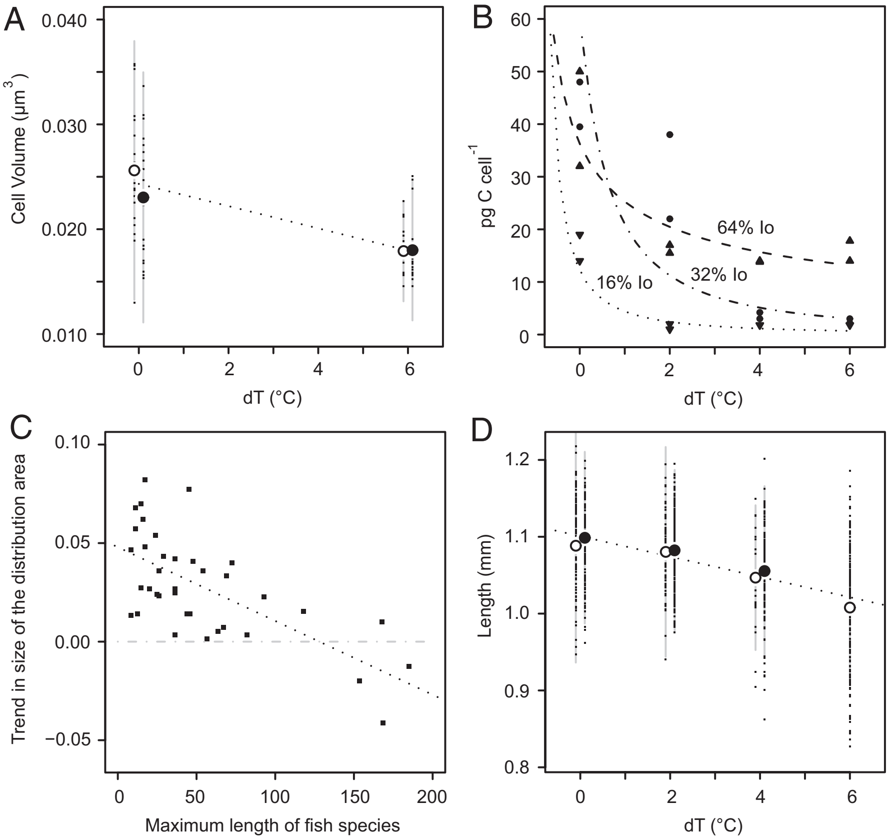

Fig. 4. Change in size structures under warming. (*A*) Cell size of bacteria subjected to different level of warming (+0, +2, +4, and +6 °C) compare to a reference thermal regime (dT) [means (open and closed circles), standard errors (gray lines), and raw data (closed rectangles) in the different replicates are represented]. (*B*) Mean cell size of phytoplankton at different level of warming (dT) and different light conditions [percentage of the natural light intensity above cloud cover (Io); 16% Io: hanging triangles; 32% Io: circles; 64% Io: standing triangles] (after figure 3c of ref. 16). (*C*) Effect of maximum length on distribution trends (expansion or shrinkage) of fish species in the North Sea during the past 20 y (after figure 4b of ref. 17). (*D*) Size of female adult *Pseudocalanus* sp. at different level of warming (dT) (symbols as for *A*).

increase in proportion of juveniles (Fig. 3), emphasizing the change in age structure of the populations. No specific response of herring and sprat populations was found (coefficient $Qb = 2.1$, $P = 0.15$).

Size-At-Age Shift. Finally, long-term analysis of fish populations highlighted a significant decrease in size-at-age (Fig. 3) with a significantly stronger effect for herring and sprat populations in the Baltic Sea (coefficient $Qb = 20.4$, $P = 6.34 \times 10^{-6}$). The decrease in size-at-age was also detected in experimental data. We observed a decrease in size of adult females of *Pseudocalanus* sp. with temperature in the temperature-controlled mesocosms (extended LME model, coefficient estimate = −13.46, *t* value = −8.41, number of observations = 807, $P = 4.00 \times 10^{-4}$; Fig. 4*D*).

Discussion

Observed patterns are consistent with our hypotheses, emphasizing a negative effect of global warming on the body size of aquatic ectotherms from the individual to community structure levels. Given that the biota and ecosystems considered in this study were diverse with regard to the potential confounding factors affecting body size, our results suggest that a common mechanism (or set of mechanisms) links size structure and thermal energy at all biological scales considered. Of course, other factors may have additive or multiplicative effects on size. For instance, it has been shown that body size of fish decreases with fishery activities (18, 19). By targeting large individuals, fisheries are considered as a selective pressure favoring early maturation at smaller size. Thus, fishery activities could explain the observed decrease in size of herring and sprat in the Baltic Sea. On the other hand, regarding freshwater ecosystems, recreational fishing tended to decrease over the study periods in France (e.g., −38,703 fishermen/year from 1993 to 2008; data source: Federation Nationale de la Peche en France et de la Protection du Milieu Aquatique, www.federationpeche.fr). In addition, the species considered in our study (mostly cyprinid species) are not specially targeted by fishermen who prefer top predators. Similarly, the commercial fishing mostly concerns migratory species, eels, and top predators that accounted together for 79% of the total catches over the 1999–2001 period (data source: Suivi National de la Peche aux Engins/Resultats Professionnels; Conseil Superieur de la Peche/Office National de l'Eau et des Milieux Aquatiques, www.onema.fr). Finally, the number of professional fisherman was low at our study areas (e.g., on average 4, 3, 0, and 3 in the vicinity of site 1, 2, 3, and 4, respectively, over the 1999–2002 period; data source: Suivi National de la Peche aux Engins/Resultats Professionnels). Therefore, fishery pressure can be considered of minor impact in the freshwater areas studied in this article. Thus, the impact of fisheries cannot fully explain the decrease in size observed in rivers. In addition, obviously, fisheries cannot explain the patterns observed in the mesocosms. As a consequence, the overall consistency of the observed patterns emphasizes the role of temperature. Such results underline the importance of taking into account thermal constraints as potential confounding factors when studying changes in size structures. Indeed, early maturation at smaller size has systematically been attributed to fishery activities in marine ecosystems (18, 19), although this pattern perfectly fits the TSR in environments that have been subjected to gradual warming. Although not underestimating the impact of fisheries, our results stressed that fishery pressure cannot be considered as the unique trigger of observed changes in size structures in marine ecosystems. Further analyses would be needed to evaluate the relative merit of global warming and fisheries in explaining changes in body size of marine fish. The TSR predicts a negative effect of warming on size at maturity but a positive effect on growth rate. As a consequence, warming leads to smaller sizes late in the ontogeny but to larger sizes early in the ontogeny. Thus, by only considering young-of-the-year individuals fish in rivers when testing the size-at-age shift hypothesis, we probably underestimated the impact of climate change on individual body size under no or low fishery pressure.

Cascading effects could also contribute to the changes in size. For instance, we cannot exclude that the decrease in size of *Pseudocalanus* sp. is partially due to low food quality of small-sized phytoplankton in warmer mesocosms (Fig. 4*B*). However, this effect can, again, not fully explain the observed changes because the other decreases in size (e.g., for freshwater fish or for phytoplankton) were not observed under decreasing food quality. Regarding fish communities, top-down constraints can also influence size structures. In particular, change in the abundance of predators can influence the abundance of smaller prey (20, 21). Nevertheless, in the large rivers studied, no special change in the predator abundance was observed (22, 23). Thus, we can safely conclude that temperature clearly negatively impacts body sizes at all biological scales.

One of the most surprising results of our analysis is the increase in proportion of young age classes under warming. Actually, to our knowledge, such a pattern has never been suggested before to explain the decrease in mean body size at the population scale under warming. However, it is important to note that this hypothesis has been mostly tested by using European freshwater fish populations where cyprinidae was the dominant family. The positive effect of high temperature and/or low flow conditions on recruitment is well known for many cyprinidae (24–30). Even though the underlying mechanisms are unclear, we cannot exclude that this effect is specific to cyprinids. From this viewpoint, we may agree that it is necessary to test the population age-structure shift hypothesis with other biota to consider it as a rule.

We have shown that ecological rules represent important tools when evaluating the ecological impacts of climate change. Symmetrically, climate change provides a good opportunity to test for the relative contribution of temperature in explaining ecogeographical rules. Explanations of Bergmann's and James' rules have invoked also latitude-related factors other than temperature, e.g., food availability, predation risk, distance from low-latitude refuges during ice ages, migration availability, and resistance to starvation (31). Although not negating the role of other factors, our study provides strong evidence that temperature actually plays a major role in driving changes in the size structure of populations and communities. More generally, it would be interesting to consider the impacts of global warming when studying any ecological rules based on size variability. For instance, according to Elton's rule [which states that body size decreases with decreasing trophic levels (32)], our results suggest that upper trophic levels could be more sensitive to climate warming than lower ones.

To conclude, we provide evidence that reduced body size is the third universal ecological response to global warming besides the shift of species ranges toward higher altitudes and latitudes and the seasonal shifts in life-cycle events. Further analyses would be necessary to identify the possible mechanism linking temperature and size across the different biological scales. If such a mechanism exists, it should be linked to general theories in ecology. For instance the metabolic theory of ecology [MTE (33)] could help to understand at least part of the involved mechanisms. Indeed, according to this theory, the equilibrium number of individuals in a population (K) is predicted to vary as $K \propto [R]M^{-3/4}e^{E/kT}$, where R is the supply rate of the limiting resource, M is the mean mass of an individual, E is the activation energy of metabolism, k the Boltzmann's constant and T is the Kelvin temperature. Thus, $KM^{3/4}$ varies as $KM^{3/4} \propto [R]e^{E/kT}$. As a consequence, warming should lead to a decrease in the mean body mass and/or a decrease in abundance at equilibrium if $[R]$ does not concomitantly increase. In this way, the MTE could explain the population body size shift hypothesis and/or the species shift hypothesis. Further analyses of the relative sensitivity of the decreases in abundance and size to the species maximum size should help to evaluate the extent to which the MTE explains both hypotheses. Finally, we want to point out that it is critical to assess the evolutionary nature of the observed changes. Indeed, evolutionary responses to disturbances can be difficult (or impossible) to reverse and can lead to loss of genetic diversity (19). From this viewpoint, it would be important to distinguish evolutionary responses from plastic changes for conservation and management purposes (18, 19). Overall, knowing the triggers of changes in size with temperature from individual to community could greatly increase our understanding of ecosystem structuring and our ability to forecast impacts of anthropogenic pressures on biota.

Materials and Methods

Long-Term Data. ***Large river fish communities.*** We used data that were collected each year from 4 different study areas located on large French rivers (the Rhône and the Seine rivers) and over periods ranging from 14 to 27 years (22, 34). Fish were sampled 1–4 times per year [supporting information (SI) Table S1], from a boat, along banks and by using electrofishing techniques. Electrofished individuals were identified to species, measured and released. All study areas experienced a significant increase in temperature due to climate change (22, 34) (Table S2). We used yearly mean size (all individuals included) to test the community body size shift hypothesis. To test the species shift hypothesis, we used time series of proportion of small species in terms of (*i*) number of individuals (abundance) and (*ii*) number of species (species richness). For each study area, small species were defined as species with a maximum size (35) below the first quartile of the maximum size of all of the species in the community. The yearly mean sizes of the most abundant species were used to test the population body size shift hypothesis. At each study area, the most abundant species were defined as the species accounting for >5% of the total abundance. We used time series of the proportion of juveniles (young-of-the-year individuals) to test the population age-structure shift hypothesis. Each year, young-of-the-year individuals were identified by analysis of size-class frequencies. Finally, we used the yearly mean size of young-of-the-year individuals of the most abundant species to test the size-at-age shift hypothesis. We only considered the most abundant species having high juvenile numbers (i.e., on average >50 young-of-the-year individuals per year) to test the population age-structure shift hypothesis and the size-at-age shift hypothesis. For all time series, values were calculated for biological (i.e., not calendar) years fitted on the biological cycle of cyprinids. This enabled comparison of similar year class individual among sites (22, 34). Times series are provided in Table S3.

Brown trout populations. We used data that were collected yearly over 15 years from 3 French streams. The sites experienced a significant increase in water temperature during the 1985–2005 period (Table S2, Mann–Kendall trend tests, $n = 21$ for each test, P values ranging from 2.6×10^{-6} to 1.7×10^{-2}). Each site consisted of a stream section of 140–200 m closed by upstream and downstream nets. Trout were sampled by using 2-pass removal electrofishing, and each individual was measured and weighed before being released. For each sample, young-of-the-year individuals were identified by analysis of size-class frequencies. We used the time series of yearly mean individual size in the 3 populations to test the population body size shift hypothesis. The population age-structure shift hypothesis was tested by using the yearly proportions of young-of-the-year fish. Times series are provided in Table S4.

Herring and sprat populations. We used fishery data provided by the International Council for the Exploration of the Sea (ICES; available online at www.ices.dk/reports/ACFM/2005/WGBFAS/directory.asp). Data consisted of time series of yearly total catch in numbers and mean weight in the catch for different age classes (1 y old to >8 y old) over 31 years in the Baltic Sea. Sprat data were available for the whole Baltic Sea except the Kattegat area (i.e., for ICES subdivisions 22–32). Herring data were available for the whole Baltic Sea except zones located to the east of $\approx$15°05′00″ E (i.e., for ICES subdivisions 25–32 and Gulf of Riga). Baltic Sea surface temperature during the sampling period significantly increased (36) (Table S2). We used yearly mean weights to test the population body size shift hypothesis. Mean weights were calculated as the ratio of total biomass to total catch per year. Total biomasses were estimated as the product of weights-at-age and catches in numbers per age, summed over all ages. The population age-structure shift hypothesis was tested by using the yearly proportions of fish <3 years old. Time series of weight-at-age were used to test the size-at-age shift hypothesis.

Published data. To test the species shift hypothesis, we used observed changes in fish community structure in the North Sea under global warming (17). The study was based on the North Sea IBTS (International Bottom Trawl Survey) fishery data from DATRAS (Database of Trawl Surveys) and were provided by the ICES to the authors.

Experimental Data. Experimental data were obtained by sampling female adults of the copepod *Pseudocalanus* sp., bacteria, and phytoplankton communities in indoor mesocosms that simulated early spring (February–April) environmental conditions in the Kiel Bight (Baltic Sea) under different climatic scenarios (15, 16). Eight mesocosms were exposed to 4 temperature regimes (i.e., 2 mesocosms per chamber). The reference regime (+0 °C) corresponded to the 1993–2002 average temperature regime observed in the Kiel Bight, whereas the +2, +4, and +6 °C regimes corresponded to different levels of warming. Percentage of the natural light intensity above cloud cover (Io) was controlled to simulate different cloud cover and underwater light attenuation. Phytoplankton was sampled at 16%, 64%, and 32% Io (2005, 2006, and 2007 experiments). *Pseudocalanus* sp. and bacteria were only sampled at 64% and 16% Io, respectively.

Bacteria communities. Data were collected in the +0 and +6 °C mesocosms during the 2005 experiment (37). Bacteria were sampled on average 1.33 times a week in the reference mesocosms and on average 1.5 times a week in the +6 °C mesocosms. For each sample, mean cell volume of the community was derived from length and width cell measurements. Measurements were assessed by means of a new Porton grid—G12 after DAPI staining in an epifluorescence microscope by vision. We used mean cell volume in the different mesocosms to test the community body size shift hypothesis. Times series are provided in Table S5.

Pseudocalanus sp. individuals. Female adults of *Pseudocalanus* sp. were sampled and individually measured at the end of the 2006 experiment. The size-at-age shift hypothesis was tested by using mean length of individuals in the different mesocosms. Copepods are particularly suitable for testing this hypothesis because they do not increase in length after having molted to the adult stage. Times series are provided in Table S6.

Published data. We used a study dealing with changes in size structure of phytoplankton communities in Kiel mesocosms (16) to test the community body size hypothesis and the species shift hypothesis.

Statistical Analysis. Hypotheses were tested with the time series data by using a weighted metaanalysis (38). The ''effect sizes'' in the metaanalysis were S statistics from Mann–Kendall trend tests (39) (see Fig. S1, *SI Text*, and Table S7 and S8). Variances of S were corrected for temporal autocorrelation when they occurred (40). Mean temporal trends were considered significant if their 95% confidence intervals did not contain 0 (38). To test whether fishery pressure on herring and sprat populations can influence the response of organisms to warming (population body size shift, population age-structure shift, and size-at-age shift hypotheses), we defined a categorical variable that discriminated marine vs. freshwater populations. Fishery effect was evaluated by checking for significant between-group heterogeneity (Qb) in the effect size (38).

For experimental data, we used LME models (41) to evaluate the effect of temperature on the dependent variables (see Fig. S1, *SI Text*, and Table S7 and Table S8). This allowed the potential differences in variance among mesocosms to be considered when evaluating the coefficients of the models and their confidence intervals. All statistical analyses were performed by using R (42).

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Climate change impacts on structure and diversity of fish communities in rivers

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Abstract

It is widely accepted that climate change constrains biota. Yet, because of the lack of consistent multisite and multitaxon surveys, few studies have addressed general rules about how climate change impacts on structure and diversity of animal communities. Especially, the relative influence of nonclimatic anthropogenic disturbances on this impact is fairly unknown. Here, we present for the first time a meta-analysis assessing the effect of global warming on stream organisms. Fish communities of large rivers in France undergoing various anthropogenic pressures showed significant increase in proportions of warm-water species and of specific richness during the last 15–25 years. Conversely, the equitability decreased, indicating a gradual decrease of the number of dominant species. Finally, the total abundance increased, coupled with rejuvenation and changes in size-structure of the communities. Interestingly, most of these effects were not depressed by the strength of nonclimatic anthropogenic disturbances. Conversely, geographical location of communities and especially closeness of natural barriers to migration could influence their response to climate change. Indeed, increase in the proportion of southern species seemed hindered at sites located close to the southern limit of the European species' geographical ranges. This work provides new evidence that climate change have deep impacts on communities which, by overtaking the effects of nonclimatic anthropogenic disturbances, could be more substantial than previously thought. Overall, our results stress the importance of considering climate change impacts in studies addressing community dynamics, even in disturbed sites.

Keywords: anthropogenic disturbances, climate change, community, diversity, fish, France, large rivers, long-term studies, meta-analysis, time series

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Introduction

Understanding the effects of climate change on plant and animal population, community structure and ecosystem functioning is one of the main challenges of modern ecology. Since the beginning of the 1990s, the literature concerning climate change has noticeably increased. The first studies were mostly predictive (Pastor & Post, 1988; Melillo *et al.*, 1993; Vitousek, 1994) and stimulated the emergence of long-term surveys which, in turn, allowed for a new generation of descriptive studies (Walther *et al.*, 2002; Parmesan & Yohe, 2003; Root *et al.*, 2003; Harley *et al.*, 2006). However, the majority of the latest studies were performed at single study sites, making the results sensitive to site-specific confounding factors, and hindering the quest for general rules in the ecological impacts of global warming. To tackle this problem, some authors have put efforts on publishing reviews combining the results of different studies (Walther *et al.*, 2002; Harley *et al.*, 2006) and/or performed meta-analyses of data sets collected in the literature (Parmesan & Yohe, 2003; Root *et al.*, 2003). Their results have strengthened the case that climate warming is a cause for biological changes, highlighting the trend of spring biological events to occur earlier and showing clear spatial shifts in species geographical ranges

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towards higher altitudes or latitudes. However, these studies have focused on single species, and have not attempted to assess the relative importance of climate change and anthropogenic habitat alterations as causes of biological change (Jensen, 2003; Parmesan & Yohe, 2003). Hence, it is still difficult to evaluate the relative effects of climate changes vs. nonclimatic disturbances on ecosystems, and to estimate how climate change might affect community structure and biodiversity.

In an attempt to fill these gaps and tackle these technical difficulties, we present here a meta-analysis on a multisite and multifish species data set. Stream fish are poikilotherms whose movements are significantly constrained by the spatial structure of their environment. This makes them suitable biological models for studying the impact of climate change on biota (Daufresne *et al*., 2004). Surprisingly, as far as we know, few studies based on actual data have assessed the influence of climate warming on large river communities (Daufresne *et al*., 2004; Mouthon & Daufresne, 2006), probably because available data sets are scarce. However, we showed in a previous study that southern and warm-water species have gradually come to dominate the fish community of the upper Rhône River (France) over the last 20 years (Daufresne *et al*., 2004). As observed for many organisms (Walther *et al*., 2002; Parmesan & Yohe, 2003; Root *et al*., 2003), these patterns were correlated with thermal constraints during spring. This period coincides for most of the cyprinids (dominating fish communities in large European rivers) with the reproduction, a key period for fish population dynamics (Mills & Mann, 1985; Cushing, 1995; Daufresne *et al*., 2004).

We propose, here, to evaluate the effect of climate change, especially spring warming, on global abundance, structure (proportion of southern and warm-water species to the total abundance) and diversity [specific richness, Shannon equitability index (or evenness; Begon *et al*., 1990)] of fish communities in large rivers. We used data collected each year on seven different study areas located along large French rivers (the Rhône, the Loire and the Seine rivers) on periods ranging from 15 to 25 years (Fig. 1, Table 1), making up a set of 24 study area × sampling site combinations to our knowledge unique in stream ecology. Using meta-analysis technique, we first analyse the common patterns shared by all communities. Then, we examine whether the strength of the local nonclimatic disturbances can weighted the responses of the different communities to the global warming. Our study stresses the impacts of climate change on fish communities.

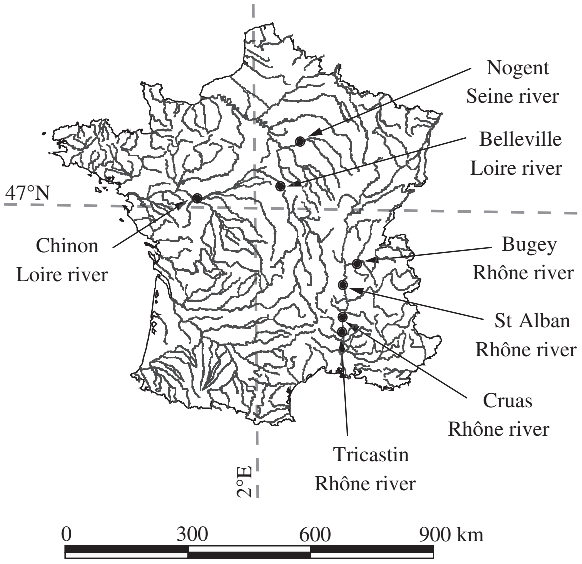

Fig. 1 Location of the study areas.

Table 1 Main properties of the study areas

Study area	River	Sampling period	Latitude (°N)	Catchment area (km^2)	No. of sampling sites: Upstream from NPS	No. of sampling sites: Downstream from NPS	No. of samples per year
Belleville	Loire	1989–2003	47.5	34 883	1	1	1
Bugey	Rhône	1979–2003	45.8	15 830	3	4	4
Chinon	Loire	1988–2003	47.2	60 024	1	1	1
Cruas	Rhône	1983–2003	44.6	68 878	1*	2*	4
Nogent	Seine	1987–2003	48.5	8939	1	2	1
Saint-Alban	Rhône	1985–2003	45.4	50 736	1	3*	4
Tricastin	Rhône	1982–2003	44.3	74 256	1*	2*	4

*Sampling sites located along (i.e. <5 km from) hydropower schemes.
NPS, nuclear power station.

Materials and methods

Data

We used data that were collected each year on seven different study areas located along large French rivers (the Rhône, the Loire and the Seine rivers; Fig. 1) over periods ranging from 15 to 25 years (Table 1). These data were collected in the vicinity of nuclear power stations (NPS) operated by Electricité de France. At each study area, fish sampling sites were located either upstream from the NPS (control sampling sites) or downstream from the NPS (sampling sites potentially disturbed by thermal discharges). Nine sampling sites were located along (i.e. <5 km from) hydropower schemes. Hydropower schemes include a bypassed section, closed by an upstream diversion dam, and a diversion canal ended by a hydropower dam. Finally, this represented a set of 24 study area × sampling site combinations. Fish were sampled one to four times per year (Table 1), from a boat, along banks and using electrofishing techniques. Fish data were obtained by point abundance sampling (Persat & Copp, 1990) at Saint-Alban and continuous sampling at the other study areas. Size of the sampling sites depended on sampling duration but was ca. 300–400 m long and 2–4 m wide for a 30 min continuous sampling period (Daufresne *et al*., 2004). For each sample, the species abundance was expressed as catch per unit effort (CPUE), (i.e. the number of fish of a given species captured in a 20 min fishing period; Cattanéo *et al*., 2001; Grenouillet *et al*., 2001; Daufresne *et al*., 2004). According to Grenouillet *et al*. (2001), we used the equivalence found between point abundance and continuous sampling (Pont *et al*., 1993) to evaluate CPUEs at Saint-Alban. For each species, CPUEs were then averaged by biological year × sampling site (called site-years afterwards). Biological year (from 1 July to 30 June of the next calendar year) was defined according to the 'average' hatching date of most of the cyprinid species present in large rivers (Daufresne *et al*., 2004). This allowed comparing similar year classes across the different study areas. Because part of its life cycle is completed in the sea, the eel (*Anguilla anguilla*, L.) was not included in the study. The fish abundance data set finally contained 47 columns (species) and 503 rows (study area × sampling site × biological year combinations).

We defined a set of five biological variables to study changes in total abundance, structure, and diversity of fish communities. For each site-year, we first calculated the total abundance (as the sum of the average CPUE of the different species), the specific richness (i.e. the number of species with a non-null average CPUE) and the Shannon equitability index (Begon *et al*., 1990). These first three variables were calculated using all the species sampled each site-year. Then, we calculated the proportions (to the total abundance) of southern and warm-water species. We calculated both variables to determine if changes in proportions of southern species could be linked to thermal preferences. For a given study area, we only considered the most common species, excluding those accounting for $<5\%$ of the total number of fish sampled. We focused on dominant species because affinities for temperature and geographical ranges were not available for all the less-abundant species. We first calculated the relative position of the study area in the geographical range of the most common species (x values; Daufresne *et al*., 2004; Fig. 2). Species with the upper 25% x values were considered as southern species. The proportion of southern species time series was calculated by dividing the CPUE of southern species by the total CPUE for each site-year. Then, we calculated the proportion of warm-water species time series. To do so, we first coded affinities for high temperatures of all the most common species sampled in the different study areas (18 species). We defined a group of six species with the highest affinity for high temperature: tench (*Tinca tinca* L.), bream (*Abramis brama* L.), white bream (*Blicca bjoerkna* L.), rudd (*Scardinius erythrophthalmus* L.), wels catfish (*Silurus glanis* L.) and black bullhead (*Ameiurus melas* R.).

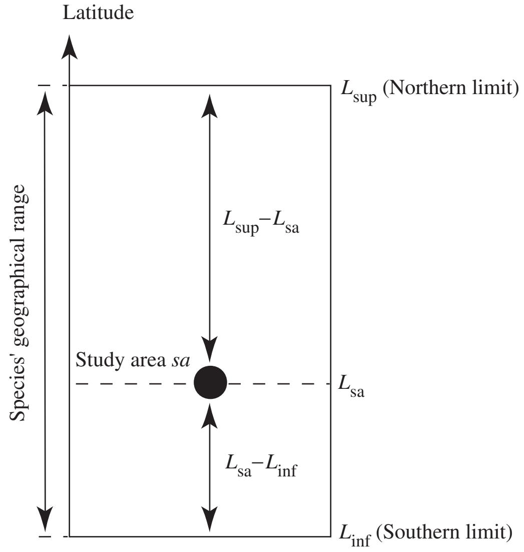

Fig. 2 Relative position (x) of a study area sa in the geographical range of a species. x is coded on the basis of the geographical range of European fish species (Bruslé & Quignard, 2001; Keith & Allardi, 2001) as a function of: $x = (L_{sa}–L_{inf})/(L_{sup}–L_{sa})$, where L_{sa}, L_{sup} and L_{inf} are the latitudes (°N) of the study area, the northern limit of the species' geographical range and the southern limit of the species' geographical range, respectively (Daufresne *et al*., 2004).

Tench, bream and rudd, were assigned to this group according to Philippart & Vranken (1983) (i.e. lethal temperature: $>34\,^{\circ}$C; optimal growing temperature: 20–28 °C; reproduction temperature: 20 °C). White bream, was added to this group according to Küttel *et al.* (2002) (e.g. critical thermal maximum for larvae, juveniles and adults = 28, 34 and 36 °C, respectively, maximum reproduction temperature = 23 °C), as well as black bullhead (e.g. critical thermal maximum for adults = 38 °C, maximum reproduction temperature = 20 °C). Wels catfish was included because this species is known to grow faster at high temperature ($>20\,^{\circ}$C; Proteau *et al.*, 1996) and to reproduce at temperatures above 20 °C (Bruslé & Quignard, 2001). According to Philippart & Vranken (1983) we then define a group of six species with medium affinity for high-temperature (lethal temperature, 28–34 °C; optimal growing temperature, 14–23 °C; reproduction temperature, 7–15 °C): Bleak (*Alburnus alburnus* L.), stream bleak (*Alburnoides bipunctatus* B.), barbel (*Barbus barbus* L.), chub (*Leuciscus cephalus* L.), roach (*Rutilus rutilus* L.) and bitterling (*Rhodeus sericeus* P.). Stream bleak and barbel were considered as slightly stenothermic by Philippart & Vranken (1983). However, stream bleak was assessed to this group because of its abilities to grow at high temperature (optimal range: 18–27 °C for adults; Küttel *et al.*, 2002) and to reproduce in warm water (optimal range: 12–24 °C; Küttel *et al.*, 2002). Similarly, barbel has some affinities for warm water (Kraiem, 1979; Bruslé & Quignard, 2001) and can reproduce at high temperature (maximum: 29 °C; Küttel *et al.*, 2002). Finally, we defined a group of six species with the lowest affinity for high temperature: pike (*Esox lucius* L.), perch (*Perca fluviatilis* L.), pumpkinseed (*Lepomis gibbosus* L.), gudgeon (*Gobio gobio* L.), nase (*Chondrostoma nasus* L.) and dace (*Leuciscus leuciscus* L.). According to Philippart & Vranken (1983) pike, nase and dace have the same affinities for temperature than the species of the previous group but are defined has slightly stenothermic. Indeed, reproduction and larval growth of pike are optimal at low temperature (optimal range: 5–10 and 8–15 °C, respectively; Küttel *et al.*, 2002). Perch presents similar properties (optimal range: 6–15 and 12–18 °C for reproduction and larval growth, respectively; Küttel *et al.*, 2002) and eggs are sensitive to high temperature (lethal temperature: 16 °C; Elliott, 1981). Larvae of nase and dace have also affinities for cold water (lethal temperature: 19 and 18 °C, respectively; Küttel *et al.*, 2002) and both species reproduce at low temperature (maximum: 16 and 14 °C for nase and dace, respectively; Küttel *et al.*, 2002). Gudgeon was assessed to this group because of its optimal temperature for reproduction (optimal range: 12–17 °C; Küttel *et al.*, 2002), lower than those of the species of the second group (Küttel *et al.*, 2002). Finally, despite the ability of the adults of pumpkinseed to face high temperatures (lethal temperature: 35 °C; Küttel *et al.*, 2002), this species was conservatively assessed to this group because of its thermal optimum for growth (ca. 20 °C, Scott & Crossman, 1973) and the sensitivity of the juveniles to warm water [lethal temperature: 25 °C (Küttel *et al.*, 2002); high mortality rate in warm environment (Dembski *et al.*, 2006)]. Heterogeneity of data available for the different species did not allow computing affinities for high temperature using mathematical procedures. Thus, we assigned an affinity of 0.75, 0.5 and 0.25 for the species of the three different groups, respectively. For each site-year, the proportion of warm-water species was calculated by first summing the products of species abundance with species affinity. Then this sum was divided by the total CPUE.

Water temperatures were recorded right upstream from the NPS by Electricité de France. To evaluate the potential effect of temperature on fish communities (biological year n), we calculated the average temperature during reproduction (April–June, biological year n–1) for each study area (Daufresne *et al.*, 2004).

Statistical analysis

We performed a weighted meta-analysis on data using Mann–Kendall trend statistics (S) and the slope of the linear regression of biological variables on reproduction temperature (b) as ‘effect sizes’ (Gurevitch & Hedges, 1993). S provides information on long-term changes of biological variables, whereas b provides information on the interannual relationship between the biological and temperature variables. Variances of S and b were corrected for temporal autocorrelations before the analysis (Hamed & Rao, 1998; Pyper & Peterman, 1998). For each biological variable, we first evaluated the overall magnitude of S and b (called grand mean effect sizes) and calculated their 95% confidence intervals using the bootstrap procedure. To test whether effect sizes depended on nonclimatic anthropogenic constraints (NPS and hydropower schemes), we defined two categorical variables. The first one discriminated whether or not sampling sites were located downstream from a NPS. The second one discriminated whether or not they were located along hydropower schemes. We used a mixed effect model (Gurevitch & Hedges, 1993) to test whether grouping variables explained significant heterogeneity in the effect size, whereby the overall heterogeneity was divided into within-group heterogeneity and between-group heterogeneity (Q_b). The effects of categorical variables on effect sizes were evaluated using 10 000 randomizations and Bonferroni's corrections (Sokal & Rohlf, 1995). Because such corrections are known to be conservative (Sokal & Rohlf, 1995), significant results at the 0.1 (and lower) level were discussed (Hillebrand, 2004). All statistical analyses were performed using *R* (R Development Core Team, 2006).

Results

During the whole study periods a total number of 362 692 fish of 47 species were captured, representing an average of 51 813 ± 14 111 (average ± 95% confidence interval) fish and 32 ± 3 (average ± 95% confidence interval) species for each study area. The upstream water temperature of our study areas during reproduction has gradually increased over the last 25 years [modified Mann – Kendal trend test (Hamed & Rao, 1998), $P < 0.01$, $n = 25$, Fig. 3]. We observed a good synchronism of water temperature time series at large spatial scale (narrow 95% confidence intervals of mean water temperature; see Fig. 3). In addition, the water temperature and the maximum air temperature measured by Météo France at the closest sampling sites from the study areas (i.e. 60, 36, 170, 8, 14, 83 and 26 km from Belleville, Bugey, Chinon, Cruas, Nogent, Saint-Alban and Tricastin, respectively) were significantly correlated [modified Pearson's correlation test (Pyper & Peterman, 1998), P-values < 0.01].

Analysis of grand mean effect sizes revealed significant increasing temporal trends (*S*) in total abundance and proportion of warm-water species at all the sampling sites and a significant decreasing trend in equitability (Figs 4a and 5). Body size measurements at Bugey highlighted that the increase in abundance could be coupled with a drastic increase in the proportion of small individuals (Fig. 6a and b). The specific richness also significantly increased at all the sampling sites but this pattern was more pronounced at the sites located along hydropower schemes ($Q_b = 10.7$, Bonferroni corrected $P < 0.1$). This increase was partially due to appearance of some species, including non native species (*Carassius gibelio* B., *Pseudorasbora parva* T. & S., *Silurus glanis* L.), but was mainly due to a global increase in occurrence of local species (Table 2). Finally, the proportion of southern species significantly increased at the sampling sites which were not located along hydropower schemes but did not show any significant trend

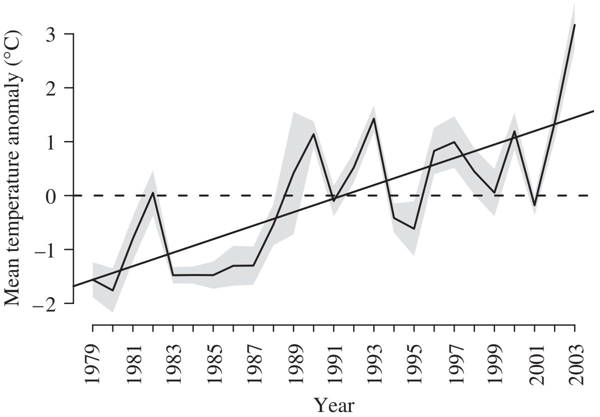

Fig. 3 Annual mean anomalies (±95% confidence intervals) of temperature during reproduction (April–June). Temperatures were recorded upstream from the study areas. The trend is shown ($Y(t) = 0.12t - 1.56$).

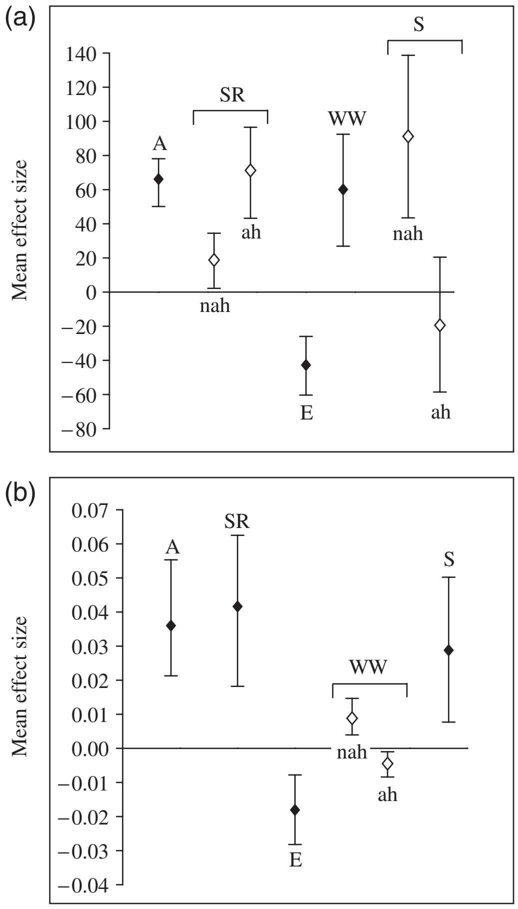

Fig. 4 Mean effect sizes (±95% confidence intervals). (a) Mean for the trend *S* of the total abundance of fish (A), the specific richness (SR), the Shannon equitability index (E), the proportion of southern species to the total abundance (S) and the proportion of warm-water species to the total abundance (WW) at the different sampling sites. Significantly different means at sampling sites located along hydropower schemes (ah) or not (nah) are represented (open diamonds). (b) Mean for the slope of the linear regression with temperature during reproduction *b* of A, SR, E, S and WW at the different sampling sites. Significantly different means at sampling sites located along hydropower schemes (ah) or not (nah) are represented (open diamonds). To increase readability, *b* values (and confidence intervals) of A and SR were divided by 1000 and 10, respectively. Nonoverlapping of confidence intervals with 0 indicates significant effects.

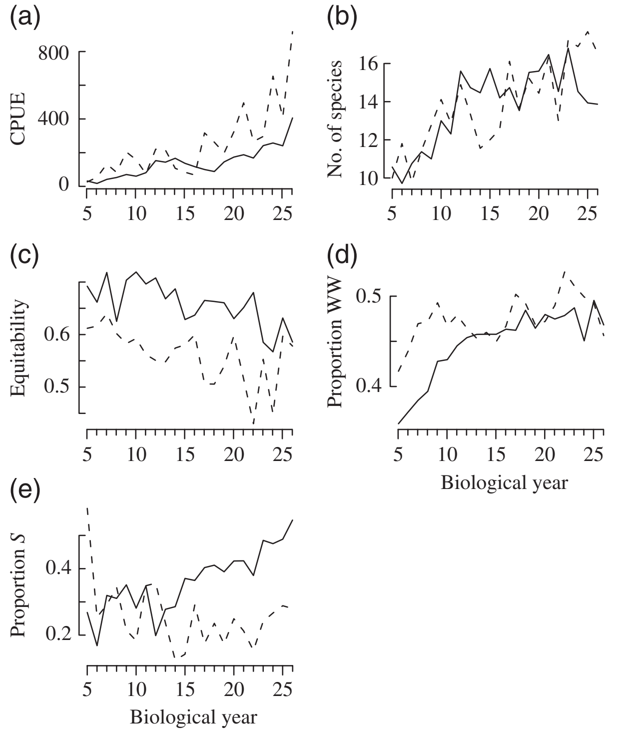

Fig. 5 Temporal changes (annual means) in total abundance, community structure and diversity at the sampling sites located along hydropower schemes (dotted lines) or not (continuous lines). (a) Total abundance (expresses as CPUE). (b) Specific richness (expresses as number of species). (c) Shannon equitability index. (d) Proportion of warm-water species (WW) to the total abundance. (e) Proportion of southern species (S) to the total abundance. CPUE, catch per unit effort.

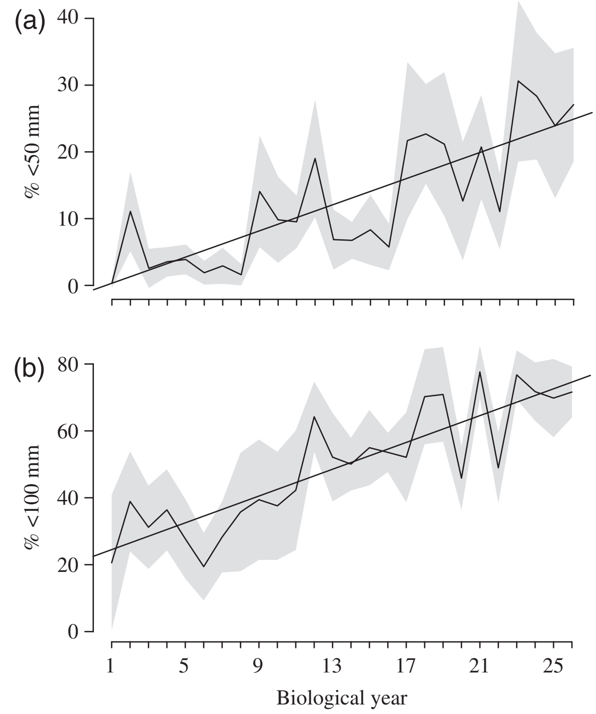

Fig. 6 Annual mean percentages (±95% confidence intervals) of small fish sampled at Bugey. (a) Fish smaller than 50 mm. (b) Fish smaller than 100 mm. Biological year (from 1 July to 30 June of the next calendar year) was defined according to the 'average' hatching date of most of the cyprinid species present in large rivers (Daufresne *et al*., 2004). The trend are shown ($Y(t) = 0.98t - 0.66$ for fish smaller than 50 mm and $Y(t) = 2.00t + 22.49$ for fish smaller than 100 mm).

at the other sampling sites ($Q_b = 13.04$, Bonferroni corrected $P < 0.1$).

Total abundance, species richness and the proportion of southern species were all positively related to temperature during reproduction (*b*), whereas equitability was negatively related (Fig. 4b). Proportion of warm-water species was positively related to temperature at sites without hydropower influences, but negatively related at sites located along hydropower schemes (Fig. 4b; $Q_b = 13.4$, Bonferroni corrected $P < 0.05$).

All patterns were highly consistent among sampling sites located either upstream or downstream from a NPS (Q_b ranging from 4.8×10^{-4} to 0.9). Beside temporal aspect, ANOVAs performed on total abundance, equitability, specific richness and proportions of warm water and southern species did not neither highlighted any significant overall differences among sampling sites located upstream or downstream from a NPS (Bonferroni corrected $P > 0.1$ in all cases). Conversely, ANOVAs highlighted an overall detrimental effect of hydropower schemes on equitability (lower at sites located along schemes; Bonferroni corrected $P < 0.01$; Fig. 5c) and proportion of warm-water species (higher at sites located along schemes; Bonferroni corrected $P < 0.01$; Fig. 5d).

Discussion

General long-term changes

The consistent increase in water temperature at large spatial scale, correlated with air temperature, confirmed the results of former studies about the impact of climate change on temperature of European rivers (Webb, 1996; Daufresne *et al*., 2004; Mouthon & Daufresne, 2006). Concomitantly, we observed important changes in total abundance, structures and diversity of fish communities, significantly linked to the temperature during reproduction (Fig. 4). These changes, mostly consistent across communities that were submitted to similar climate warming but to different kind and strength of

Table 2 Comparison of species occurrences between the first 5 biological years vs. the last 5 biological years of study periods at each study area

	Belleville	Bugey	Chinon	Cruas	Nogent	St Alban	Tricastin
Appearing (number of species)	–	2 (Barbt, Silg)	–	3 (Cotg, Carg, Psep)	–	3 (Gymc, Psep, Rhos)	2 (Psep, Rhos)
Disappearing (number of species)	–	–	1 (Amem)	–	–	–	1 (Chot)
Increasing occurrence (number of species)	7	13	7	15	11	11	14
Decreasing occurrence (number of species)	4	6	7	4	6	5	8

Species occurrence was calculated as the total number of biological year when the species has been sampled at the study area. Amem, *Ameiurus melas*; Barbt, *Barbatula barbatula*; Carg, *Carassius gibelio*; Chot, *Chondrostoma toxostoma*; Cotg, *Cottus gobio*; Gymc, *Gymnocephalus cernuus*; Psep, *Pseudorasborra parva*; Rhos, *Rhodeus sericeus*; Silg, *Silurus glanis*.

nonclimatic constraints, highlight the specific impacts of climate change on communities.

Some of the observed changes were quantitatively extremely important. For instance, the mean change in total abundance (±95% confidence interval) was of +232% (±103%) when comparing average values for the first 5 years vs. the last 5 years of the study periods. We can propose two hypotheses to explain such increase. First, since recruitment of cyprinids is favoured by warm water (Mills & Mann, 1985; Mann, 1996), we can hypothesize that the increase in abundance was mostly the fact of small young fish of the year. For instance, the percentage of fish smaller than 50 mm at Bugey (a study area for which fish size was available) presented a drastic increase (Fig. 6a). At these sizes, fish are considered as young of the year for most of European cyprinids (Bruslé & Quignard, 2001; Keith & Allardi, 2001; Daufresne *et al.*, 2004; Froese & Pauly, 2006). Second, we can hypothesize that this increase in abundance may underline a global increase in abundance of small size species such as stream bleak (maximum size = 160 mm; Froese & Pauly, 2006) and bitterling (maximum size = 100 mm; Froese & Pauly, 2006) as it has already been suggested in large French rivers (Daufresne *et al.*, 2004; Daufresne & Boët, 2005). Hence, the increase in total abundance may be mostly due to rejuvenation and/or an increase in the abundance of small size species. For instance, fish smaller than 100 mm actually came to dominate the fish community at Bugey (Fig. 6b). Interestingly, this fits the ecogeographical rule highlighted by Knouft (2004), which states that the average body size within fish communities tends to increase with decreasing latitude (i.e. increasing temperature).

Beside the trend of the total abundance, we observed a general significant increase in specific richness and decrease in equitability. According to Connell (1978) and Wilson (1990) the increase in specific richness is not surprising because gradual climate change would be one of the most convincing explanations for Hutchinson's 'paradox of the plankton' (1961; i.e. 'How [it is] possible for a number of species to coexist in a relatively isotropic or unstructured environment, all competing for the same sorts of materials?'). The initial hypothesis is based on the assumption that communities seldom reach an equilibrium state. Under gradual climate change no species would have time to eliminate (by the way of competitive exclusion) the others before being itself constrained by the environment. As a consequence, the communities always include a mixture of species favoured by the current vs. previous climate. The latter species do not necessarily disappear suddenly but can be eliminate only after several generations (Wilson, 1990). Regarding the current global warming this would explain why species seem to invade faster from lower latitudes than resident species recede pole ward, ultimately leading to an increase of the specific richness (Sagarin *et al.*, 1999). However, this phenomenon could be only transitional (Walther *et al.*, 2002). Actually, Connell (1978) suggested that too quick environment changes will not allow communities to change and, thus will not promote diversity. From this view point, the fact that we observe very few new comers within the communities (Table 2), as well as the decrease in equitability, matches the hypothesis of a detrimental effect of climate change on biodiversity (Sala *et al.*, 2000). Indeed, if we observe a gradual increase of specific richness, very few species seem to take advantage of the warming. For instance, over the last 2 biological years, the communities were dominated by bleak and stream bleak at Belleville (accounting for 26.4% and 22.0% of the total abundance, respectively), stream bleak and chub at Bugey (accounting for 50.8% and 18.7% of the total abundance, respectively), bleak

Table 3 Median (25–75% percentiles) of chemicals in the vicinity of six of the seven study areas during the 1985–2005 period

	O_2 *(6)*	pH	BOD_5 *(6)*	NH_4 *(0.5)*	NO_2 *(0.3)*	NO_3 *(50)*	P *(0.5; 0.2*)*
Chinon	10.1	8.3	4.4	0.1	0.08	11.1	0.22*
	(9.4–10.7)	(8.2–8.5)	(3.8–5.0)	(0.1–0.2)	(0.07–0.09)	(10.0–13.0)	(0.15–0.29)
Cruas	9.8	8.0	1.5	0.2	0.09	6.4	0.19
	(9.7–10.0)	(8.0–8.1)	(1.1–1.6)	(0.2–0.3)	(0.09–0.11)	(6.1–6.6)	(0.18–0.41)
Bugey	10.1	8.1	1.2	0.1	0.06	3.9	0.10
	(10.0–10.3)	(8.0–8.1)	(1.0–1.4)	(0.1–0.1)	(0.06–0.07)	(3.7–4.2)	(0.08–0.15)
Nogent	10	8.0	1.9	0.1	0.07	19.7	0.10*
	(9.1–10.3)	(8.0–8.1)	(1.7–2.9)	(0.1–0.1)	(0.06–0.09)	(17.8–21.2)	(0.07–0.14)
St. Alban	10.0	8.0	1.6	0.2	0.08	6.2	0.20
	(9.7–10.2)	(8.0–8.1)	(1.4–2.0)	(0.2–0.3)	(0.07–0.09)	(5.8–6.5)	(0.17–0.23)
Tricastin	9.8	8.0	1.5	0.1	0.10	6.5	0.16
	(9.5–10.0)	(8.0–8.0)	(1.3–1.8)	(0.1–0.2)	(0.09–0.12)	(6.2–6.9)	(0.15–0.38)

Values in italics represent the upper or lower (for O_2) limits currently defining the good ecological state for French rivers according to the European Water Framework Directive (Direction de l'eau 2005).

O_2, oxygen; BOD_5, 5-day biological oxygen demand; NH_4, ammonium; NO_2, nitrite; NO_3, nitrate; P, phosphate (or total phosphorus*). All values but pH are expressed as $mg\,L^{-1}$. Statistics are derived from data collected by the Agences de l'eau (http://www.lesagencesdeleau.fr) at Chinon, 27 km upstream from Cruas, 11 km downstream from Bugey, at Nogent, 27 km upstream from St Alban and 33 km downstream from Tricastin. No data were available at the vicinity of Belleville for the 1985–2005 period.

and stream bleak at Chinon (accounting for 24.0% and 23.1% of the total abundance, respectively), roach and bleak at Cruas (accounting for 34.0% and 25.2% of the total abundance, respectively), roach and bleak at Nogent (accounting for 22.8% and 16.6% of the total abundance, respectively), roach and bleak at Saint Alban (accounting for 49.9% and 17.5% of the total abundance, respectively) and chub and roach at Tricastin (accounting for 45.5% and 15.8% of the total abundance, respectively). Note that these results tend to confirm the gradual domination of communities by small size species (e.g. bleak and stream bleak).

Finally, the proportion of southern species significantly increased at the sampling sites that were not located along hydropower schemes and we also observed a general increase in the proportion of warm-water species for all sites. Such results are consistent with the general hypotheses about the ecological effects of climate change, as well as with results of previous single-site studies and meta-analysis (Walther *et al.*, 2002; Parmesan & Yohe, 2003; Root *et al.*, 2003). With regard to fish communities in large rivers, these results are to our knowledge the first to generalize the pattern that was highlighted by a single-site study performed on the Upper Rhône River (Daufresne *et al.*, 2004).

Interestingly, according to the theory of ramp disturbance (i.e. disturbance with a steadily increasing strength in space and time; Lake, 2000), the observed trends were currently mostly continuous (van Nes & Scheffer, 2004). However, such trends may reduce the basin of attraction around the present community states (van Nes & Scheffer, 2004). This could result in sudden shifts in the community structures in the next few years, especially if the frequency of extreme climatic events tends to increase due to climate change. Actually, such pattern has already been observed in large rivers for invertebrate communities (Mouthon & Daufresne, 2006; Daufresne *et al.*, 2007).

Thus, our results match the general hypothesis of an effect of climate warming on fish communities. In addition, such consistent changes across different ecosystems can only be due to a common disturbance, playing at large geographical scale. Nonetheless, besides climate warming, one may argue that large rivers in Europe also underwent a general improvement in water quality (Aarts *et al.*, 2004). However, the water quality at the study areas during the study periods was globally good according to the European Water Framework Directive and did not presented major variations (Table 3). In addition, Daufresne *et al.* (2004) showed that changes in fish community structure at Bugey were not consistent with the potential effects of a water improvement. For instance, the dace, which is known to be highly sensitive to water quality (Verneaux, 1981; Bruslé & Quignard, 2001) tended to decrease in abundance whereas eury-tolerant species such as chub (Verneaux, 1981; Bruslé & Quignard, 2001) came to dominate the community. More generally, the species currently dominating the communities at the different study areas (e.g. bleak, chub and roach) are known to be not sensitive to water quality (Verneaux, 1981). In addition, even in rivers undergoing water-quality improvement the recovery of fish community seems low, probably because of a more important impact of other anthropogenic constraints (Aarts *et al*., 2004).

Impacts of climate change vs. nonclimatic anthropogenic disturbances

NPS and hydropower schemes could be defined as press disturbances (Bender *et al*., 1986) whereas climate warming mainly refers to a ramp disturbance (Lake, 2000). Press disturbances arise sharply and are maintained in time at a rather constant level. Many studies have clearly showed the influences of press disturbances on biota, especially on aquatic ecosystems (Bravard, 1987; Petts *et al*., 1989; Fruget, 1992; Kinsolving & Bain, 1993; Phillips & Johnston, 2004). Such disturbances typically rapidly modify the structure and diversity of communities, defining a new state until any other major changes occur. On the other hand, recent works highlighted that ramp disturbances generally induce gradual changes in community structures disrupted in times by rapid shifts (van Nes & Scheffer, 2004).

We did not find any significant impact of NPS neither on long-term trends (Fig. 4a) nor on grand means ($P > 0.1$) of total abundance, community structure and diversity. At the interannual scale NPS not either impacted the relationship between these variables and the reproduction temperature. These results are consistent with some previous studies highlighting marginal effects of NPS on fish at the community level (Kirchmann *et al*., 1985; Daufresne *et al*., 2004, 2005). In fact, the impacts of NPS on water temperature are rather punctual and fish can probably easily escape from this disturbance (Daufresne *et al*., 2004, 2005). Nonetheless, our results only refer to impacts of NPS on communities and do not imply that such disturbance has no effects at other biological scales (individual, population).

In the same way, the hydropower schemes had an insignificant overall influence, but they clearly muted the effect of climate change on proportion of southern species and warm-water species while intensifying the effect on specific richness (Figs 4a and 5). The latter effect, significant at the 0.1 level, did not seem quantitatively important (Fig. 5b) and was probably due to a more gradual increase of the specific richness at the sites located along hydropower schemes. Regarding southern species, we can propose two nonexclusive hypotheses explaining why their proportion did not increase at sites located along hydropower schemes. First, these sites were the most southern sites and were located close to the Mediterranean Sea that can be considered as a natural southern barrier to migration. Second, these sites were located close to dams, which are barriers to migration as well. As a consequence, fish communities at these sites may have suffered from a reduced flow of southern individuals. Interestingly, we observed inconsistent long-term changes of southern vs. warm-water species at sites located along hydropower schemes (Figs 4a, 5d and e). This result is not surprising because we assigned thermal preferences at the species level. In this way, a given species had the same thermal preference at all the sites (e.g. tench was always considered as a warm-water species) but was not necessarily considered as southern at all sites. Thus, if warm-water species were favoured at all the sites, the most southern ones did not come to dominate the community at sites located along hydropower schemes because of natural and/or anthropogenic barriers to migration. Finally, we observed no correspondence between the long-term trend of warm-water species with increasing temperature and the increase in warm-water species with increasing within-year temperature at sites located along hydropower schemes (Fig. 4). Hydropower schemes actually reversed the relationship between temperature and proportion of warm-water species at long-term vs. interannual scale. This can be due to different processes. First, water temperature was negatively correlated [modified Pearson's correlation test (Pyper & Peterman, 1998), P-values < 0.001] to discharge measured at the vicinity of these sites [32, 17 and 18 km from the NPS (i.e. temperature-recording sites) of Saint Alban, Cruas and Tricastin, respectively]. Most of the warm-water species considered in the study deposit their eggs on aquatic macrophytes (tench, white bream, rudd and wels catfish) or either on macrophytes or on gravel (bream), often in the backwater areas of the river (Mann, 1995). As a consequence, the recruitment of such species is highly sensitive to environmental constraints (Mann, 1995) and particularly to dewatering. In this way, low proportion of warm-water species could be due to low recruitment the warm and dry years. Beside such negative effect of temperature at the interannual scale, the positive long term trend of proportion of warm-water species can be attributed to contradictory effects of an environmental factor on different development stages. Indeed, high temperatures may punctually decrease the recruitment but concomitantly increase the survival of juveniles and/or adults (knowing that these species have strong affinities for warm water) maintaining, at least transitionally, a positive growth rate of populations. Such processes could be of course enhanced by density dependent survivals. A similar process (but with opposite patterns) has already been observed for dace population at Bugey (Daufresne, 2004; Daufresne *et al.*, 2004). Despite a positive effect of temperature on recruitment at the interannual scale, the population tended to collapse, probably because of an extra mortality rate of adults after reproduction.

These results do not question the influence of land use changes on community structures. Many studies have clearly showed the influences of such pressures, especially on aquatic ecosystems (Bravard, 1987; Petts *et al.*, 1989; Fruget, 1992; Kinsolving & Bain, 1993; Phillips & Johnston, 2004). Actually, we highlighted an overall detrimental effect of hydropower schemes on equitability (Fig. 5c). Similarly, the proportion of warm-water species was significantly higher at the sampling sites located along hydropower schemes (Fig. 5d). The latter pattern is probably due to the general decrease in current velocity along the schemes, favouring eury-tolerant organisms and especially species with positive affinities with low current velocities and warm water (Fruget, 1992). Thus, our results do not exclude an effect of press disturbances but rather suggest that the effects of nonclimatic anthropogenic disturbances and climate change on communities are generally additives. Overall, these results have profound implications regarding management policy. Global change probably did not really undermine the progress in environmental management over the past 30 years. However, it sounds crucial not considering past conditions to evaluate the effect of such improvement. It is probably more accurate to consider, as a reference, sites submitted to climate change but not to the studied anthropogenic constraint, as it has been done in this study.

Limits of the study

First of all, patterns regarding the proportion of southern and warm-water species were only evaluated using a restricted number of species. We considered the most abundant species and we thus evaluated the most important quantitative effects. However, this study again underlines the crucial need of a better knowledge of the ecology of European freshwater fish, and especially of their thermal preferences (Elliott, 1981; Küttel *et al.*, 2002).

Another potential caveat in our analysis is the use of several sampling sites within the different study areas. This may hinder the statistical independence of the different dynamics and in turn the meta-analysis itself. The mean distance between two consecutive sampling sites (along an upstream–downstream gradient) within a study area was of 5.1 km. In addition, the sampling of a study area was generally achieved within few hours. This should have ensured the independence of the samples between the different sampling sites. However, significant effects of the study areas were found for two of the 10 effect sizes: the *b* value of the proportion of warm-water species (ANOVA, Bonferroni corrected $P<0.05$) and the *S* value of the proportion of southern species (ANOVA, Bonferroni corrected $P<0.05$). The effects were mainly due to the homogeneity of the effect size at Bugey, where the mean distance between two consecutive sampling sites was only of 2.1 km. Indeed, effects of the study area were no longer significant when we only considered the most upstream and most downstream sampling sites at Bugey (18 km distant from each other). Rerunning the meta-analyses for these two effect sizes by only considering the most distant sampling sites for Bugey gave similar results than those found when considering all the sampling sites. Thus, our results did not strongly depend on the homogeneity of these two effect sizes among the different sampling sites at Bugey.

Finally, all the sites located along hydropower schemes were on the Rhône River. In this way, the characteristics of that river could have influenced the results. The increase in proportion of southern species, as well as the positive correlation between the proportion of warm-water species and temperature (i.e. the patterns observed in the most natural sites) were consistent with the patterns previously observed at Bugey (Daufresne, 2004; Daufresne *et al.*, 2004), a site located on the Rhône River. This, together with the general independence of the effect sizes across study areas, underline that the river effect *stricto sensu* could be weak. However, sites submitted to the influence of hydropower schemes were also located close to the Mediterranean Sea, a natural southern barrier to migration. Thus, it is still difficult to disentangle the effects of geographical location vs. hydropower schemes on community changes. Nevertheless, (1) these effects appeared to be weak and (2) there was no significant effect of the distance to the sea on *S* values of proportion of southern species at these specific sites (Pearson' correlation test, $P>0.1$).

Conclusion

Our results show that species composition, diversity, global abundance and size structure of fish communities exhibited important trends related to water warming in large rivers. Most of the observed patterns did not seem strongly sensitive to the effects of hydropower schemes and NPS. Further studies, involving additional study areas and/or taxa, would be useful to quantify more clearly the relative influence of nonclimatic anthropogenic stressor and geographical location (e.g. closeness to natural southern barriers to migration) on the responses of communities to climate change. Nevertheless, our results suggest that it is crucial to more systematically include climate change as a potential confounding factor when studying long-term ecological patterns, even in sites undergoing non-climatic anthropogenic disturbances.

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Long-term changes within the invertebrate and fish communities of the Upper Rhône River: effects of climatic factors

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Abstract

There is increasing evidence that the global climate change is already having measurable biological impacts. However, no study (based on actual data) has assessed the influence of the global warming on communities in rivers. We analyzed long-term series of fish (1979–1999) and invertebrate (1980–1999) data from the Upper Rhône River at Bugey to test the influence of climatic warming on both communities. Between the periods of 1979–1981 and 1997–1999, the average water temperature of the Upper Rhône River at Bugey has increased by about 1.5 °C due to atmospheric warming. In the same period, several dams have been built from 12.5 to 85 km upstream of our study segment and a nuclear power plant has been built on it. Changes in the community structure were summarized using multivariate analysis. The variability of fish abundance was correlated with discharge and temperature during the reproduction period (April–June): low flows and high temperatures coincided with high fish abundance. Beyond abundance patterns, southern, thermophilic fish species (e.g. chub, and barbel) as well as downstream, thermophilic invertebrate taxa (e.g. *Athricops*, *Potamopyrgus*) progressively replaced northern, cold-water fish species (e.g. dace) and upstream, cold-water invertebrate taxa (e.g. *Chloroperla*, *Protoneumura*). These patterns were significantly correlated with thermal variables, suggesting that shifts were the consequences of climatic warming. All analyses were carried out using statistics appropriate for autocorrelated time series. Our results were consistent with previous studies dealing with relationships between fish or invertebrates and water temperature, and with predictions of the impact of climatic change on freshwater communities. The potential confounding factors (i.e. dams and the nuclear power plant) did not seem to influence the observed trends.

Keywords: climate change, fish, freshwater, invertebrate, long-term series, Rhône River

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Introduction

The average air temperature of the Earth has increased by 0.06 °C per decade over the last century with two main periods of warming: between 1910 and 1945 and since 1976 (IPCC, 2001). Many studies have attempted to understand and predict how this warming influences ecosystems. They cover a wide range of ecosystems and taxa (e.g. plants, zooplankton, marine and terrestrial invertebrates, birds, mammals and fish; Hughes, 2000; Walther *et al.*, 2002). The two major consistent results of these studies are a change in the timing of life cycle events and spatial shifts towards higher altitudes and higher latitudes, according to thermal preferences.

Temperature is of major importance for poikilotherm aquatic organisms. It controls their physiology and behavior (Coutant, 1987) and can be considered as an ecological resource (Magnuson *et al.*, 1979). The potential impacts of global warming on aquatic organisms have been mainly documented in marine environments (Beamish, 1995; Sagarin *et al.*, 1999; Cabral *et al.*, 2001; Attrill & Power, 2002; Brooks *et al.*, 2002).

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However, the influence of global climate change could be particularly marked in freshwater bodies where movements are constrained by the environment (Shuter & Post, 1990). Many studies of climate change impacts in freshwater were predictive (Coutant, 1990; Regier *et al*., 1990; Carpenter *et al*., 1992; Meyer *et al*., 1999), and those based on actual data essentially dealt with lakes (George, 2000; Gerten & Adrian, 2000; Straile & Adrian, 2000; Scheffer *et al*., 2001; Sorvari *et al*., 2002). Few studies have assessed impacts of climate change on stream organisms (Elliott *et al*., 2000; Bradley & Ormerod, 2001), probably due to the lack of long-term data sets. This paper involves fish and invertebrate data collected between 1979 and 1999 in a segment of the Upper Rhône River at Bugey. During this period, Europe has warmed by 0.8 °C, with a greater warming rate (2 °C per century) in southern and central France (IPCC, 1997). Therefore, this data set gives us the opportunity to study the changes in stream communities at different trophic levels under climatic warming. It includes a wide range of organisms, with a large range of ecological, biological traits and niches, potentially reflecting major modifications of the aquatic ecosystem.

Since 1979, the Upper Rhône River has undergone other types of anthropogenic impacts. Hydropower schemes were built upstream from our study segment. They could affect sediment structure and discharge patterns, particularly during dam releases performed every 3 years (Bravard, 1987; Petts *et al*., 1989; Calow & Petts, 1992). On the Rhône River, schemes built before 1979 have led to an increase in winter and spring discharge rates and a decrease in summer and autumn discharge rates (Bravard, 1987). In addition, a nuclear power plant was built in 1978 in the middle of our study segment, potentially warming part of it. Such potentially confounding effects had to be considered when studying the influence of the global warming on freshwater communities at Bugey.

In this paper, we analyzed the temporal changes of fish and invertebrate communities at Bugey between 1979 and 1999 and tried to identify their causes. For this purpose, we used the fish and invertebrate data sampled at Bugey each year, as well as relevant temperature and discharge data. We first evaluated the relationships between climate, discharge rate and water temperature. Then, we analyzed the structural modifications of fish and invertebrate communities, using multivariate analysis to summarize community changes. We related community patterns to environmental modifications, using statistical analyses appropriate for time series. The results were compared with previous studies dealing with the biological impacts of human activity to identify the potential influence of confounding factors.

Materials and methods

Study area

The study segment of Bugey is a 13 km long segment of the French Upper Rhône River (45.8°N; 5.2°E), upstream from the confluence of the Ain River (Fig. 1). The segment has a slope of 0.35%, a mean width of 120 m, a mean depth of 3 m and a mean annual discharge of $500\,\mathrm{m^3\,s^{-1}}$. Five hydropower schemes were built 12.5–85 km upstream from the study segment in the last 20 years (the Chautagne hydropower scheme, completed in 1981; the Belley hydropower scheme, completed in 1982; the Brégnier Cordon hydropower scheme, completed in 1984 and the Sault Brénaz hydropower scheme, completed in 1986), and a nuclear power plant (CNPE Bugey) was built in 1978 in the segment. The nuclear power plant discharges water on an average 10 °C warmer than the input water. The warmed effluent stream cools on an average of 1 °C per kilometer and only affects a narrow area 15–25 m wide along the right bank (Ginot *et al*., 1996).

The study segment was a morphologically unimpacted section, not subjected to a minimum flow and with an overall good water quality (Agence de Bassin Rhône Méditerranée Corse, 1999; see Table 1). Phosphorus (PO_4), Ammonium (NH_4) concentrations, suspended and organic matter tended to decrease during the study period. Nitrate (NO_3) concentrations were stable and relatively low (Table 1). All these data are available online on the Agence de l'eau Rhône Méditerranée Corse web site at http://rdb.eaurmc.fr.

Temperature and discharge data

During the study period, the mean daily water temperature and discharge were recorded by Electricité de France at the Bugey nuclear power plant. The temperature recorded was the input water temperature (upstream from the power plant). The mean daily air temperature (1979–1999) at Ambérieux en Bugey (20 km north from the study segment) was recorded by Météo France. To evaluate the potential water temperature and discharge modifications induced by the climatic change, for each calendar year (from daily measurements) we calculated the mean annual discharge, the mean annual water temperature and the mean annual air temperature. To evaluate the potential modification of the hydrological regime at the study segment by the hydropower schemes built during the study period, for each year (from daily measurements) we calculated the mean, the maximum and the minimum discharges during winter/spring (December–May) and summer/autumn (June–September).

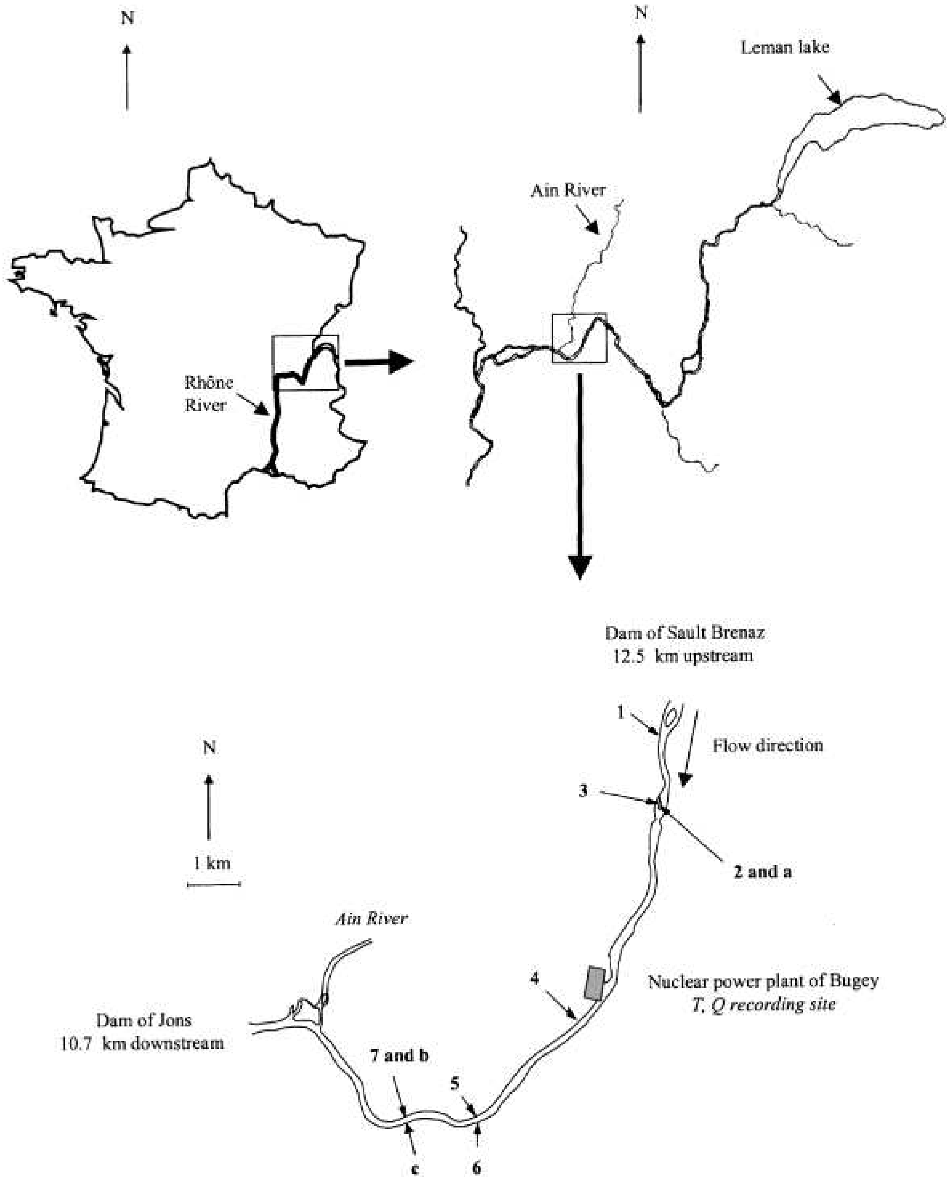

Fig. 1 Study area and location of the fish sampling sites (1–7), the invertebrate sampling sites (a–c) and the temperature (*T*) and discharge (*Q*) recording sites.

Finally, to link environmental changes to fish and invertebrate community changes, for each year (from daily measurements) we calculated the mean water temperature and mean discharge during limiting periods of the life cycles of fish and invertebrate. Periods considered for fish were the reproduction (from April to the end of June; Mann, 1996) and early growth of the Young of the Year (YOY) (from July to the end of October; Cattanéo *et al.*, 2001). Both directly influence the recruitment and, as a consequence, the strength of cohorts (Cragg-Hine & Jones, 1969; Mann, 1974; Philippart, 1981; Mills & Mann, 1985). The growth period includes summer and could also influence the survival of juveniles and adults. For invertebrates, the large number of different invertebrate taxa made it impossible to define the key periods influencing the whole invertebrate community (Hynes, 1970). A biological year was defined beginning on 1 October of the calendar year n and ending on 30 September of the calendar year $n + 1$. These dates were chosen according to the ecology of most of the taxa present in the invertebrate community of the Upper Rhône River (Hynes, 1970). In summary, we used the six seasonal variables defined above to link environmental changes to the changes in fish and invertebrate communities (Table 2): the mean annual reproduction temperature;

the mean annual reproduction discharge; the mean annual growth temperature; the mean annual growth discharge; the mean temperature calculated for the invertebrate biological year; and the mean discharge calculated for the invertebrate biological year.

Table 1 Mean, median and standard deviation of BOD5, conductivity and NH_4, NO_2, NO_3, PO_4, chlorophyll *a* and O_2 concentrations at Jons (10.7 km downstream the study segment)

	Mean	Median	SD	Trend
NH_4 ($mg\,L^{-1}$)	0.13	0.10	0.11	–
NO_2 ($mg\,L^{-1}$)	0.07	0.06	0.04	–
NO_3 ($mg\,L^{-1}$)	3.74	3.50	1.36	~
O_2 ($mg\,L^{-1}$)	10.16	10.20	1.17	~
BOD5 ($mg\,L^{-1}$)	1.80	1.60	0.96	–
PO_4 ($mg\,L^{-1}$)	0.15	0.12	0.1	–
Chlorophyll *a* ($\mu g\,L^{-1}$)	3.95	2.65	3.92	–
Conductivity ($\mu S\,cm^{-1}$)	312.73	310.00	41.90	~

BOD5, 5-day biological oxygen demand; ammonium, NH_4; nitrite, NO_2; nitrate, NO_3; phosphorus, PO_4; dissolved oxygen, O_2; SD, standard deviation.
Statistics are derived from the data collected by the Agence de l'eau Rhône Méditerranée Corse (available at http://rdb.eaurmc.fr). Data were collected on average 12.5 times a year (SD = 8.5), except for chlorophyll *a*, which were sampled 12, 6 and 6 times during 1981, 1987 and 1988, respectively, and on average 7.1 times a year (SD = 2.7) since 1993. Values strictly below the detection limit were considered as equal to the detection limit (Helsel, 1990). Trend refers to the decrease (–) or stability (~) of values since 1971 (1981 for chlorophyll *a*).

Fish data

Fish were sampled at seven sites at different seasons, on average 3.7 times a year (SD = 1.2). Four sites (4, 5, 6 and 7, Fig. 1) were from 0.250 to 13 km downstream from the nuclear power plant. The other sites were from 4.5 to 6.25 km upstream from the nuclear power plant (Fig. 1). Descriptions of site morphology are given by Ginot *et al.* (1996). The sampling of site 6 began in 1980, whereas the other sites were sampled since 1979.

Fish were collected at each site by continuous electrofishing from a boat, drifting downstream along banks, always starting at the same point (Allardi *et al.*, 1975; Carrel & Rivier, 1996). The average sampling duration was 27.6 mn (SD = 6.9). A site was 300–400 m long (depending of the sampling duration) and 2–4 m wide. Sampling was performed using a Dream Electronics apparatus (type: Heron DC; 300–400 V; 2–3 A, Pessac, France). Captured fish were identified to the species, measured to the nearest millimeter, and released.

The species abundance was expressed as catch per unit effort (CPUE) calculated for each sample (site × season), i.e., the number of fish of a given species captured for a 20 mn period (to be consistent with other studies on the Rhône River using the same protocol; Cattanéo *et al.*, 2001; Grenouillet *et al.*, 2001). Fish

Table 2 Trend test (trend *P*) for air temperature (T_{air}) water temperature (T_{water}) and discharge (*Q*) seasonal variables used in the paper

Variables			Trend *P*	Use
Annual	Mean	T_{air}	*	Detecting the influence of climate change on water temperature and discharge
		T_{water}	*	
		Q	NS	
Summer	Min.	*Q*	NS	Detecting discharge modification at Bugey due to hydropower schemes
	Mean	*Q*	NS	
	Max.	*Q*	NS	
Winter	Min.	*Q*	NS	
	Mean	*Q*	NS	
	Max.	*Q*	NS	
Reproduction	Mean	T_{water}	**	Detecting the influence of environment on fish community
		Q	*	
Growth	Mean	T_{water}	*	
		Q	NS	
Invertebrate	Mean	T_{water}	*	Detecting the influence of environment on invertebrate community
		Q	NS	

Annual, Summer, Winter, Reproduction, Growth and Invertebrate refer to, respectively, calendar year (January–December), summer (June–September), winter (December–May), fish reproduction period (April–June), growth period of young of the year fish (July–October) and invertebrate biological year (October–September). Min. and Max. designate minimum and maximum values, respectively. We indicate how the different variables were used.
$*P < 0.05$, $**P < 0.01$, NS, non-significant.

species accounting for less than 5% of sampled fish CPUE were excluded from the study. CPUE were $\ln(x+1)$ transformed to normalize their distributions, and averaged by year × site. Note that year was defined from the fish biological year (from 1 July of the calendar year *n* to 30 June of the calendar year $n+1$). These dates were defined according to the 'average' hatching date of most of the cyprinid species (Spillmann, 1961; Mann, 1996; Bruslé & Quignard, 2001; Keith & Allardi, 2001) present in fish community of the upper river Rhône (Ginot *et al.*, 1996). An ANOVA performed on $\ln(\mathrm{CPUE}+1)$ data (before yearly averaging) indicated that only 3.1% of the total variance was due to different sampling seasons (vs. 19% for years) justifying our averaging by year × site. The fish abundance data set finally contained eight columns (species) and 146 rows (site × year combinations). The proportion of YOY in the abundance data set was evaluated for each species. We used size limits derived from analysis of general books or papers dealing with the ecology of European freshwater fish (Pattée, 1988; Persat, 1988; Bruslé & Quignard, 2001; Keith & Allardi, 2001). Individuals with a length strictly below 50 mm for bleak, barbel and roach, below 60 mm for stream bleak, gudgeon and chub, below 65 mm for nase and below 80 mm for dace were considered as YOY.

Invertebrate data

Invertebrates were sampled at three sites at different seasons, on average 3.5 times a year (SD = 0.7), from March 1980 to September 1999. Two sites (b and c, Fig. 1) were 6.8 km downstream from the nuclear power plant, and the other site was 5.25 km upstream from the nuclear power plant (Fig. 1). Sites were sampled using cylindrical artificial substrates (30 cm diameter × 15 cm high) according to the IQBP protocol (Verneaux *et al.*, 1976). At each site, a pair of artificial substrates was left on the streambed along banks, one in a lotic zone, the other in a lentic zone. They remained 21 days to allow macro-invertebrates to colonize. Because of hydrological conditions (floods), this time could be extended by up to 4 days. Then, substrates were washed and invertebrates were collected in a 0.5 mm mesh net. All specimens were identified to the genus level except chironomids, Limnephilidae, other Diptera (identified to the subfamily or family level) and the species *Dugesia tigrina*. Oligochaeta and Hydracarina (accounting, respectively, for 3.6% and 0.01% of the total abundance) were not included in the analysis because of difficulties in their identification. One taxon (*Gammarus*) largely dominated the community (it could account for more than 90% of the abundance of a sample; Roger *et al.*, 1991). Thus, in our analysis we included all taxa with more than five individuals sampled during the whole study. CPUE values were not calculated for invertebrate data because the sampling effort was constant across samples. The abundance of taxa was $\ln(x+1)$ transformed to normalize their distributions, and averaged by invertebrate biological year × site. An ANOVA performed on $\ln(\mathrm{abundance}+1)$ data (before yearly averaging) indicated that only 5.7% of the total variance was due to different sampling seasons (vs. 19.7% for years), justifying our averaging by year × site. The invertebrate abundance data set finally contained 74 columns (taxa) and 60 rows (site × year combinations).

Summarizing interannual biological variations

We first calculated the proportion of variance due to the year and site for fish and invertebrate abundance data and tested the significance of the effects using permutation tests (ADE4 software; Thioulouse *et al.*, 1997). Then, we studied the annual variations of data in the different sites. To reduce the number of analyses, we summarized separately fish and invertebrate changes in all sites as multivariate axes. A centered principal component analysis was performed on fish abundance data (ADE4 software; Thioulouse *et al.*, 1997). The resulting axes are independent linear combinations of mean species $\ln(\mathrm{CPUE}+1)$. The temporal changes in fish community structure were represented by time series of annual factorial scores at each site. For invertebrates, the large domination in the abundance of *Gammarus* led us to choose correspondence analysis rather than principal component analysis. Instead of dealing with variability in the abundance of taxa, this allowed us to focus on the variability of community structure (relative abundances). As for fish, the temporal changes in invertebrate community structure were represented by the time series of annual factorial scores at each site. All community trends were compared between sites located downstream and upstream of the nuclear power plant to assess the potential impact of the warm effluent.

Trend analysis

To detect trends in time series, we used a modified Mann–Kendall trend test developed by Hamed & Rao (1998). This non-parametric test (based on ranks) looks for temporal trends once autocorrelation effects are removed. Tests were performed using S-plus software (S-plus2000, 2000).

Link between variables

The potential relations between temperature and discharge variables were assessed using Pearson's correlation coefficients. Then, we estimated the correlation between biological axes and environmental variables. To cope with autocorrelation effects in our correlation tests, we adjusted the number of degrees of freedom by a modified Chelton method, using the autocorrelation estimator proposed by Chatfield (1989). Unlike smoothing or prewhitening, this method deals with the increase of type I error rate without increasing the type II error rate (see Pyper & Peterman, 1998 for details). For each factorial axis and each environmental variable, we used a global Fisher's test (Fisher, 1950) (combining all sites) to assess the influence of environmental conditions on communities. The proportion of community variability explained by each environmental variable was evaluated by the Pearson's correlation coefficient between mean scores by site × year and the environmental variable. All tests were performed using S-plus software (S-plus2000, 2000), and corrected for their multiplicity using Bonferroni procedures (Sokal & Rohlf, 1998).

Results

Temperature and discharge data

Between 1979 and 1999, the mean annual air at Ambérieux en Bugey and water temperatures upstream the nuclear power plant increased, respectively, from 10.7 °C to 11.7 °C and from 11.3 °C to 11.9 °C. Three years were warm for both air and water temperatures: 1982, 1989 and 1994 (Fig. 2a). In the same period, discharge fluctuated around its mean annual discharge (500 $m^3 s^{-1}$) without exhibiting particular cycles or trend (Fig. 2b).

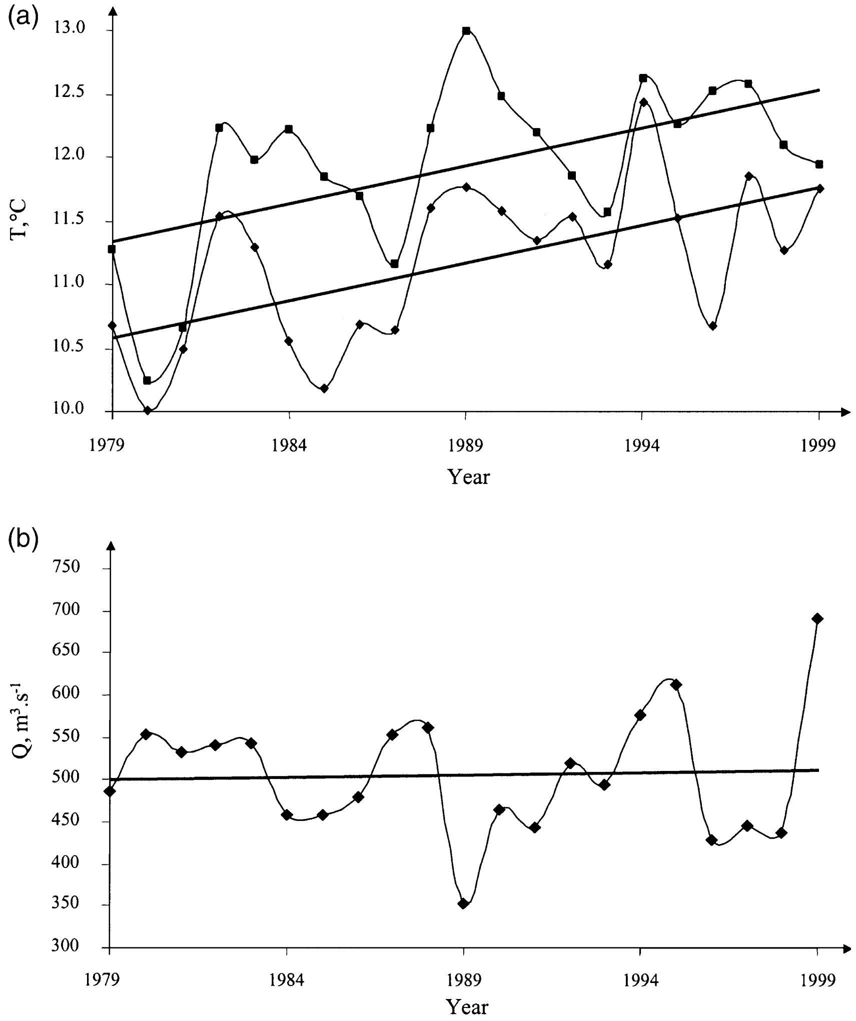

Fig. 2 Mean annual daily water (◆) and air (■) temperature (a) and mean annual daily discharge (b) at Bugey between 1979 and 1999. Trends are shown ($Y(t) = at + b$).

Fish data

Between 1979 and 1999, a total number of 58 090 fish of 37 species were caught (from 12 to 25 species per year). The fish community was dominated by a set of eight species accounting for 95.3% of sampled fish CPUE: chub (*Leuciscus cephalus* Linnaeus, 1758), stream bleak (*Alburnoides bipunctatus* Bloch, 1782), dace (*Leuciscus leuciscus* Linnaeus, 1758), bleak (*Alburnus alburnus* Linnaeus, 1758), barbel (*Barbus barbus* Linnaeus, 1758), roach (*Rutilus rutilus* Linnaeus, 1758), nase (*Chondrostoma nasus* Linnaeus, 1758) and gudgeon (*Gobio gobio* Linnaeus, 1758), which accounted, respectively, for 26.7%, 17.2%, 16.4%, 10.9%, 7.8%, 6.0%, 5.2% and 5.1% of sampled fish CPUE. 66.9% of the sampled fish CPUE were caught in the sites 4, 5, 6 and 7 downstream of the nuclear power plant. Most fish were more than 1 year old even though the percentage YOY represented about 1/3 of the nase CPUE, about 1/4 of the stream bleak CPUE and about 1/5 of the bleak and dace CPUE. The total proportion of YOY was high in the warm years (e.g. proportion of YOY in the total abundance = 45.6% the biological year 11 (1988–1989) and 30.4% in biological year 12 (1989–1990) compared with only 17% in biological year 2 (1979–1980)).

Invertebrate data

A total of 1 282 230 invertebrates (92 taxa) were captured during the study period. The proportion of *Gammarus* averaged across sites was 87.4%. Chironimidae (4% of the total invertebrate abundance), Oligochaeta (3.6%), *Hydropsyche* (2.3%), *Heptagenia* (1.1%), molluscs such as *Theodoxus*, *Potamopyrgus* and *Corbicula* (0.4%, 0.2%, 0.1%, respectively), *D. tigrina*, *Ephemerella* and Simulidae (0.2%, 0.1%, 0.1%, respectively) were the other most abundant taxa. Invertebrate abundance was higher in site a (42% of the total invertebrate abundance) than in sites b (30%) and c (28%), located downstream of the nuclear power plant.

Interannual variability of fish and invertebrates data

41.3% ($P < 0.001$) of the total variance in fish abundance data was due to temporal (between year) effects (compared with 20.1% ($P < 0.001$)) for spatial, between-site effects). Similarly, for invertebrate abundance data, interannual variance represented 51.5 % ($P < 0.001$) of the total variance (compared with 7.2% ($P < 0.001$) for spatial effects).

Temporal changes of fish community structure

The first two axes of the principal component analysis accounted for 59% of the total variability of fish abundance data (37% for the first axis and 22% for the second axis). Time series of multivariate scores were calculated for each site. However, to clarify figures, we only represented scores averaged across all sites, across sites downstream of the nuclear power plant and across sites upstream of the nuclear power plant (Fig. 3a, c).

The first axis reflected the overall abundance variability. For each site, there was a strong correlation between factorial scores and the sum of species CPUE per year (r^2 values from 0.86 to 0.95). Therefore, factorial scores of each site for each year represented the overall fish abundance sampled at the site. As a consequence, the first axis did not discriminate fish species (Fig. 3b). Site scores confirmed that fish density was higher in sites downstream of the nuclear power plant, especially between the biological years 8 and 11 (i.e. from July 1985 to June 1989) (Fig. 3a). However, temporal changes of the site factorial scores were comparable in all sites, including those upstream the nuclear power plant.

The second axis opposed in each site species with increasing relative CPUE over time (such as chub and barbel) to those with decreasing relative CPUE over time (such as dace and bleak) (Fig. 3d). Between biological years 1 and 21, the mean annual CPUE of chub increased by three times and that of barbel by 5.6 times (Fig. 4). Meanwhile, the mean annual CPUE of dace decreased by 2.9 times. The mean annual CPUE of stream bleak was stable until biological year 19 and rapidly increased between biological years 19 and 21 (multiplied by 2.1). This last change was not revealed by the principal component analysis. Site factorial scores on the second axis reflected the relative CPUE of chub and barbel compared with the relative CPUE of dace and bleak in each site (Fig. 3c). Temporal changes of the site factorial scores were comparable in all sites, including those upstream from the nuclear power plant (Fig. 3c).

Temporal changes in invertebrate community structure

The first axis of the correspondence analysis accounted for (18%) of the invertebrate data inertia. This axis opposed taxa that tend to appear and increase during the study period (e.g. *Athricops*, *Potamopyrgus*, *Corixa* and *Lepidostoma*) to taxa that instead tended to decrease or disappear (e.g. *Chloroperla*, *Protonemura* and *Nemoura*) (Fig. 5). As for fish, we only represented factorial scores time series averaged across all sites, across sites downstream of the nuclear power plant and across sites upstream of the nuclear power plant (Fig. 6). The factorial scores of sites located upstream and downstream the nuclear power plant showed similar temporal patterns.

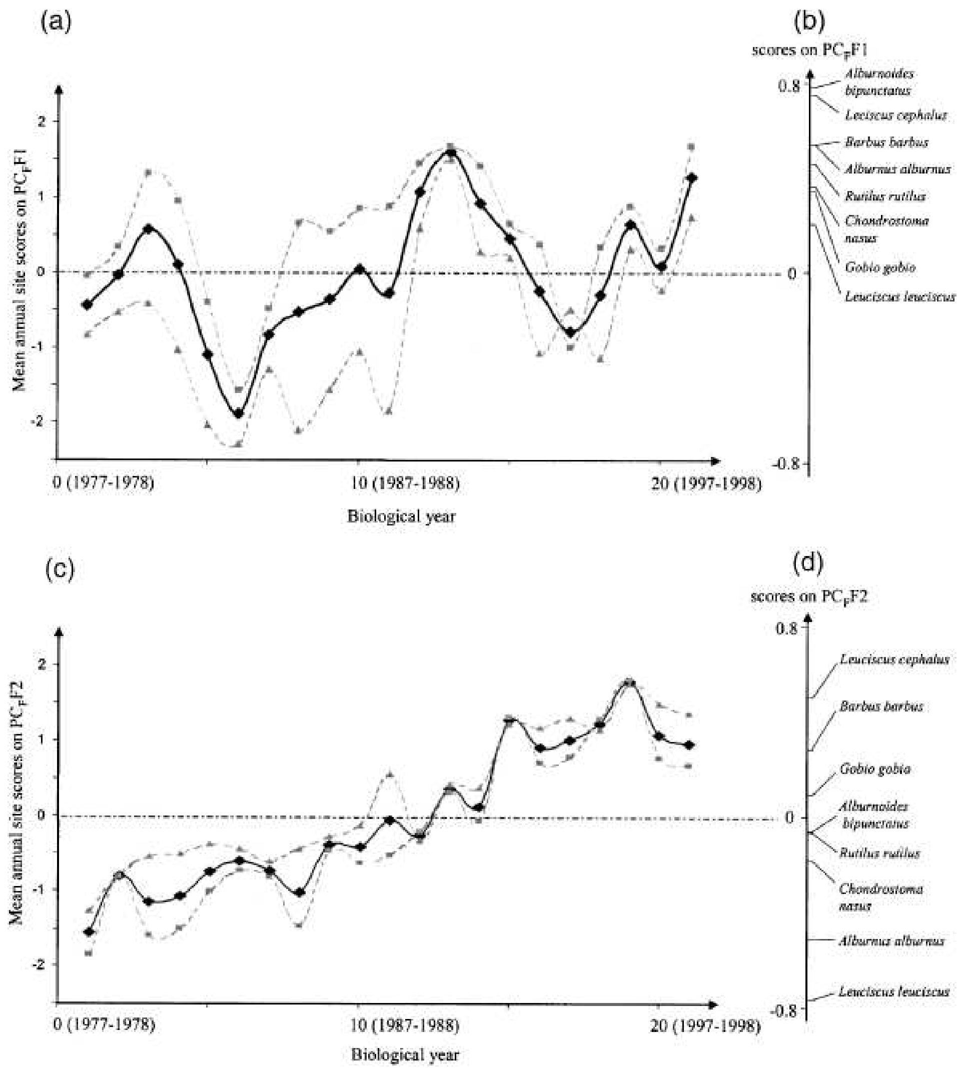

Fig. 3 Time series of sites scores on the two first axes of the principal component analysis of fish data ($PC_F F1$ (a) and $PC_F F2$ (c)). Scores are averaged across all sites (◆, continuous line), across sites downstream from the nuclear power plant (■, dashed line) and across sites upstream from the nuclear power plant (▲, dashed line). Species contributions on $PC_F F1$ (b) axis and $PC_F F2$ (d) axis are given. Note that *Y*-axis scales of (a) and (b) ((c) and (d), respectively) are different.

Trend analysis

The mean annual air and water temperature time series showed significant positive trends ($P < 0.05$) (Fig. 2, Table 2). These trends were not sensitive to extreme years: they remained significant at the 0.05 level when we removed the 1982, 1989 and 1994 warm years. Similarly, the mean temperature during fish reproduction period, fish growth period and invertebrate biological year increased significantly during the study. Discharge variables show weak trends. Only mean discharge during the reproduction period decreased ($P < 0.05$) (Table 2).

For fish, the factorial scores on the first axis of the principal component analysis of five sites (1, 2, 3, 6, 7) showed significant positive trends (Table 3). However, trends of factorial scores of sites 1 and 3 did not remain significant when we removed the extreme biological years 3, 13 and 21. Two sites located downstream from the nuclear power plant (4, 5) did not exhibit significant trend. On average, the total fish abundance did not show any major increase (see Fig. 3a). Factorial scores of all sites on the second axis showed strong significant positive trends ($P < 0.05$ for site 4, $P < 0.01$ for site 7, $P < 0.001$ for all other sites) (Table 3). Chub and barbel thus progressively replaced bleak and dace in all the sites including those upstream from the nuclear power plant. This pattern was continuous throughout the study period (Fig. 3).

With respect to invertebrates, time series of site scores on the first axis of the correspondence analysis also

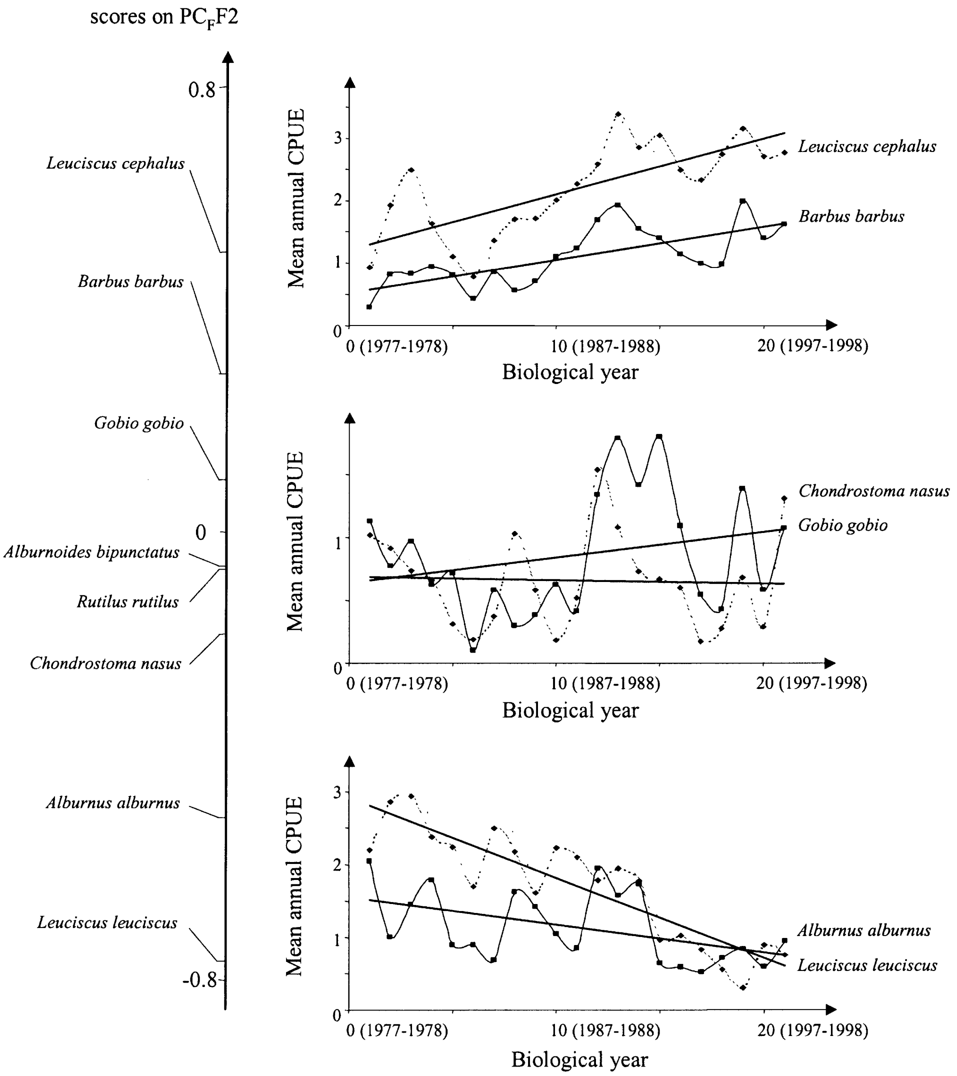

Fig. 4 Examples of mean annual catch per unit effort (CPUE) time series of species discriminated by the second axis of the principal component analysis realized on fish abundance data (PC$_F$F2). Trends are shown ($Y(t) = at + b$).

exhibited positive trends ($P < 0.001$ for sites b and c and $P < 0.05$ for site a) (Table 3). In all sites, the abundance of taxa such as *Athricops*, *Potamopyrgus* and *Lepidostoma* gradually increased, whereas the abundance of taxa such as *Protonemura*, *Chloroperla* and *Nemoura* decreased (Fig. 5b).

Correlation among the seasonal environmental variables (temperature, discharge)

The mean annual air and water temperatures were significantly correlated ($P < 0.01$). The mean annual air temperature was correlated with the mean water temperature during the fish reproduction period and the invertebrate biological year ($P < 0.01$ and $P < 0.05$, respectively). No relationship was found between the mean annual discharge and mean annual air or water temperature. The mean annual water temperatures during the reproduction period and discharge were significantly correlated ($P < 0.01$), as was the mean annual water temperature during the growth period and discharge ($P < 0.05$) (Table 4).

Link between community structure variability and environmental changes

For fish, site scores on the first axis of the principal component analysis were significantly correlated with the mean annual temperature and mean annual discharge during the reproduction period ($P < 0.05$ and $P < 0.001$, respectively). However, these two environmental variables were redundant (see above). On the

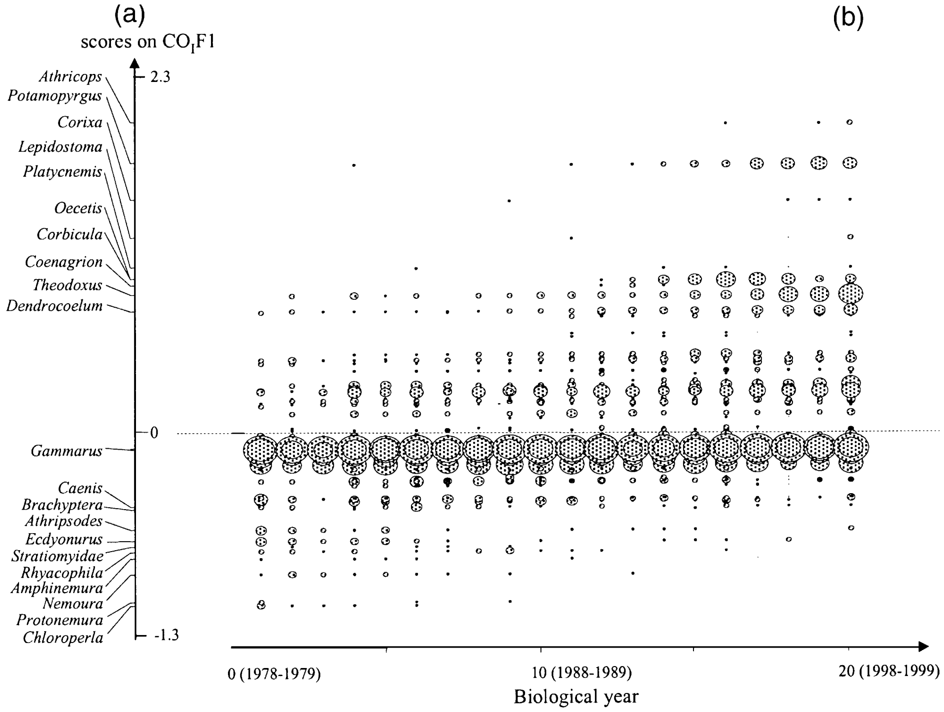

Fig. 5 Mean annual abundance per sample of invertebrate taxa (circle area and abundance are proportional) classified according to the first axis of the correspondence analysis of invertebrate data (CO_IF1) (b). Contribution of the 10 taxa with highest factorial scores, *Gammarus* and the 10 taxa with lowest factorial scores are given (a).

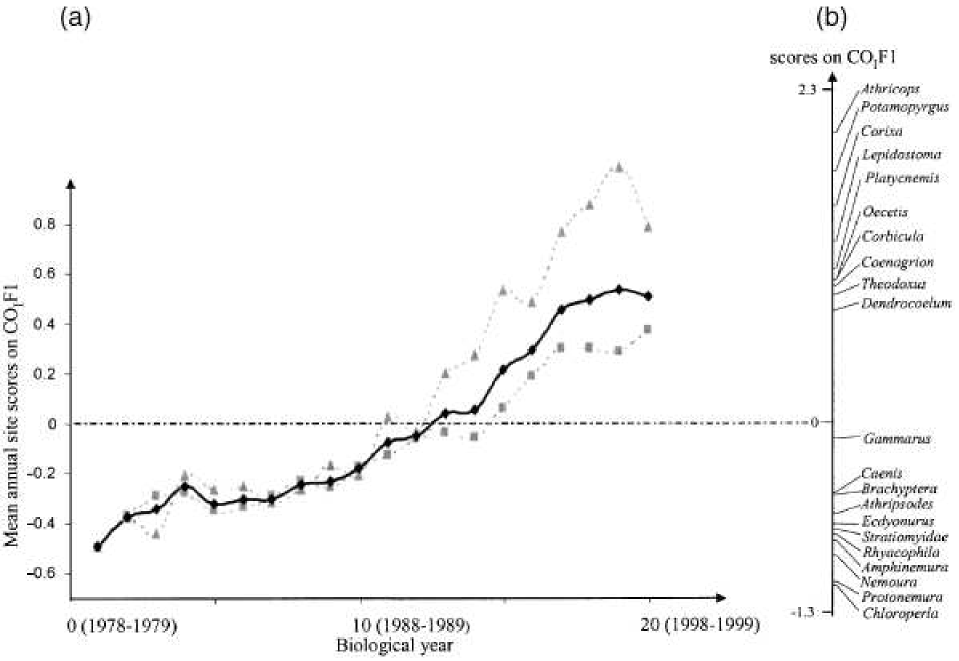

Fig. 6 Time series of site scores on the first axis of the correspondence analysis of invertebrate data (CO_IF1) (a). Scores are averaged across all sites (▲, continuous line), across sites downstream from the nuclear power plant (■, dashed line) and across sites upstream from the nuclear power plant (▲, dashed line). Contribution of the 10 taxa with highest factorial scores, *Gammarus* and the 10 taxa with lowest factorial scores are given (b). Note that *Y*-axis scales of (a) and (b) are different.

Table 3 Trend probabilities (*P*) of factorial sites scores time series

Data set	Axis	Site	*P*
Fish data	First axis of the principal component analysis	1	**
		2	*
		3	*
		4	NS
		5	NS
		6	*
		7	**
	Second axis of the principal component analysis	1	***
		2	***
		3	***
		4	*
		5	***
		6	***
		7	**
Invertebrate data	First axis of the correspondence analysis	a	**
		b	***
		c	***

$^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, NS, non-significant.

first axis, these two variables explained, respectively, 20% and 46% of the variability in the mean annual site scores. No relationship was found between fish abundance in a sample and temperature or discharge on the day of sampling, indicating that this pattern was not a sampling protocol effect. Site scores on the second axis were significantly correlated with the mean annual temperature during the reproduction period ($P<0.05$). On the second axis, this variable explained 45% of the mean variability in annual site scores (Table 5).

For invertebrates, site scores on the first axis of the correspondence analysis were significantly correlated with the mean annual temperature calculated on the invertebrate biological year ($P<0.05$). On the first axis, this variable explained 29% of the mean variability in annual site scores.

Discussion

Since 1979, the Upper Rhône River has warmed up under the influence of climate warming. This pattern is consistent with the thermal change of many rivers in the northern hemisphere and especially in Europe (Webb, 1996). Punctual discordance between air and water temperature (e.g. 1996) are difficult to explain and could be due to the influence of factors such as snow melt. Water temperature was recorded continuously only upstream from the nuclear power plant. However, Ginot *et al.* (1996) have shown that the nuclear power plant discharges water 10 °C warmer on average than the input water. The warmed effluent stream cools of 1 °C on average per kilometer and only affects a narrow area (15–25 m wide) along the right bank, independent of the input water temperature (Ginot *et al.*, 1996). Therefore, we can reasonably assume that the relative increase of water temperature under climatic warming was the same in all sites since 1979. During the same period, we observed significant trends in both fish and invertebrate community structures.

With respect to fish, we observed an increase of chub and barbel abundance and a decrease of dace and bleak abundance. Considering the relative position of the study segment in the geographical range of fish species (Table 6), chub, barbel, stream bleak and gudgeon are southern species (Bugey is close to the center of the species latitudinal range). By contrast, Bugey is close to the southern limit of the geographical range of bleak, nase, roach and especially dace (Table 6). According to Bruslé & Quignard (2001), dace is the most northerly European cyprinid species. Therefore, the observed shift in fish community structure reflected the gradual displacement of northern species by southern species. Most of the eight studied species are eurytherme (Bruslé & Quignard, 2001; Keith & Allardi, 2001). However, barbel has a clear affinity for warm water (Kraiem, 1979; Bruslé & Quignard, 2001) and chub is attracted by warm water (Bruslé & Quignard, 2001). By contrast, dace prefers cold water (Bruslé & Quignard, 2001). With respect to invertebrates, we also observed a gradual disappearance of cold-water taxa as *Chloroperla*, *Protonemura*, *Nemoura* and *Amphinemura* (Tachet *et al.*, 2000) opposed to a gradual increase in abundance of warm-water taxa as *Corbicula* (Tachet *et al.*, 2000). However, developing taxa (*Athricops*, *Potamopyrgus*, *Corixa*, *Lepidostoma*, *Platycnemis*, *Oecetis*, *Corbicula*, *Coenagrion*, *Theodoxus*, *Dendrocoelum*) also prefer null or low current velocity and downstream zones (Tachet *et al.*, 2000). By contrast, decreasing taxa (*Chloroperla*, *Protonemura*, *Nemoura Amphinemura Rhyacophila*, *Stratiomyidae*, *Ecdyonurus*, *Athripodes*, *Brachyptera*, *Caemis*) prefer fast current velocity and upstream zones (Tachet *et al.*, 2000).

The overall increase of thermophilic and southern fish species and of thermophilic invertebrate taxa was consistent with the predictions or observations of the consequences of the global warming. Coutant (1990) and Carpenter *et al.* (1992) predicted a geographical shift of freshwater fish geographical range towards higher latitudes and higher altitudes. Hauer *et al.* (1997) and Mulholland *et al.* (1997) supposed that similar patterns could occur for freshwater invertebrates. Such patterns have already been observed in the marine environment. Attrill & Power (2002) and Sagarin *et al.* (1999) have locally observed an increase in the

Table 4 Air temperature (T_{air}) water temperature (T_{water}) and discharge (Q) seasonal variables correlation matrix

			Annual			Summer			Winter			Reproduction		Growth		Invertebrate	
			Mean			Min.	Mean	Max.	Min.	Mean	Max.	Mean		Mean		Mean	
			T_{air}	T_{water}	Q	Q	Q	Q	Q	Q	Q	T_{water}	Q	T_{water}	Q	T_{water}	Q
Annual	Mean	T_{air}	1.00														
		T_{water}	0.71**	1.00													
		Q	0.17	−0.32	1.00												
Summer	Min.	Q	0.44	0.01	0.73**	1.00											
	Mean	Q	−0.05	−0.46*	0.81***	0.68***	1.00										
	Max.	Q	−0.17	−0.26	0.31	0.20	0.67**	1.00									
Winter	Min.	Q	0.22	0.06	0.47	0.55*	0.38	0.06	1.00								
	Mean	Q	0.12	−0.06	0.71**	0.49	0.43	−0.08	0.64**	1.00							
	Max.	Q	0.09	0.01	0.32	0.27	0.35	0.19	0.15	0.28	1.00						
Reproduction	Mean	T_{water}	0.65**	0.61*	−0.13	0.34	−0.08	0.04	−0.11	−0.19	0.23	1.00					
		Q	−0.42	−0.05	−0.09	−0.31	−0.06	0.03	0.21	0.18	−0.01	−0.61**	1.00				
Growth	Mean	T_{water}	0.07	0.39	−0.27	−0.15	−0.18	0.14	−0.37	−0.16	0.18	0.55*	−0.06	1.00			
		Q	0.29	0.20	0.21	0.21	−0.07	−0.27	0.50	0.35	−0.17	−0.24	0.20	−0.52*	1.00		
Invertebrate	Mean	T_{water}	0.57*	0.95***	−0.48*	−0.14	−0.60**	−0.29	−0.06	−0.13	−0.05	0.64*	−0.03	0.52*	0.00	1.00	
		Q	0.23	−0.19	0.84***	0.72**	0.75***	0.23	0.77**	0.79***	0.24	−0.10	-0.03	−0.40	0.41	−0.35	1.00

Annual, Summer, Winter, Reproduction, Growth and Invertebrate refer to, respectively, calendar year (January–December), summer (June–September), winter (December–May), fish reproduction period (April–June), growth period of young of the year fish (July–October) and invertebrate biological year (October–September). Min. and Max. designate minimum and maximum values, respectively.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Table 5 Correlation between site scores on factorial axes and seasonal environmental variables

	R		Site scores on PC_FF1	Site scores on PC_FF2	Site scores on CO_IF1
Reproduction	Mean	T_{water}	**0.45***	**0.67***	
		Q	**−0.68*****	−0.39	
Growth	Mean	T_{water}	0.06	0.36	
		Q	−0.30	0.01	
Invertebrate	Mean	T_{water}			**0.54***
		Q			−0.01

r, Pearson's correlation coefficient between mean annual site scores and environmental variables. PC_FF1 and PC_FF2 design, respectively, the first and second axis of the principal component analysis of fish data. CO_IF1 is the first axis of the correspondence analysis of invertebrate data. Reproduction, Growth and Invertebrate refer to fish reproduction period (April–June), growth period of young of the year fish (July–October) and invertebrate biological year (October–September), respectively. T_{air} and T_{water} design air and water temperature, respectively.
Bonferroni corrected global Fisher's test probability: $^*P<0.05$, $^{***}P<0.001$.

Table 6 Relative position of the study segment in the geographical range of fish species

Fish species	Latitude of the nothern limit of the species' geographical range (°N)	Latitude of the southern limit of the species' geographical range (°N)	X
Alburnus alburnus	67.0	41.0	0.23
Alburnoides bipunctatus	62.0	36.0	0.61
Barbus barbus	57.0	42.0	0.34
Chondrostoma nasus	63.0	42.5	0.19
Gobio gobio	67.0	36.0	0.46
Leuciscus cephalus	63.5	36.5	0.52
Leuciscus leuciscus	70.0	41.5	0.18
Rutilus rutilus	66.0	41.0	0.24

This variable (x) is coded on the basis of the geographical range of the species (Keith & Allardi, 2001) as a function of: $x = L_{Bugey} - L_{inf}/L_{sup} - L_{Bugey}$ where L_{Bugey}, L_{sup} and L_{inf} are the latitudes (°N) of the study segment, of the northern limit of the species' geographical range and the southern limit of the species' geographical range, respectively.

abundance of southern taxa in invertebrate and fish marine communities. More generally, shifts of species range to upper latitude or altitude due to climate change have been observed in zooplankton, invertebrates, fish, plants, butterflies, birds and mammals (Hughes, 2000; Walther *et al.*, 2002).

Hydrology and temperature widely influence the freshwater fauna (Magnuson *et al.*, 1979; Poff *et al.*, 1997). On the Upper Rhône River (45 km upstream from the study segment), Dolédec *et al.* (1996) found a gradual increase of lentic and thermophilic invertebrate taxa between 1980 and 1991, correlated with increasing water temperature. This pattern was continuous during the study period and was not an artifact due to the warm 1989 year. On the lower Rhône River, Fruget *et al.* (2001) showed that discharge and water temperature were the major factors controlling the long-term dynamics of invertebrate and fish communities. According to Fruget *et al.* (2001), low discharge and high temperature positively influenced the breeding success of fish. Such a positive influence has already been shown for many cyprinid species (Hellawell, 1974; Mann, 1974; Philippart, 1981, 1989; Mills & Mann, 1985; Araújo *et al.*, 1999), including in the Rhône River (Grenouillet *et al.*, 2001). Consistently, we found that low flow and high temperature during the reproduction period were associated with high total fish abundance and high proportions of YOY. In addition, water temperature during the reproduction period explained shifts in community structure. Generally, dace recruitment is positively influenced by high water temperatures (Philippart, 1981; Mann & Mills, 1985; Araújo *et al.*, 1999). However, Mann & Mills (1985) showed that a warm summer (especially a warm June; Mills & Mann, 1986) can result in a very low egg size and very low fecundity for the next spring. According to the authors, the energy investment in growth was at the expense of gonad development. The reproduction period that we defined included June. Thus, the dace population could have suffered from the increase in mean annual temperature in the reproduction period because of a gradual decrease in the species' breeding success. Moreover, Philippart (1981) has shown that dace has a high mortality rate after reproduction, from April to May. This mortality, due to the energy investment in gonad development and spawning, has probably been reinforced by the shift from thermal optima for cold-water and northern species.

Changes in invertebrate and fish communities could be complicated by interactions between the two trophic

Table 7 Evolution of the relative proportion of the eight species considered in our study

Fish species	During the study period at Bugey	Before completion vs. by-passed section (Persat, 1988)	Before completion vs. diversion canal (Persat, 1988)
Alburnus alburnus	–	–	–
Alburnoides bipunctatus	**+**	**–**	**–**
Barbus barbus	**+**	**~**	**–**
Chondrostoma nasus	**~**	**–**	**–**
Gobio gobio	**~**	**+**	**+**
Leuciscus cephalus	+	+	+
Leuciscus leuciscus	–	–	–
Rutilus rutilus	**~**	**+**	**+**

Comparison with the effects of hydropower schemes on fish structure of the Upper Rhône River (from Persat, 1988). An increase, a decrease and stability are indicated, respectively, by ' + ', '–'and ' ~ '. Rows in bold font highlight different variations in the two studies.

levels (Petchey *et al.*, 1999; Hughes, 2000; Walther *et al.*, 2002). Chub, dace, barbel and bleak are omnivorous (Bruslé & Quignard, 2001). These fish species eat invertebrate taxa that were either disappearing, appearing or stable in abundance during the study period. For example, chub and barbel do not preferentially eat invertebrates such as *Chloroperla* or *Protonemura*. Therefore, there was probably no cause and effect trophic relationship between fish and invertebrate community changes at Bugey.

The true effects of climatic change may be confounded by the effects of hydroelectric schemes built upstream of the study segment and by the nuclear power plant. The effects of flow regulation and urbanization on stream ecology has been well documented and includes a slower current, sedimentation, periodic releases and pollution (Bravard, 1987; Petts *et al.*, 1989; Calow & Petts, 1992). In the study segment, no trend was detected for the mean annual discharge variables during the study. Since neither the width nor the depth of the study segment has been modified, no slowing in current has occurred since 1979. As far as the possible sedimentation of the study segment is concerned, visual observations did not indicate any gradual siltation since 1979. With respect to the potential influence of water releases from dams, the temporal changes in communities (represented by site factorial score time series) did not show any triennial cycles (the frequency of the releases on the Upper Rhône River). Similarly, Dolédec *et al.* (1996) has shown that such releases have no clear impact on the invertebrate community, probably because the substrate is cleaned after releases in a few months (Bournaud *et al.*, 1987). Finally, water quality remained good at Bugey during the study (Table 1). Although the study segment was not regulated in terms of the river itself, an overall effect of the hydropower schemes could have influenced the local dynamics of communities. Distinguishing the effects of climatic factors and hydropower schemes on invertebrate data was complicated by the fact that taxa were only identified to the genus level. We observed an increase in both thermophile taxa and lentic taxa. In fact, at the generic level, taxa with warm water preferences always have preferences for lentic and downstream zones (Tachet *et al.*, 2000). This could explain for example the paradoxical increase in eutrophic taxa (e.g. *Potamopyrgus*, *Drendrocoelum*) in an oligotrophic zone (chlorophyll *a* concentration $<10\,\mu g\,L^{-1}$; Table 1). In this way, the change in the invertebrate community observed at Bugey is consistent with the hypothesis of a combined effect of both climate change and hydropower scheme construction. But neither in our study nor in that of Dolédec *et al.* (1996) was the smooth change in the invertebrate community structure disrupted by a sudden switch at the completion of the schemes (1984, 1986). Moreover, the same community changes were observed in both studies under the same climatic influence but at different locations, with different numbers of upstream schemes and different distances between the study segment and upstream schemes. With respect to fish, Persat (1988) studied changes in fish community structure before and after the completion of schemes, in the by-passed section and in the diversion canal. These changes were quite different than those observed here (see Table 7). In particular, barbel, nase and stream bleak are sensitive to stream regulation (Lamouroux *et al.*, 1999; Bruslé & Quignard, 2001), but their abundance increased or remained stable at Bugey.

Another factor that could have influenced changes in the communities was the presence of the nuclear power plant in the study segment. However, the temporal changes of communities in sites downstream and upstream from the nuclear power plant were very similar. The warmed effluent affected only a small area of our study segment and did not locally influence community trends. In addition, the consistency of the observed changes in the invertebrate community at Bugey and 45 km upstream (Dolédec *et al.*, 1996) suggests that the power plant did not play a central role in the temporal changes of the community structure. More generally, studies dealing with the influence of nuclear power plants on freshwater fauna have shown that qualitative differences between warmed and non-warmed sampling sites were small (Kirchmann *et al.*, 1985; Fruget *et al.*, 1999) and essentially due to velocity, substrate, deep or bank morphology differences (Ginot *et al.*, 1996).

To conclude, our results support that shifts in fish and invertebrate community structure towards thermophilic and southern taxa at Bugey were the consequence of global warming. The observed trends were significant, correlated with thermal variables and consistent with the predicted and observed consequences of climate change on many organisms (Walther *et al.*, 2002). However, the possibility that there was a cumulative effect of hydropower scheme construction on invertebrates could not be rejected. Our study is the first assessing the influence of the climate warming on fish and invertebrate communities in large streams. Most studies dealing with the influence of the climate change, including ours, have been conducted on a single site. To deal with the influence of confounding factors, there is nowadays a need for long-term studies on several sites. Identifying which species trait are impacted in comparative studies of multiple sites can improve our understanding of the effects of climate change on a wide range of organisms and environmental conditions. Such studies would be particularly helpful in large streams that have undergone many anthropogenic disturbances during the last century. This implies the production of new monitoring programs focusing on the consequences of global warming (Hughes, 2000), and the analyses of various existing data sets (Blenckner & Hillebrand, 2002). It is essential to take into account confounding factors in studies of the impacts of climate change. On the other hand, ecologists and other scientists working on temporal patterns must keep in mind that air temperature and atmospheric composition have been modified in the last century. Therefore, the possible effects of thermal and atmospheric changes have to be taken into account before making any conclusions on the causes of all kinds of temporal change.

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A user's guide to functional diversity indices

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Abstract. Functional diversity is the diversity of species traits in ecosystems. This concept is increasingly used in ecological research, yet its formal definition and measurements are currently under discussion. As the overall behavior and consistency of functional diversity indices have not been described so far, the novice user risks choosing an inaccurate index or a set of redundant indices to represent functional diversity.

In our study we closely examine functional diversity indices to clarify their accuracy, consistency, and independence. Following current theory, we categorize them into functional richness, evenness, or divergence indices. We considered existing indices as well as new indices developed in this study. The new indices aimed at remedying the weaknesses of currently used indices (e.g., by taking into account intraspecific variability). Using virtual data sets, we test (1) whether indices respond to community changes as expected from their category and (2) whether the indices within each category are consistent and independent of indices from other categories. We also test the accuracy of methods proposed for the use of categorical traits.

Most classical functional richness indices either failed to describe functional richness or were correlated with functional divergence indices. We therefore recommend using the new functional richness indices that consider intraspecific variability and thus empty space in the functional niche space. In contrast, most functional evenness and divergence indices performed well with respect to all proposed tests. For categorical variables, we do not recommend blending discrete and real-valued traits (except for indices based on distance measures) since functional evenness and divergence have no transposable meaning for discrete traits. Nonetheless, species diversity indices can be applied to categorical traits (using trait levels instead of species) in order to describe functional richness and equitability.

Key words: categorical variables; functional divergence; functional evenness; functional richness; morphological traits; species richness.

INTRODUCTION

Biodiversity is commonly expressed through indices based on species richness and species abundances (Whittaker 1972, Lande 1996, Purvis and Hector 2000). Recently, however, studies focused on diversity have begun to incorporate the concept of functional diversity. In contrast to species diversity, functional diversity measures the distribution and the range of what organisms do in communities and ecosystems and thus considers the complementarity and redundancy of co-occurring species (Díaz and Cabido 2001, Petchey and Gaston 2006). Functional diversity is commonly assumed to be a better predictor of ecosystem productivity and vulnerability than species diversity (Tilman et al. 1997, Hulot et al. 2000, Díaz and Cabido 2001, Heemsbergen et al. 2004).

Including species' functions in the measurement of biodiversity is a relatively recent approach. Since 1990, the number of publications based on functional diversity has been steadily increasing (Fig. 1). Although the concept of functional diversity itself is relatively simple to understand, its increasing importance in biodiversity studies has revealed that measuring it is a complex endeavor: while studies focused on species diversity only need to count individuals from different species (i.e., sort them into several categories), functional diversity studies have to describe a multidimensional cloud of points in trait space (i.e., each coordinate corresponds to a measured trait), each point representing an individual or a species. Several methods have recently been proposed to help identify the necessary measures of functional diversity (reviewed in Ricotta 2005, Petchey and Gaston 2006, Podani and Schmera 2007, Villéger et al. 2008). Two main approaches have emerged: on the one hand, functional groups can be defined based on few behavioral/morphological characteristics (e.g., diet affinities, food acquisition methods, preferred habitat) and the observed species are assigned to different functional categories (Bremner et al. 2003, Stevens et al. 2003, Petchey and Gaston 2006). These data can be further processed with conventional species diversity indices (functional group richness, Shannon index, Simpson diversity index, etc.; e.g., Stevens et al. 2003). This approach is suitable for macro-ecological studies since

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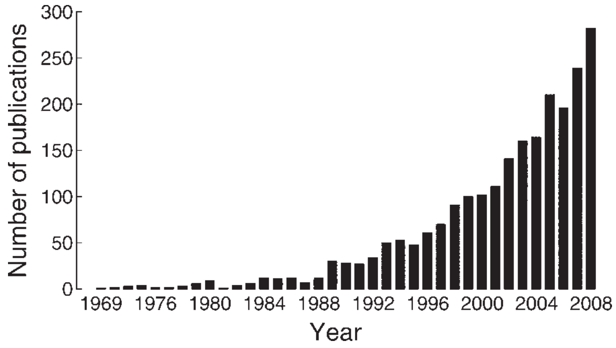

FIG. 1. Number of publications containing the term "functional diversity" in title, abstract, or key words. Source: Scopus ⟨http://www.scopus.com/scopus/search/form.url⟩ to 31 December 2008.

information on species assignment to functional groups is available for a broad range of species and generally easy to obtain. Furthermore, such studies only need a low level of detail in contrasting species traits. On the other hand, functional diversity can be calculated based on specific functional traits measured for each species. This approach promises a finer resolution (Bremner et al. 2003, Petchey and Gaston 2006), but trait values are more difficult to obtain than information on functional group memberships. For instance, it is easier to categorize fish species by their general diet than to obtain measurements on their size, gape width, stomach length, etc. Functional traits can be morphological traits that represent adaptations to different diets or habitats, physiological traits (e.g., temperature tolerance), reproductive traits (e.g., number of eggs and egg diameter), or behavioral traits (e.g., migratory behavior or parental care) (Bremner et al. 2003, Dumay et al. 2004, Lepš et al. 2006). Because most of these measurements are real-valued (i.e., not discrete) and more than one trait is used to describe the different functions, the indices commonly used to measure species diversity cannot be applied (e.g., Simpson diversity index).

To make use of multiple trait measurements, Bremner et al. (2003) compared functional trait compositions between sites using principal components (PCA) or co-inertia analyses. However, this approach is comparative and not based on functional diversity per se and therefore does not give absolute insight into the distribution of traits within a specific site. Alternatively, species diversity indices have now been transposed to functional diversity measurements, and several new indices have been proposed (e.g., Mason et al. 2005, Ricotta 2005, Petchey and Gaston 2006, Villéger et al. 2008). These indices usually describe two broad aspects of functional diversity: (1) how much of the functional niche space is filled by the existing species (functional richness) and (2) how this space is filled (functional evenness, functional divergence/variance).

Using functional diversity indices, however, entails several methodological problems. The first difficulty is the selection and the treatment of the traits, e.g., how many and which traits to use, how to weigh them, and how to combine them (Lepš et al. 2006, Petchey and Gaston 2006). Some solutions to these problems have been discussed and proposed by Lepš et al. (2006). The second set of problems is related to the indices themselves, i.e., do the indices measure exactly what the user wants to describe? Are the chosen indices independent from one another? Will diversity be measured for a single trait only or for a multivariate trait data set? Does the data set contain categorical and continuous variables? It is particularly important that these problems are considered carefully because ecological theories are developed and confirmed based on these results.

Some properties of selected indices were specified by Petchey and Gaston (2006) and Ricotta (2005), but new indices have been published since then (e.g., Cornwell et al. 2006, Podani and Schmera 2007, Villéger et al. 2008), and although the importance of intraspecific specialization and variability is clearly acknowledged (Bolnick et al. 2003), it has rarely been considered in the formalization of functional diversity. Moreover, a direct comparison of the different indices and their correlations with one another is still missing, and the user of functional diversity still faces the problems described here when selecting an index. The aims of this study were therefore: (1) to describe the main properties of the different functional diversity indices; (2) to propose new indices that enhance and supplement existing ones (e.g., accounting for intraspecific variability); (3) to test and compare the accuracy of all these indices in defined scenarios; (4) to measure the correlations among all these indices; (5) to summarize the results of 1–4 in a table to facilitate the selection of an appropriate index for the user.

METHODS

Functional diversity indices

The functional diversity of a community approached through the measurements of traits is usually described by three kinds of indices that can be combined to calculate different facets of functional diversity (Mason et al. 2005, Villéger et al. 2008; examples for application, Mason et al. 2007, 2008): functional richness (FR), functional evenness (FE), and functional divergence (FD). The FR indices generally measure how much niche space is filled, while FE and FD indices describe how this space is filled. Defining functional diversity indices, however, is not a simple task since there is no natural way of describing richness, evenness, or divergence when individuals are not assigned to classes (i.e., species), but rather described by their traits. First, any index should reflect the verbal definition of its properties. Second, FR, FE, and FD indices aim at measuring different aspects of functional diversity and should therefore be uncorrelated (independent) in a random community.

There are nine indices available in the literature to calculate functional diversity on the basis of measured traits, which we describe and test in this study: three FR indices (first described by Petchey and Gaston 2002, Mason et al. 2005, Cornwell et al. 2006), two FE indices (Mouillot et al. 2005, Villéger et al. 2008), and four FD indices (first described by Rao 1982, Mason et al. 2003, Lepš et al. 2006, Villéger et al. 2008). Each index group contains one- and multidimensional indices. Despite their multiplicity, these indices still miss some important points, e.g., FR indices do not consider individual variability. Indeed, individual variability in functional diversity has been approached through the expansion of existing indices for the use of individuals (Lepš et al. 2006, Cianciaruso et al. 2009). However, indices that specifically account for the use of intraspecific variation (using means and intraspecific variability as input, not individual trait values) have only been proposed for two FD indices (Lepš et al. 2006). To fill these gaps, we propose three additional indices (two FR indices and one FD index), which are also described and tested in this study.

Functional richness.—The FR indices measure how much of the niche space is occupied by the species present. They are usually interpreted by ecologists as an indicator for potentially used/unused niche space and thus, e.g., for productivity, buffering against environmental fluctuations, or vulnerability to invasion (Mason et al. 2005). Functional richness is naturally positively correlated with the number of species present (the more species there are, the larger the functional space occupied when species traits are somewhat randomly distributed). However, two communities with the same number of species may have different FR when functional traits of species are more closely clustered in one community than in the other. Functional richness is not weighted by species abundance.

1. *One-dimensional indices*.—Mason et al. (2005) suggested using the functional range (FR_R) as a measure of FR (Table 1: index number [IN] 1.1). Functional range is the relative range of a trait that is filled by a community at a site compared to the range of the trait for all communities together. In this way FR_R is restricted between 0 and 1 and becomes comparable for differently scaled traits. Please note, however, that the value for an individual site is not absolute but might change when the overall range changes (e.g., by the addition of a new site with species that differ in trait values). The community's range is calculated based on species' mean trait values by simply subtracting the lowest from the highest mean trait value at a site. The absolute range is calculated accordingly for all sites together. If more than one trait is used, the mean community range is then the mean of all traits.

Neither individual variability nor gaps in trait space are accounted for by FR_R. This is an issue when individual variability between sites differs and when functionally exceptional species are added to the community or when species with trait values within the range are missing. We therefore propose a new one-dimensional functional richness index (FR_{Is}) (Table 1: IN 1.2). The FR_{Is} is based on species' trait variability: instead of the community's trait range, FR_{Is} is based on the union of the species' trait ranges (and thus considers individual variability). It is calculated as the union of species' trait ranges at one site, relative to the union of species' trait ranges for all sites together (see FR_R). When calculating FR_{Is} the species' trait range can be calculated using two species/trait matrices as input tables, containing the species' minimum and maximum trait values, respectively. With this method, however, the range depends on the number of individuals measured. We therefore suggest using more conservative values, e.g., the 10th and 90th percentiles, confidence intervals, or the mean species trait value ± SD. For further details see Appendix A. A script in R for this index is *available online*.[2]

2. *Multidimensional indices*.—The multivariate counterpart of FR_R is the functional volume FR_V (Cornwell et al. 2006; Table 1: IN 1.3). The FR_V calculates the volume of trait space with the convex hull volume, which represents the smallest convex hull that encloses all species. With a complex algorithm, the most extreme points (vertices) can be determined and the volume encompassed by these vertices is calculated (TraitHull programmed in Python [Cornwell et al. 2006], *available online*;[3] and for R [Villéger et al. 2008], *available online*).[4] To calculate this index, the number of species must always exceed the number of traits.

A second existing multidimensional FR index is the dendrogram-based index FR_D (Petchey and Gaston 2002) (Table 1: IN 1.4). This index measures the extent of species complementarity based on a trait distance matrix (Petchey and Gaston 2002), a property equivalent to FR (Mouillot et al. 2005). A dendrogram is computed by hierarchical clustering; the functional richness is then the sum of the branch lengths of species present. There has been discussion in the literature on which distance measure and cluster method is best at calculating this index (Podani and Schmera 2006, 2007, Petchey and Gaston 2007, Mouchet et al. 2008). Here, we used the method developed by Mouchet et al. (2008; R program *available online*, see footnote 4) that computes dendrograms based on two distance matrices and seven clustering methods, which belong to the family of hierarchical agglomerative classifications and then selects the combination (including a consensus tree) that best represents the species distribution in functional trait space.

As for FR_R, FR_V does not consider gaps in functional trait space. We therefore developed a multivariate

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[2] ⟨http://www.cemagref.fr/le-cemagref/lorganisation/les-centres/aix-en-provence/ur-hyax/scripts/ecology_schleuter2010/⟩

[3] ⟨http://www.pricklysoft.org/software/traithull.html⟩

[4] ⟨http://www.ecolag.univ-montp2.fr/software⟩

TABLE 1. Index number (IN), index abbreviation (IA), name, formula, and references for the different functional diversity indices.

IN	IA	Name	Formula	Source
1.1	FR_R	functional range	$\dfrac{\max\limits_{s\in S_c}(X_{ts}) - \min\limits_{s\in S_c}(X_{ts})}{\max\limits_{s\in \cup S_c}(X_{ts}) - \min\limits_{s\in \cup S_c}(X_{ts})}$	Mason et al. (2005)
1.2	FR_{Is}	functional richness (one-dimensional)	$\dfrac{\int \max\limits_{s\in S_c}[\mathbf{1}_{st}(x)]\, dx}{\int \max\limits_{s\in \cup S_c}[\mathbf{1}_{st}(x)]\, dx}$ where $\mathbf{1}_{st}(x)$ is 1 if x is between min and max, else it is 0 for categorical variables, L_{tc}/L_t	this study
1.3	FR_V	functional volume	the volume inside the minimum convex hull that encloses all species in functional space	Cornwell et al. (2006)
1.4	FR_D	functional dendrogram	distance matrices: Euclidean, Gower; clustering methods: single linkage, complete linkage, UPGMA, WPGMA, UPGMC, WPGMC, Ward's method	Petchey and Gaston (2002), modified by Mouchet et al. (2008)
1.5	FR_{Im}	functional richness (multidimensional)	$\int \max\limits_{s\in S_c}\left(f_s(\mathbf{Z})\right) d\mathbf{Z}$ where $f_s(\mathbf{Z}) = \exp[-0.5(\mathbf{Z} - \mathbf{X}_s)^\top\, \mathbf{\Sigma}_s^{-1}(\mathbf{Z} - \mathbf{X}_s)]$	this study
2.1	FE_s	functional evenness (one-dimensional)	$\sum\limits_{s=1}^{\lvert S_c\rvert-1} \min\left[\dfrac{\lvert X_{ts+1} - X_{ts}\rvert/(A_{ts+1} + A_{ts})}{\sum\limits_{s'=1}^{\lvert S_c\rvert-1} \lvert X_{ts'+1} - X_{ts'}\rvert/(A_{ts'+1} + A_{ts'})}, \dfrac{1}{\lvert S_c\rvert - 1}\right]$ where species subscripts, s, are ranked by ascending order of trait value, t; for categorical variables: $\sum\limits_{l=1}^{L_t} \min\left[\dfrac{A_l}{A}, \dfrac{1}{L_t}\right]$	Mouillot et al. (2005)
2.2	FE_m	functional evenness (multidimensional)	$\dfrac{\sum\limits_{e\in E} \min\left[\dfrac{\mathrm{dist}(e)/(A_e/A)}{\sum\limits_{e'\in E}\mathrm{dist}(e')/(A_{e'}/A)}, \dfrac{1}{\lvert S_c\rvert - 1}\right] - \dfrac{1}{\lvert S_c\rvert - 1}}{1 - \dfrac{1}{\lvert S_c\rvert - 1}}$	Villéger et al. (2008)
3.1	FD_{var}	functional logarithmic variance	$\dfrac{2}{\pi}\arctan\left[5\left(\dfrac{1}{T}\sum\limits_{t=1}^{T}\sum\limits_{s\in S_c}\dfrac{A_s}{A}\left(\ln X_{ts} - \overline{\ln X_{ts}}\right)^2\right)\right]$ where $\overline{\ln X_{ts}}$ is the mean of $\ln X_{ts}$ over all species present	Mason et al. (2003)
3.2	FD_σ	functional variance (FD_{var} modified)	$\sum\limits_{s\in S_c}\dfrac{A_s}{A}\left(X_{ts} - \overline{X_{ts}}\right)^2$	Lepš et al. (2006)
3.3	FD_{cat}	functional unalikeability	$FD_{cat} = 1 - \sum\limits_{l=1}^{L_t}\left(\dfrac{A_l}{A}\right)^2$	Kader and Perry (2007)
3.4	FD_s	functional divergence (one-dimensional)	$\dfrac{Q_1(Y_t) - Q_3(Y_t)}{\max_{s\in S_c}(X_{ts}) - \min_{s\in S_c}(X_{ts})}$ where Y_t is a dummy variable that takes values X_{ts} with frequency A_s	this study
3.5	FD_Q	Rao's quadratic entropy	$\sum\limits_{s\in S_c}\sum\limits_{s'\in S_c}\dfrac{A_s A_{s'}}{A^2}\,\mathrm{dist}(s, s')$	Rao (1982), Champely and Chessel (2002)

TABLE 1. Continued.

IN	IA	Name	Formula	Source
3.6	FD_m	functional divergence (multidimensional)	$\frac{\Delta d + \overline{dG}}{\Delta \lvert d \rvert + \overline{dG}}$ where $\Delta d = \sum_{s \in S_c} \frac{A_s}{A} (dG_s - \overline{dG})$, $\Delta \lvert d \rvert = \Sigma_{s \in S_c} (A_s/A) \lvert dG_s - \overline{dG} \rvert$, dG_s is the distance between species s and the gravity center of the convex hull (coordinates $G_t = (1/\lvert V \rvert) \Sigma_{s \in V} X_{ts}$) and $\overline{dG}$ is the mean value of dG_s over all present species	Villéger et al. (2008)

Notes: IN starting with 1, 2, or 3 and IA starting with FR, FE, or FD refer to functional richness, functional evenness, and functional divergence index groups, respectively. Subscripts s and m of index acronyms refer to single (one-dimensional) or multidimensional indices. Abbreviations: i, s, c, l, and t are individual, species, community, level of trait (for categorical variables), and trait subscripts, respectively; A_s, abundance of species s; A_{ts}, abundance of species s when species are sorted following trait t ascending ranking; A, total abundance of all individuals; A_l, abundance of trait level l; I_s, set of individuals of species s; L_{tc}, number of levels of categorical trait t covered by community c; L_t, total number of levels of trait t; L, total number of cross-trait levels; S_c, set of species present in community c ($\lvert S_c \rvert$, number of species present in community c); T, number of traits studied; V_c, set of vertex species from the convex hull in community c ($\lvert V_c \rvert$, the corresponding number of vertices); x_{tsi}, value of trait t in individual i from species s; X_{ts}, mean value of trait t in species s; $\mathbf{X}_s$, mean value of all traits in species s arranged in a vector; $\mathbf{Z}$, sample vector of all traits, used for the purpose of computing integrals over trait space; $\mathbf{\Sigma}$, variance/covariance matrix of traits; dist(s, s'), distance between species pairs based on mean trait values (for continuous variables, Euclidean distance is used; for discrete variables, the Gower distance is used); E, set of edges connecting species pairs in the minimum spanning tree; e, subscript of an edge; dist(e), distance between endpoint species of edge e; A_e, sum of the abundances of the endpoint species of edge e; Q_1, lower quartile; Q_3, upper quartile; UPGMA, unweighted pair group method using arithmetic averages; WPGMA, weighted pair group method using arithmetic averages; UPGMC, unweighted pair group centroid method; WPGMC, weighted pair group centroid method.

counterpart to FR_{Is}: FR_{Im} (Table 1: IN 1.5). The FR_{Im} index is specifically designed to account for individual variability and for gaps in the multidimensional functional trait space. The idea is to compute an equivalent "range union" (as for FR_{Is}) across species present in a community. To do so, each species s is assigned a function on trait space (f_s) that indicates whether a particular point in trait space is (or is not) occupied by species s (that is, whether species s trait values encompass this point). Here, we chose a simple form for f_s functions that uses information on both mean trait values and trait variance–covariance matrices for each species. FR_{Im} is then obtained as the integral of the maximum of all f_s functions (integrated over studied trait space). For further details see Appendix B. A script in Mathematica to calculate this index is *available online* (see footnote 2).

Functional evenness.—Functional evenness indices measure whether mean species traits are distributed regularly within the occupied trait space, i.e., with equal distances between nearest neighbors and equal abundances (a high FE index usually means a very regular distribution; a low FE index indicates the existence of separate clouds of species and/or abundances). Functional evenness indices are generally used to indicate under- or overutilization of resources and thus again productivity, reliability, and vulnerability to invasion (Mason et al. 2005). This index group includes species' abundances in its calculation.

1. *One-dimensional index.*—Based on Bulla's index for the measurement of species' evenness (Bulla 1994), the FE_s index (Mouillot et al. 2005) measures for each trait separately how evenly the trait values of all species present are distributed (Table 1: IN 2.1). First, the absolute, relative distances between the mean species trait values, in order of increasing values, is calculated and weighted by the sum of the relative species abundances. Second, if the distance is greater than $1/(S-1)$ (with S representing species richness), it is replaced by $1/(S-1)$, the distance, which is obtained for an optimal even distribution (Mouillot et al. 2005). FE_s is 1 if the distance between nearest neighbor species is exactly $1/(S-1)$ and all species have the same abundances. The more a community differs from the optimal distribution in terms of abundance and trait difference, the lower the FE_s. When trait space is multidimensional, the community's evenness is the average of the FE_s calculated for each trait.

2. *Multidimensional index.*—The multivariate equivalent of FE_s is FE_m (Villéger et al. 2008; Table 1: IN 2.2). Instead of using the absolute distances between the species' trait values for each trait separately, this index uses the abundance-weighted distances between all species pairs to calculate first the minimum spanning tree (MST) that links all the species in a multidimensional trait space. The index then measures the regularity of the MST branch lengths, according to FE_s (i.e., comparison with the optimal branch length distribution).

Functional divergence.—The FD indices, finally, measure the variance of the species' functions and the position of their clusters in trait space (a high FD is caused by the clustering of species and/or abundances at the edges of the traits' space). The FD indices find application for indicating the degree of resource differentiation and thus competition (Mason et al. 2005), but they can also indicate a predominance of extreme species. As FE, FD includes species' abundances in its calculation.

1. *One-dimensional divergence*.—Functional divergence can be calculated as the abundance-weighted functional variance using mean species values (Mason et al. 2003). Mason et al. (2003) suggested log transforming the trait values before calculating the variance (FD_{var}; Table 1: IN 3.1) and using species relative abundances as abundance weight. If more than one trait is used, FD_{var} is calculated for each trait separately and then averaged over traits. They recommend then using an arctangent transformation in order to restrict the index between 0 and 1. If species and/or abundances are clustered around the mean trait value, FD_{var} is low; if they are clustered at the edges of the community, FD_{var} is high.

The FD_{var} index is not applicable if the data set contains 0 values. To account for this problem, we propose using simply the abundance-weighted variance without log transformation (FD_{σ}; Table 1: IN 3.2) (Lepš et al. 2006). Since the variance is scale-dependent, traits should be standardized (e.g., centering and scaling by standard deviation) in case the trait space is multidimensional and the different traits have different scales. Another possibility is to use the coefficients of variation instead (i.e., standard deviations divided by means). Lepš et al. (2006) suggest including intraspecific variability in this index by adding the abundance-weighted intraspecific variance to the interspecific variance.

Since FD can also be understood as the relative range of the trait clustering, we propose a new one-dimensional index of functional divergence, FD_s. The FD_s index calculates the range of the zth percentiles (e.g., 25th percentile, Q_1, and 75th percentile, Q_3) relative to the overall range of each trait (Table 1: IN 3.4). The species abundance is accounted for by replicating the mean trait value of a species i times the species' abundance. This approach is conceptually different, since it is based on the relative span of the trait cluster and not on the variance. Low FD_s values indicate that half of the individuals in the community occupy a very confined functional space, independent of the position of that cluster along the trait axis; high values instead suggest that the functional space is more densely occupied at both its edges. For further details see Appendix C. A script in R to calculate this index is *available online* (see footnote 2).

2. *Multivariate divergence*.—The most common multivariate index of FD is Rao's quadratic entropy FD_Q (Rao 1982, Champely and Chessel 2002, Ricotta 2005; Table 1: IN 3.5). This index calculates the abundance-weighted variance of the dissimilarities between all species pairs. It is based on the Simpson diversity index (Simpson 1949) for the calculation of species diversity and weighs the trait-based distances between pairs of species [dist(s, s')] by the product of their relative abundances. Lepš et al. (2006) suggest calculating the species' pairwise dissimilarities through the sum of their overlaps for each trait. In this way, individual variability is included in this index and categorical and continuous variables can be mixed. Rao's quadratic entropy can also be calculated for single traits, the average of which leads to the same result as the multivariate approach, if the distance measure used is unchanged by averaging (e.g., Euclidean distance; Lepš et al. 2006). Since Walker et al.'s (1999) functional attribute diversity differs from Rao's quadratic entropy only in the way the distance matrix is calculated, it was not tested as a separate index in this study.

Villéger et al. (2008) proposed a new multivariate measure for functional divergence (FD_m). Based on the vertex species V of the convex hull (see FR_v), this index first determines the center of gravity G of the convex hull (Table 1: IN 3.6). It then computes the abundance-weighted deviances Δd of each species present from the species' mean distance to the center of gravity. In a last step, the index is restricted between 0 and 1. The FD_m index is low when species/abundances are close to the center of gravity and high when species and/or abundances are higher at the vertices of the convex hull. Since this index is based on the calculation of the convex hull, the same assumptions as for the calculation of FR_V must be met.

Transformation of trait data

Transformations of the original data should be avoided whenever possible. Nonetheless, one should always consider that some indices are not applicable to differently scaled traits (e.g., FD_{σ}). If at least one trait is scaled differently, all traits should be transformed; otherwise, this trait might have too little or too much weight in the index calculation. One possibility is to transform a trait that differs in several orders of magnitudes from the others using an algebraic function such as the logarithm (e.g., number of seeds in plants or number of eggs in fishes). Another possibility is to standardize the trait values with respect to the others, e.g., centering and scaling the trait by its standard deviation. This transformation has to be done for the overall data set and not on potential subsamples (e.g., at different study sites). The subsamples should then be drawn from the transformed trait matrix. For indices that calculate relative index values for each trait separately (e.g., FR_R, FR_{Is}, FD_s, etc.) and for one-dimensional indices, which range between 0 and 1 (e.g., FE_s, FD_{var}), the data need not to be transformed. Different transformation methods and their pros and cons are summarized in Lepš et al. (2006).

Accuracy of the indices

The first criterion for the quality of an index (accuracy) is the match between the way it actually behaves and the verbal definition of its properties (FR, FE, FD). This was tested in our study using an artificial data set (up to 25 species, two traits). We manipulated species composition and abundances in five tests (T1–

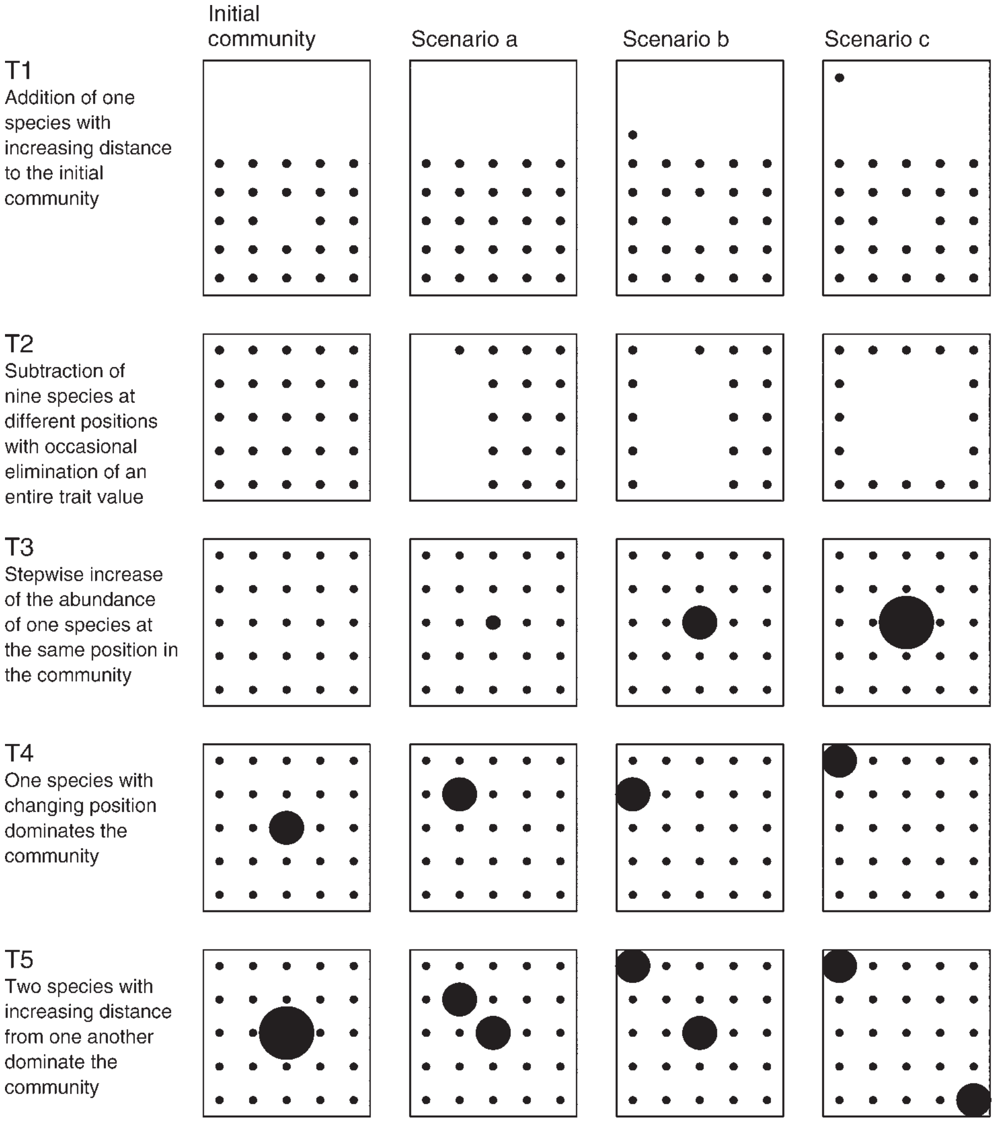

FIG. 2. Illustration of the artificial scenarios used to test the behavior of the different indices. There are five different tests (T1–T5) whereby an initial community is modified three times in different ways (scenarios a–c). Each square represents one community with two trait axes; one dot within a square represents a species. The size of the dot indicates the abundance of the species (small dots reflect one individual, and with increasing size, 2, 25, and 250 individuals).

T5; Fig. 2) to compare the observed and expected changes of index values. Trait values were integer numbers between 1 and 5 for trait 1 and between 1 and 8 for trait 2. We assumed a standard deviation of 0.2 and a maximum deviation from the mean equal to 0.4 for all species and traits. In all tests, the initial species richness was 25, apart from T1, where it was 24. In T1 and T2 and, if not stated differently, in T3–T5, species abundance was set to 1 for all species.

The main aim of T1 and T2 was to test the effect of empty space in the trait space on the behavior of functional richness indices. In T1, we tested the effect of adding one species, with varying distance between the added species and the existing community (Fig. 2, T1). In T2, we removed nine species from the initial community. We removed these species so that either one trait value was eliminated completely, once at the outer edge of the community and once within the trait space (Fig. 2, T2 scenarios a and b), or the removed species were chosen in the middle of the trait space so that each trait value was present at least once (Fig. 2, T2 scenario c).

In T3–T5, the influence of varying species abundances on functional evenness and divergence was tested. In T3, we increased the abundance of one species stepwise from 1 (initial community) to 2, 25, and 250 individuals to test for the influence of the change in the abundance of one species (Fig. 2, T3). In T4, the influence of the position in trait space of a dominating species (25 individuals) was tested (Fig. 2, T4). In T5, we increased the distance between two dominating species (25 individuals each), starting from an initial community in which the abundance of only one species was set to 50 individuals to simulate two identical species.

All indices were calculated for the initial community and for the different scenarios. The change in index values of the scenarios compared to the initial community was evaluated semi-quantitatively. We used the symbols "–," "+," and "=" to indicate whether the calculated value was higher, lower, or equal, respectively, compared to the initial community. The symbols were replicated to give a raw quantitative view of the changes (++, +++ with +<++<+++; – –, – – – with – – –<– –<–).

Indices correlation

The correlations among the different indices were tested using random communities. We computed 1000 randomizations for 14 different species richness levels (as multiples of five from five to 70) and three different numbers of traits (three, five, 10). The total number of treatments was 42 (14 × 3). Trait values were generated using uniform distributions between 0 and 1. The intraspecific standard deviation was considered to be 10% of the randomized trait value. Species abundances were randomized with a uniform distribution between 1 and 100 for each randomized trait matrix.

All indices described were calculated for each of the 42 000 random communities, apart from $\mathrm{FR}_{\mathrm{Im}}$, which was only calculated for the three-trait simulation. Actually, its computation time was very long on the computers used in this study, and it increased exponentially with the number of traits used.

To provide an overview of the relationships between different community indices, we used ordination techniques (e.g., PCA), which are known to perform well when summarizing complex data (Lebart et al. 2000). Since we had to compare a set of 42 matrices of 1000 rows (randomized communities) and 12 columns (indices), a single matrix-based approach was not appropriate. As a consequence, for each of the three trait levels, we used a multi-table ordination technique (e.g., Escofier and Pages 1994, Lavit et al. 1994, Chessel and Hanafi 1996) to assess the common structure of the 14 species richness-specific matrices. We performed a STATIS analysis (Lavit et al. 1994) to summarize the link between the different indices while removing the potential effect of species richness. This method is based on the optimization of the average ordination of the species richness-specific ordinations. The first step of STATIS consisted of calculating a matrix of scalar products between indices for each of the 14 levels of species richness. In a second step, the 14 rows × 14 columns matrix of the pairwise vectorial correlation coefficients (RV coefficients) between the scalar products' matrices was computed. The RV coefficient ranges from 0 to 1 and evaluates the extent to which two matrices share a common structure (Robert and Escoufier 1976). Then the matrix of RV values was diagonalized and the 14 coefficients of the first eigenvector were used to weight the 14 matrices of the scalar product between indices. A mean table of maximum inertia (called the reference structure) was subsequently computed as the weighted sum of the matrices of the scalar product between indices. By weighting the sum, greater importance was given to tables with similar structures, whereas lesser importance was given to the other tables. Finally, a PCA was performed on the reference structure. It provided the graphical representation of the common structure of the indices derived from the 14 species richness-specific tables.

Two groups of statistics synthesized the relevance and the efficiency of STATIS. The first statistics were the RV coefficients between two species richness-specific tables. The second statistical procedure used was the squared cosines ($\cos^2$) of the angles between the first axis scores of separate PCAs performed on each species richness-specific table and the first axis scores of the reference structure. This evaluates the match between the species richness-specific tables and the reference structure by using synthetic auxiliary variables.

Categorical variables

All the indices proposed so far (except those based on a distance matrix) cannot be computed for categorical variables. To address this problem, it is recommended in the literature to transform the data set from categorical to continuous variables via distance matrices and ordination methods, which allow a mix of continuous and categorical variables as inputs. Villéger et al. (2008) proposed calculating the Gower distance and then computing a principal coordinate analysis (PCoA). The trait values thus transformed (principal coordinate axis) can then be used to calculate the different functional diversity indices. Next to using the Gower distance with a PCoA, Hill and Smith's method can also be used (Hill and Smith 1976). This ordination technique combines discrete and continuous variables in a single analysis. If all variables are discrete, it is reduced to a simple correspondence analysis.

Although it is not really correct to transform categorical traits to continuous ones for the use of indices designed for real-valued variables, we consider these methods because they are commonly applied. Since continuous trait-based indices cannot be calculated for categorical traits it is impossible to assess the true effect of the transformation on the outputs (i.e., to compare the outputs for transformed and untransformed trait values). As a consequence, we only pseudo-tested the effects of these transformations using continuous traits as input. In this way, the normally calculated indices can serve as a reference value. As described above, we computed 1000 randomizations for six different species richness levels (in multiples of 10 from 10 to 60) with three traits. We then calculated all indices (1) with the original data set, (2) with the data set transformed via the Gower distance and PCoA (Podani

TABLE 2. Results for the five index tests, T1–T5 (as illustrated in Fig. 2): expected changes for the index categories (boldface) and observed changes of index values for the different scenarios compared to the initial community and in relation to one another.

	T1			T2			T3			T4			T5		
Index	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Richness	**+**	**+**	**+**	**−**	**−**	**−**	**=**	**=**	**=**	**=**	**=**	**=**	**=**	**=**	**=**
FR$_{\mathrm{R}}$	=	+	++	−	=	=	=	=	=	=	=	=	=	=	=
FR$_{\mathrm{Is}}$	=	+	+	−	−	=	=	=	=	=	=	=	=	=	=
FR$_{\mathrm{V}}$	=	+	++	−	=	=	=	=	=	=	=	=	=	=	=
FR$_{\mathrm{D}}$	++	+	−	−	−−	−−−	=	=	=	=	=	=	=	=	=
FR$_{\mathrm{Im}}$	+	+	+	−	−	−	=	=	=	=	=	=	=	=	=
Evenness	**+**	**−**	**−−**	**−**	**−−−**	**−−**	**−**	**−−**	**−−−**	**=**	**+**	**++**	**−−−**	**−−**	**−**
FE$_{\mathrm{s}}$	=	+	+	+	+	++	=	=	=	=	−	−−	=	−	−
FE$_{\mathrm{m}}$	=	−	−−	−	−−	=	−	−−	−−−	=	=	+	−−	−	−
Divergence	**−**	**+**	**++**	**−**	**+**	**++**	**−**	**−−**	**−−−**	**+**	**++**	**+++**	**+**	**++**	**+++**
FD$_{\mathrm{var}}$	−	+	++	−	+	++	−	−−	−−−	+	++	+++	+	++	+++
FD$_{\sigma}$	−	+	++	−	+	++	−	−−	−−−	+	++	+++	+	++	+++
FD$_{\mathrm{s}}$	=	−	−−	+	++	+++	=	−	−	+	++	+++	+	++	+++
FD$_{\mathrm{Q}}$	−	−−	−−−	−	+	++	−	−−	−−−	+	++	+++	+	++	+++
FD$_{\mathrm{m}}$	−	−−	−−	−	+	++	−	−−	−−−	+	++	+++	+	++	+++

Notes: Symbols are: −, lower than initial community; +, higher than initial community; =, no change. The number of + and − signs indicates the increase compared to the other scenarios. See Table 1 for explanations of indices.

and Schmera 2006, Villéger et al. 2008), and (3) with the data set transformed using Hill and Smith's method (Hill and Smith 1976). The common structure of the three calculation methods was then assessed for each species richness level separately, using the RV values (i.e., the correlation coefficient between two tables) obtained from the method-specific tables.

All calculations and tests were carried out using the program R (R Development Core Team 2008). The STATIS analysis and the transformation via the Hill and Smith method and PCoA were computed using the ade4 package (Chessel et al. 2004, Dray et al. 2007).

RESULTS

Accuracy of the indices

Testing whether the indices behave according to the properties of the index group to which they have been assigned (FR, FE, FD) reveals that, among the FR indices, only two of them (FR$_{\mathrm{Is}}$ and FR$_{\mathrm{Im}}$) accurately reflect the expected changes in functional richness (see Table 2: T1 and T2), because these indices consider empty space in the trait distribution of a community. However, FR$_{\mathrm{Is}}$ is only applicable to one-dimensional situations, while FR$_{\mathrm{Im}}$ well reflects functional richness in a multidimensional space. The results from scenario T1 c and scenarios T2 a and b show that when one trait value is missing in the entire data set, both indices decrease. However, in a multidimensional space, when none of the trait values of the removed species are unique, but their combination is, FR$_{\mathrm{Im}}$ is the only index that reflects these gaps properly (Fig. 2, Table 2: T1 scenario a, T2 scenario c). In contrast, indices such as functional range or volume (FR$_{\mathrm{R}}$, FR$_{\mathrm{V}}$) only reflect a decrease in functional richness when species are removed at the edge of the community. The dendrogram-based index for functional richness, FR$_{\mathrm{D}}$, is furthest from the expected results for functional richness.

Among the two FE indices, the multivariate index FE$_{\mathrm{m}}$ behaves more adequately than its one-dimensional counterpart FE$_{\mathrm{s}}$ and matches the expected results quite well. Thus, FE$_{\mathrm{m}}$ can be considered an appropriate index (Table 2). However, this index fails to respond adequately in some cases, for example when species in the center of the community are added or subtracted (T1 scenario a and T2 scenario c). Species traits are then not evenly distributed over the entire functional space, but concentrated at the edges of the community, and thus the evenness is theoretically lower than in the initial community. Yet FE$_{\mathrm{m}}$ remains equal because the distribution of the branch lengths of the minimum spanning tree (the distance to the nearest neighbors) does not change. The one-dimensional index FE$_{\mathrm{s}}$ does not represent the distribution of species in a multidimensional trait space, but it reflects the evenness of the distribution for a single trait well (results not shown) and can be used if only one trait is considered.

All FD indices adequately match expectations. Even one-dimensional indices, averaged over all traits, accurately reflect changes in a multidimensional space (Table 2). Only in T1 do FD indices react differently: as expected, FD$_{\sigma}$ and FD$_{\mathrm{var}}$ increase when a species outside of the initial community is added, whereas FD$_{\mathrm{Q}}$, FD$_{\mathrm{m}}$, and FD$_{\mathrm{s}}$ decrease.

Indices correlation

Species richness has a clear effect on the different indices, either in terms of variance or mean values (Fig. 3). Nevertheless, for all trait levels, there is a high similarity between the species richness-specific tables

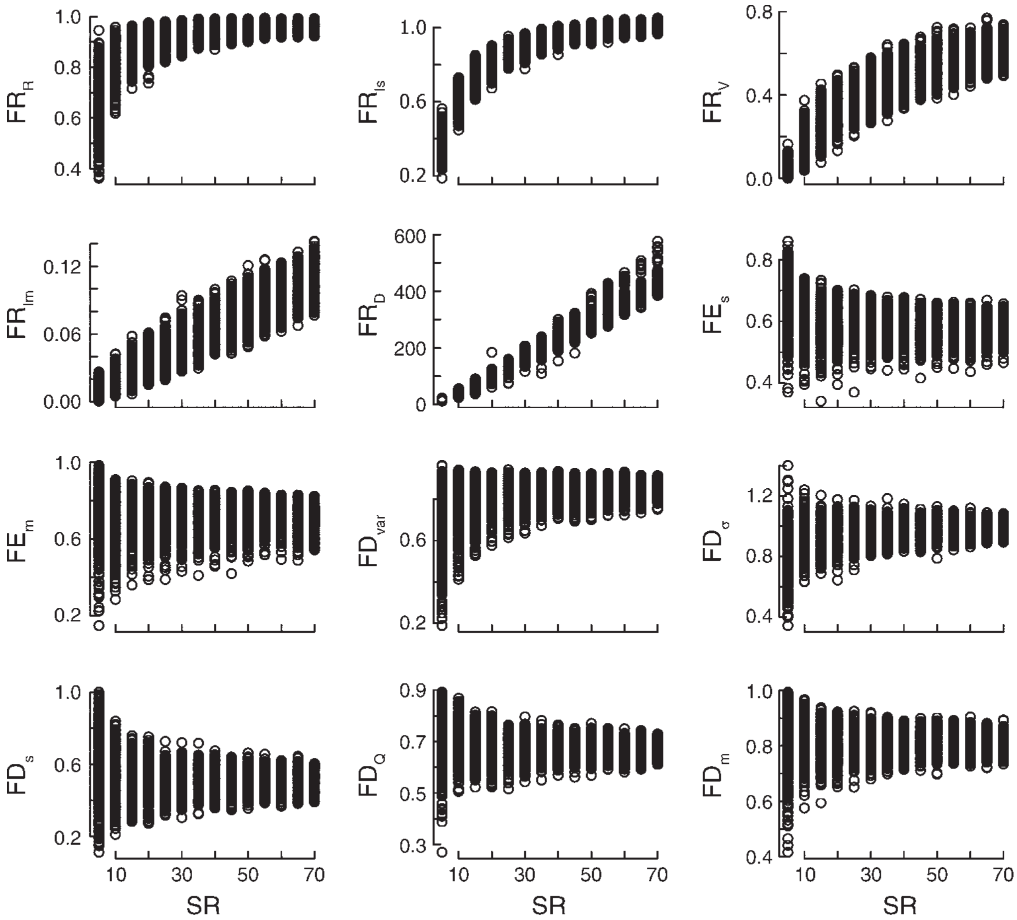

Fig. 3. Relationship of each of the 12 functional diversity indices with species richness (SR; 14 levels from 5 to 70), here for a data set with three different species traits. There are 1000 index values for each species richness level based on randomized trait and abundance data. Trait values and species abundances were generated using uniform distributions between 0 and 1 and between 1 and 100, respectively. See Table 1 for explanations of functional diversity indices.

(RV ranging from 0.885 to 0.996, from 0.945 to 0.998, and from 0.962 to 0.998 for three, five, and 10 traits, respectively). In addition, the $\cos^2$ confirms that the different reference structures reflect the structures of the individual species richness-specific tables ($\cos^2$ ranging from 0.905 to 0.944, from 0.920 to 0.940, and from 0.924 to 0.941 for three, five, and 10 traits, respectively). The lowest $\cos^2$ values are systematically found for the lowest levels of species richness (five, followed by 10) because of the higher variance of most indices at these species richness levels (see Fig. 3).

The reference structures over the different species richness levels are very similar for all trait levels (three, five, or 10; Fig. 4), confirming that there are several independent index groups that describe functional diversity. However, instead of the expected three axes corresponding to FR, FE, and FD indices, five axes are needed to explain the majority of the variance (74%) when FR_{Im} is included (three traits) and four axes without this index (77% and 78% for five and 10 traits, respectively).

The first axis is mainly correlated to the FD indices (FD_{σ}, FD_s, FD_Q, and FD_m; Table 3, Fig. 4). The fifth FD index (FD_{var}) is also correlated to the first axis, but with the second axis as well and it is overall more highly correlated to the FR indices FR_R and FR_V than to the other FD indices. Next to FD_{var} the second axis is correlated mainly to some of the FR indices (FR_R, FR_V, and partly FR_{Is}). The third axis represent functional evenness (FE_s and FE_m) and the remaining two FR indices (FR_{Im} and FR_D) are best represented by the fourth and fifth axes, respectively (Table 3, Fig. 4).

Note that FR_V is not fully independent of FD_Q and FD_s (r ranging from 0.09 to 0.59 for FD_s and from 0.21 to 0.71 for FD_Q depending on the species richness level and the number of traits), despite distance in the F1 × F2 factorial plane of the STATIS analysis. In addition, the correlation between FR_{Is} and FR_{Im} decreases with increasing species richness (from $r = 0.61$ in a community with five species to $r = 0.00$ in a community with 25 species).

Categorical variables

One- and multidimensional indices react differently to the transformation from discrete to continuous variables. Therefore, correlations between the method-

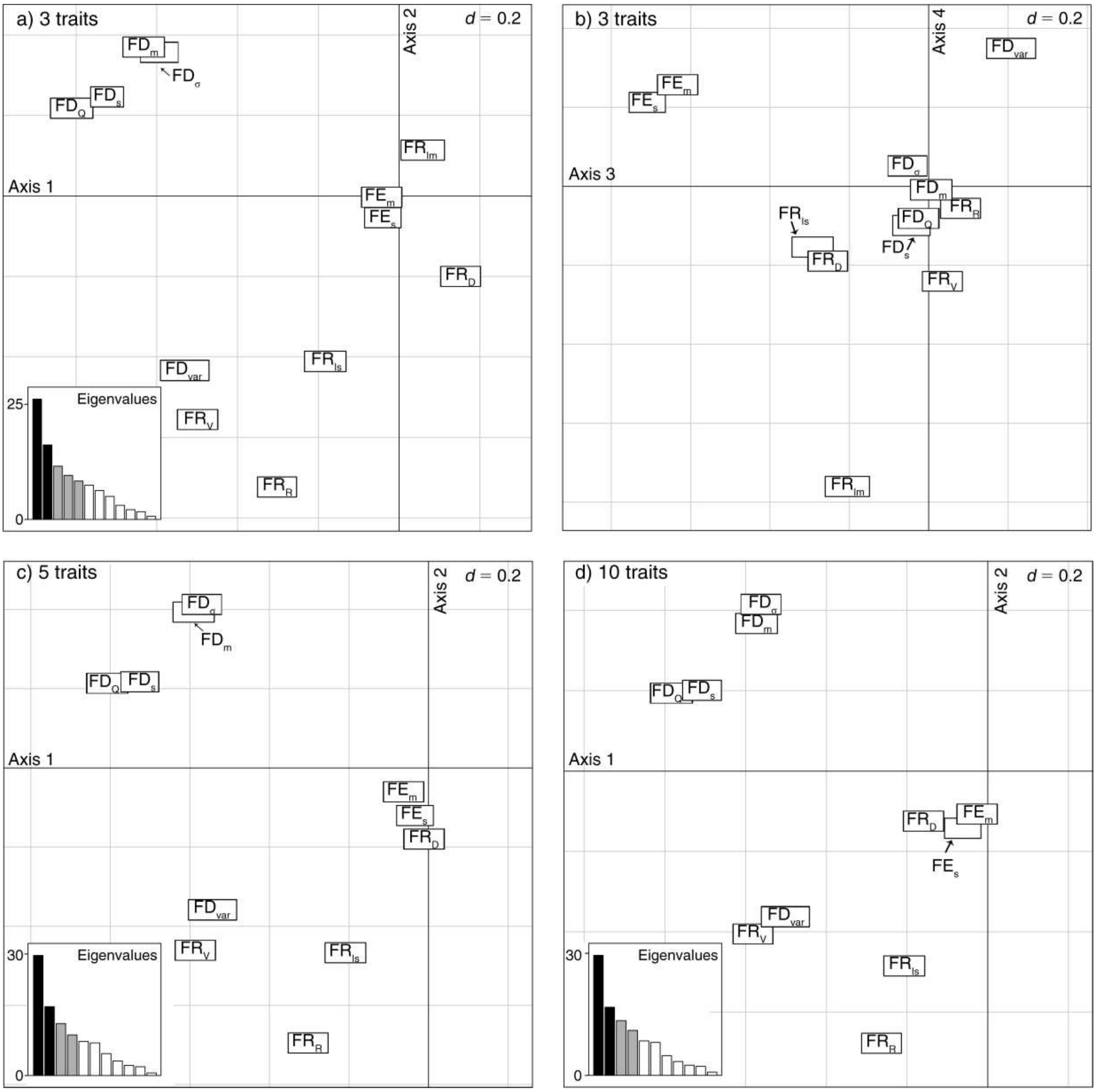

FIG. 4. Reference structures over the different species richness-specific ordinations gained from the STATIS analysis (Lavit et al. 1994). (a) Reference for three traits, first and second axes; axis 1 mainly correlates with FD indices, while axis 2 with FR indices. (b) Reference for three traits, third and fourth axes; the third axis mainly correlates with the two FE indices, axis 4 represents FR_{Im}. (c) Reference for five traits, first and second axes; axis 1 mainly correlates with FD indices, while axis 2 correlates mainly with FR indices. (d) Reference for 10 traits, first and second axes; axis 1 mainly correlates with FD indices, while axis 2 with FR indices. The insets display the eigenvalues (here expressed as percentage of contribution for the explanation of the variance, *y*-axis) of each axis (ordered from 1 to 12, *x*-axis). Shading of the bars refers to the importance of each axis, whereby white bars can be considered as statistical noise. The *d* values give the scaling of the grid. See Table 1 for explanations of functional diversity indices.

specific tables (RV values) have been computed separately for these two index groups. For multidimensional indices, we have found a high similarity between the index tables calculated with the raw data and the differently treated data sets. The transformation via the Hill and Smith method performs slightly better (RV range dependent on species richness between 0.897 and 0.956 for Gower distance and PCoA transformed data and between 0.997 and 0.999 for data transformed with the Hill and Smith method). That the results were worse for the transformation via the Gower distance and PCoA is mainly imputable to the calculation of FR_V (r with the index calculated with untransformed values ranged from 0.13 to 0.60, depending on species richness) and FD_Q ($r \sim 0.5$).

For the one-dimensional indices, neither of the transformation methods performs well (RV ranging between 0.605 and 0.894 for the transformation via

TABLE 3. Axis coordinates of the different functional diversity indices for the first five axes of the reference structure calculated with STATIS (Lavit et al. 1994) over the different species richness levels (here for a data set with three traits).

Index	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5
Richness					
FR_R	−0.30	**−0.72**	0.08	−0.06	0.09
FR_{Is}	−0.18	**−0.41**	−0.29	−0.15	**0.50**
FR_V	**−0.50**	**−0.56**	0.03	−0.24	−0.16
FR_D	0.15	−0.2	−0.26	−0.19	**−0.65**
FR_{Im}	0.05	0.11	−0.21	**−0.76**	−0.01
Evenness					
FE_s	−0.04	−0.05	**−0.71**	0.21	0.17
FE_m	−0.05	0.00	**−0.64**	0.26	−0.19
Divergence					
FD_{var}	**−0.54**	**−0.43**	0.20	0.35	−0.19
FD_σ	**−0.58**	0.36	−0.04	0.05	−0.13
FD_s	**−0.72**	0.24	−0.04	−0.10	0.05
FD_Q	**−0.80**	0.22	−0.04	−0.08	−0.04
FD_m	**−0.63**	0.37	0.01	−0.01	0.06

Note: Values lower than −0.4 or higher than 0.4 are boldface. For 1000 communities species richness trait values and species abundances were generated using uniform distributions between 0 and 1 and between 1 and 100, respectively. See Table 1 for explanations of indices.

Gower distance and PCoA and between 0.688 and 0.897 for transformation via the Hill and Smith method).

DISCUSSION

Performance of the indices

The functional diversity indices described and tested in this study performed quite differently. The quality of the indices aimed at describing the same aspect of functional diversity differed markedly, especially within the group of the FR indices.

In our view, the main criterion for the selection of an index is whether it accurately measures what it is intended to describe. This was the case for all FD indices, irrespective of whether they were one- or multidimensional, as well as for the two FE indices. However, while the multidimensional index FE_m performed quite well in describing the evenness in the two-dimensional space, its one-dimensional counterpart FE_s was only able to measure evenness when a single trait was considered. In contrast, the FR indices differed most strongly in their quality: the only indices that reflected the expected changes in FR, when species were removed within the functional trait space, were the two indices that consider gaps in the functional trait space (FR_{Is} and FR_{Im}). The FR_R and FR_V indices only partly reflected what is considered functional richness (only if there was a continuously filled trait space and species were removed or added at the edges of the community), while the results obtained from the index FR_D did not match the expected values. This index may work in some cases (Petchey and Gaston 2006), but it failed to pass the tests proposed here, perhaps due to the number of traits and/or the number of species used. The behavior of this index is thus difficult to understand, and we suggest rethinking its interpretation.

These results were confirmed by the multi-table ordination analysis (STATIS): instead of three principal component axes, which were expected to correspond to the three index groups FR, FE, and FD, we found that five axes were needed to explain most of the variance: three for FR and one for FD and FE. That three axes were needed to explain the variance of FR indicates that the existing FR indices describe independent aspects of functional diversity: FR_R and FR_V form one group, which describes the traits' range/volume; FR_{Im} measures the occupation and span of trait space; FR_D, which represents the branch length of a dendrogram, is independent of the other FR indices, but what it actually measures is difficult to determine. The FR_{Is} index was not represented by a single axis, but was partly correlated with FR_R, FR_V, and FR_{Im}, because FR_{Is} accounts for gaps in trait space (as FR_{Im}) but fails to take into account the multidimensional nature of trait space, so that gaps are severely underestimated when the number of traits is high.

The second criterion for the selection of an adequate index is that the chosen index is independent of indices describing other aspects of functional diversity. This criterion is met by the two FE indices, but not by all the FR and FD indices: FR_R and FR_V both correlated with FD_{var} and FR_V also correlated with FD_s and FD_Q (Table 4). This strongly suggests that FD_{var}, FR_R, and FR_V rather measure a mixture of functional richness and divergence.

Ricotta (2005) gave several other criteria for an index of functional diversity, which are only applicable to FR indices. For instance, an index should not decrease when a species is added (the monotonicity criterion) or should not increase when exactly the same species is added (the twinning criterion). These two criteria were also tested in

TABLE 4. Indices of functional diversity tested in this study, their properties, correlation with other indices, and their disadvantages.

Index	Source	Description	MD	*A* incl.	Use with cat. variables	Cor. with SR	Cor. with indices from other categories	Disadvantage	IN
Functional richness									
FR_R	Mason et al. (2005)	functional range	no	no	yes	yes	FD_{var}	no consideration of gaps, one-dimensional	1.1, 1.2 (cat.)
FR_{Is}	this study	individual's functional range	no	no	yes	yes	···	one-dimensional, intra-specific trait variation needed	1.2., 1.2 (cat.)
FR_V	Villéger et al. (2008)	functional volume	yes	no	no	yes	FD_{var}, FD_s, FD_Q	no consideration of gaps, SR has to exceed *N* traits	1.3
FR_D	Petchey and Gaston (2002)	sum of branch length of classification	yes	no	yes	yes	···	difficult to interpret, long computation time	1.4
FR_{Im}	this study	functional integral	yes	no	no	yes		long computation time, intra-specific trait variation needed	1.5
Functional evenness									
FE_s	Mouillot et al. (2005)	evenness of trait values	no	yes	yes	no	···	one-dimensional	2.1
FE_m	Villéger et al. (2008)	evenness of minimum spanning tree branch lengths	yes	yes	yes	no	···	···	2.2
Functional divergence									
FD_{var}	Mason et al. (2003)	logarithmic variance of traits	no	yes	yes	no	FR_R, FR_V	no 0 values possible	3.1, 3.3 (cat.)
FD_σ	Lepš et al. (2006)	variance of traits	no	yes	yes	no	···	···	3.2, 3.3 (cat.)
FD_s	this study	relative range of the distributional center	no	yes	no	no	FR_V	···	3.4
FD_Q	Rao (1982), Champely and Chessel (2002)	variance of distances between species	yes	yes	yes	no	FR_V	···	3.5
FD_m	Villéger et al. (2008)	mean deviation of the distance of the center of gravity	yes	yes	no	···	···	SR has to exceed *N* traits (see FR_V)	3.6

Notes: The table can be used to choose an index for the data set in question (dimensionality, type of variable, correlation with indices of a different category). Abbreviations are: *A*, abundance; Adapt., adaptation; cat., categorical; Cor., correlated; IN, index number; MD, multidimensional; *N*, number; SR, species richness. See Table 1 for explanations of indices.

the scenario test (Fig. 4, Table 2) and complied well with nearly all FR indices except FR_D.

In this study, we restricted our tests to classical versions of the indices without specific patterns of data distribution such as skewness. It would be of further interest to include hypotheses on the data randomizations and test how variations of a certain index (e.g., selection of the distance measure used in FD_Q) react under certain circumstances. However, we only expect slight fine-tuning and not fundamental deviations from the results presented here.

The use of categorical variables is problematic since most tested indices can only be applied to continuous variables. However, our pseudo-test with only continuous variables already revealed that the two transformations proposed (Gower distance followed by a PCoA and the Hill and Smith method) led to a loss of information. When tested for multivariate traits, this was especially true for the Gower distance transformation method followed by a PCoA; for one-dimensional indices, both index matrices calculated on the basis of transformed data correlated only weakly with the matrix based on the untransformed traits. This effect might be even worse when real discrete variables are included in the data set. In this case, the transformation methods via ordination techniques may not be the best choice for the treatment of categorical variables. One way to overcome this problem could be to use a multivariate index based on a distance matrix (e.g., FD_Q) and to transform the trait matrix only via the Gower distance (Podani and Schmera 2006). Since the second transformation step (the ordination technique) is missing in this approach, we expect less loss of information. Another possibility is the approach suggested by Lepš et al. (2006), who recommend calculating dissimilarity between species pairs via the sum of their overlaps for each trait (continuous and categorical). In both approaches (Gower distance and summed overlaps), continuous and categorical variables share the same meaning and indicate just to which extent two species are identical.

Otherwise, discrete variables cannot be transformed to continuous variables and processed further with indices designed for this type of variable since functional evenness and divergence have no transposable meaning for discrete traits (i.e., no spatial evenness and divergence of discrete variables, but evenness or variability of trait-level distribution). Since categorical and real-valued variables are of completely different character, it is difficult to find an index in which they can be mixed up (for an exception see *Material and methods: Functional diversity indices: Functional divergence: Multivariate indices: FD_Q*). A second approach would therefore be to use indices aimed at measuring the specific properties of categorical variables. At present, we propose using the (one-dimensional) indices (as follows) designed to calculate species diversity, using trait levels instead of species.

The FR index (relative richness of trait levels).— Corresponding to the relative species richness at a site, we suggest using the number of trait levels present at a site as a proportion of the number of trait levels for all communities together (see FR_{Is}; Table 1: IN 1.2).

The FE index (evenness of trait-level distribution).— We suggest using Bulla's index of species' evenness (Bulla 1994) based on the contribution of a trait level (A_l) to the overall sample size A (total number of individuals) (see FE_s; Table 1: IN 2.1).

The FD index (variability of trait-level distribution).— We suggest using the "index of unalikeability" FD_{cat} by Kader and Perry (2007; Table 1: IN 3.3), which corresponds actually to the Simpson index of species diversity (Simpson 1949) but uses trait levels instead of species. The FD_{cat} represents the proportion of possible comparisons, which are unalike, by calculating the contribution of a factor level (A_l) to the overall sample size A and subtracts the sum of the squares from 1.

Note that for categorical variables, FE and FD measure approximately the same thing, since both reflect the equitability of distribution (Smith and Wilson 1996). The proposed and other indices on species diversity have been extensively tested (e.g., Washington 1984, Smith and Wilson 1996, Beisel et al. 2003).

Recommendations for users

Based on the criteria discussed above and the tests performed, we recommend using the multidimensional index FR_{Im} to measure FR or FR_{Is} if only a single trait is considered. Both indices account for intraspecific variability and consider the existence of empty space within the functional trait space and therefore reflect the true functional richness of the community better. Besides, these indices are orthogonal to FE and FD indices. If the user decides to compute FR through FR_V, the data set should not be transformed, even in the case of differently scaled traits, since the convex hull calculated with transformed data does not properly represent the functional richness for geometrical reasons, but correlates negatively with the expected results and the other indices for functional richness (results not shown).

The FR indices are naturally correlated to species richness. When indices of different communities are compared with one another or when differences between communities are explained with predictor variables, the effect of species richness should therefore be removed from the observed pattern, in order to describe patterns of pure functional diversity. Since the observed relationships are not simply linear and differ between the FR indices and because of unequal variances, we recommend using null models to remove the effect of species richness rather than using the residuals from a constructed model (method, Gotelli and Graves 1996; example for application, Mason et al. 2007, Prinzing et al. 2008).

Both FE_s and FE_m can be used to calculate FE. The use of FE_s is, however, narrowly restricted to one-dimensional data sets. Similarly, we cannot recommend the use of a specific FD index because all indices reflect the expected changes well, irrespective of whether they are one- or multidimensional. However, it should be remembered that not all FD indices are independent of FR indices (Table 4), seemingly because FD_{var} and some FR indices measure a mixture of FR and FD properties. Further, it should be mentioned that indices based on a distance matrix allow for the use of categorical and continuous variables simultaneously, and they can include intraspecific variability when calculated via the pairwise trait overlap as suggested by Lepš et al. (2006). Specific disadvantages of all indices are further summarized in Table 4.

Concerning categorical variables, we recommend at present avoiding their use, if possible, or using indices based on a distance matrix or the one-dimensional indices of species diversity (Table 1: IN 1.2, 2.1, 3.3). In this case, however, the user should keep in mind that the indices designed for categorical variables (especially for FE and FD) do not have the same meaning as their continuous counterparts. The results for the different traits should therefore be averaged over continuous and categorical variables separately, rather than directly averaged over all traits.

In real world measurements, the user comes to face more problems apart from choosing the right index, many of which (e.g., weighing of traits) are discussed in Lepš et al. (2006) and Petchey and Gaston (2006). One frequently occurring problem is that normally not all trait values can be measured for each individual. This is, however, not a problem for the calculation of the indices, since the calculation of all indices (including the newly proposed indices that include intraspecific variability) is based on mean trait values and their variability. And how many individuals should be measured for each species? In general the more, the better. However, to restrict sampling effort, a reference value could be the amount of individuals that are necessary to describe the species' trait distribution (Cianciaruso et al. 2009).

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APPENDIX A

One-dimensional functional richness (FR_{Is}) (*Ecological Archives* M080-016-A1).

APPENDIX B

Multidimensional functional richness (FR_{Im}) (*Ecological Archives* M080-016-A2).

APPENDIX C

One-dimensional functional divergence (FD_s) (*Ecological Archives* M080-016-A3).

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Does excess carbon affect respiration of the rotifer *Brachionus calyciflorus* Pallas?

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SUMMARY

1. Herbivorous zooplankton maintain a rather constant elemental composition in their body mass as compared with the variability commonly encountered in their food. Furthermore, their high phosphorus (P) and nitrogen (N) content means that they often face an excess of carbon (C) in their diet. Regulation of this surplus of energy may occur via modulation of assimilation efficiency, or postassimilation by increased respiration (CO_2) and/or excretion dissolved organic carbon, DOC. Whereas several studies have examined the effect of elemental imbalance in the genus *Daphnia*, few have examined other zooplankton taxa.
2. We investigated whether the rotifer *Brachionus calyciflorus* uses increased respiration as a means of stoichiometrically regulating excess dietary C. Growth rate and respiration were measured under different food qualities (C : N and C : P ratios).
3. Both C : N and C : P ratios in food had strong effects on growth rate, demonstrating strong nutrient limitation of rotifer growth when nutrient elements were depleted in the diet and indicating the need for stoichiometric regulation of excess ingested C.
4. Respiration measurements, supported by a stoichiometric model, indicated that excess C was not released as CO_2 in *B. calyciflorus* and that nutrient balance must therefore be maintained by other means such as excretion of DOC or egestion in faecal material.

Keywords: *Brachionus*, food quality, growth, homeostatic regulation, stoichiometry

Introduction

The flux of carbon (C) through lake ecosystems depends on the availability of key nutrient elements such as phosphorus (P) and nitrogen (N) (Sterner & Elser, 2002; Hessen *et al.*, 2004). Herbivorous zooplankton are of particular importance, being the link between the primary producers and higher trophic levels. These consumers maintain relatively high and rather constant N and P contents compared with their food (Andersen & Hessen, 1991; DeMott, Gulati & Siewertsen, 1998), so they often face diets with excess C relative to nutrient elements. This imbalance leads to decreased C growth efficiencies and ultimately to lower food chain production (Hessen, 1992; Sterner & Hessen, 1994; Sterner *et al.*, 1998). Reduced growth efficiencies implies that the grazers have cope with the excess C, which may be released as faeces, dissolved organic carbon (DOC) or CO_2. These different fates have important consequences for C cycling and sequestration in ecosystems. An understanding of homeostatic regulation in zooplankton is therefore important when trying to link organism to ecological processes.

Zooplankton may use different strategies in homeostatic regulation. The acquisition of resources can be regulated by adjusting the uptake of carbon relative to nutrients through changed food selectivity, ingestion rates or assimilation efficiencies (DeMott, 1995; DeMott *et al.*, 1998; Plath & Boersma, 2001). Zooplankton

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may also release assimilated excess C postabsorption by the gut by excretion of carbon rich organic compounds (Darchambeau, Færøvig & Hessen, 2003), or by respiring the excess (Anderson *et al.*, 2005). This potential role of respiration as a regulatory mechanism for homeostasis in zooplankton is not well understood. The phenomenon was emphasised by Sterner (1997), who advocated the need for estimates of specific dynamic action (SDA; the increase in respiration associated with feeding) and how it relates to food quality. Recent stoichiometric modelling approaches have accordingly distinguished between basal metabolism and the costs of assimilation and growth (Anderson *et al.*, 2005; Anderson & Hessen, 2005). As well as measuring SDA, obtaining estimates of basal metabolic rates (BMR) at different food qualities is also important (Anderson & Hessen, 2005). Basal metabolism depends not only on food quantity, food quality may also be important. Elevated BMRs may play a role in the disposal of excess C in some animals (Curcio *et al.*, 1999; Even *et al.*, 2003; Fu & Xie, 2004). The time of acclimation to a given food quality could also be important for which mechanism zooplankton use to obtain nutrient balance. Increased respiration could be just a transient phenomenon in response to excess dietary C, but it could also be important after long-term acclimation to food of high C, although regulating food uptake might also be important in this situation.

A large number of stoichiometric studies have focused on *Daphnia*, a particularly important genus in the ecology of freshwater systems. However, the response of *Daphnia* to elemental imbalance in its food may not be representative of those of other taxa. Rotifers may also play an important ecological role at some periods of the year (Walz, 1995) and in particular lakes when environmental factors, such as fish predation pressure, becomes unfavourable for larger crustacean zooplankton (Jeppesen *et al.*, 1990). Although daphnids and rotifers belong to the same functional group, they are distant taxonomic relatives, and there appear to be important physiological differences between the two groups. Recent modelling work by Anderson *et al.* (2005) suggested that *Daphnia* regulates excess C in the diet primarily postabsorption by the gut, either by respiration of CO_2 or by excretion of DOC. In common with *Daphnia*, suspension feeding rotifers of the genus *Brachionus* show reduced growth on P-deficient diets (Rothhaupt, 1995; Jensen & Verschoor, 2004). However, in contrast to *Daphnia*, N-limitation may also be prevalent in this rotifer (Sterner, 1993; Rothhaupt, 1995; Jensen & Verschoor, 2004). Rotifers may therefore experience different challenges when dealing with excess carbon in the many lakes where food reaches high C : nutrient ratios (Elser *et al.*, 2000) and may use different mechanisms than *Daphnia* in order to regulate their C balance.

We studied stoichiometric regulation in a cosmopolitan, commonly occurring zooplankter, the rotifer *Brachionus calyciflorus* and in particular examined the role of respiration in the disposal of excess C in the diet. Respiration of *B. calyciflorus* was measured when feeding on different food qualities (C : N and C : P ratios) at high food concentrations. The food treatments included nutrient sufficient and phosphorus- and nitrogen-depleted algae. Respiration measurements were conducted on animals after both short- and long-term acclimation to the respective food treatments. Finally, we used the stoichiometric model of Anderson *et al.* (2005), parameterised for *B. calyciflorus*, in order to study the experimental results in greater detail.

Methods

Cultures

The green alga *Selenastrum capricornutum* was grown in continuous cultures (dilution rate 0.2 day^{-1}) in 2 L Nalgene polycarbonate vessels (Nalge Company, Rochester, NY, U.S.A.) with a magnetic stirring device receiving a nominal light of 70 μmol quanta m^{-2} s^{-1} from 25 W blue-white fluorescent tubes. Algal cultures were allowed to run for 2 weeks before the start of the experiments in order to obtain stable cultures. The algae were cultured on the same three types of COMBO medium (Kilham *et al.*, 1998), as described by Jensen & Verschoor (2004): full nutrient sufficient medium (F) with a phosphate concentration of 50 μmol L^{-1} and a nitrate concentration of 1000 μmol L^{-1}; P-depleted medium (−P) with phosphate concentration reduced to 2 μmol L^{-1}; and N-depleted medium (−N) with a nitrate concentration of 40 μmol L^{-1}.

As a proxy of cell density, daily measurements of optical density at 663 nm were performed using a spectrophotometer (UV-210A, Shimadzu Seisakusho

LTD, Kyoto, Japan). Samples were taken twice a week for phytoplankton size distribution and for samples for particulate C, N and P. Those for size distribution of the algae were fixed with Lugol's iodine (1% final concentration), inspected visually using a microscope and analysed for cell number and cell volume on a CASY TTC1 Cell Counter (Schärfe Systems, Reutlingen, Germany). Subsamples from the cultures were filtered on precombusted, acid-washed GF/F filters for analysis of particulate C, N, and P. C and N were analysed on a Flash EA^{TM} 1112 automatic elemental analyser (Thermo Finnigan, Milan, Italy). Particulate P was analysed following the persulphate method according to Hessen, Færøvig & Andersen (2002). Algae from the different cultures were used as food in the zooplankton experiments. Harvested algae were diluted with nutrient free COMBO medium to a standardised food concentration of 6 mg C L^{-1}. This concentration was calculated from pre-established regressions for each food type relating absorbance and the carbon content of the cultures.

Brachionus calyciflorus were hatched from dormant eggs (MicroBioTest inc., Nazareth, Belgium). This ensured identical egg quality and synchronised age of the population. Rotifers were cultured in COMBO medium without phosphate and nitrate in order to avoid uptake of N and P from the medium by the food algae. The ionic strength of the nutrient-free zooplankton medium was maintained by the addition of KCl at a concentration of 100 μmol L^{-1}. All experiments were carried out at constant temperature (19 °C) in dim light.

Zooplankton experiments

Brachionus calyciflorus for the experiments were hatched from cysts placed in nutrient-free COMBO medium in shallow dishes. Hatching started after 36 h of incubation at 19 °C. Because of the small individual size of *B. calyciflorus*, large numbers were needed for the respiration experiments. To obtain sufficient numbers, newborns hatched from the same batch of cysts within 36–48 h were collected, divided into three groups and transferred to large glass beakers and fed F, −P or −N algae at a food concentration of 6 mg C L^{-1} for 48 h before the respiration experiment. The food was refreshed daily.

A previous study by Olsen, Reinertsen & Vadstein (2002) indicates that *Brachionus* species can have a relatively flexible stoichiometry. Thus, the stoichiometric composition of *B. calyciflorus* fed different algal qualities in terms of C : N : P was measured in order to determine the extent to which body C : N : P was affected by food quality. Rotifers from cultures grown under the same conditions as for the respiration experiment were rinsed in nutrient-free COMBO medium in several steps. Three hundred individuals were then transferred to small acid-washed glass vials in 10 mL of distilled water and the samples with animals were filtered on precombusted acid-washed GF/F filters, before being analysed for C, N and P as described above. Samples for the determination of dry masses were also taken from the same rotifer cultures. For this purpose 70–100 rotifers were transferred to preweighed silver cups, dried for 24 h at 60 °C, cooled in a desiccator and weighed to the nearest microgram on a Mettler Toledo MX5 microbalance (Mettler Toledo, Greifensee, Switzerland).

We measured somatic growth rates of the juvenile *B. calyciflorus* that were fed different food qualities of algae. Newborn individuals (up to 2 h old) were transferred into clear polystyrene 96-well microtiterplates, with one individual per well in 250 μL food suspension (6 mg C L^{-1}). Animals were transferred from the food suspension after 24 h and preserved in an isotonic salt solution (CASYTON, Schärfe Systems, Reutlingen, Germany) that killed them quickly. The animals were measured in an inverted microscope and body volumes calculated following Ruttner-Kolisko (1977). Measured values were increased by 10% in order to account for the volume of the foot. Volume-based somatic growth rates were calculated as $r = (\ln b_1 - b_0)/t$, where r = somatic growth rate, b_1 = body volume at the end of the experiment, b_0 = body volume at the beginning and t = duration of experiment in days.

The respiration rate of *B. calyciflorus* was determined by measuring oxygen consumption by the 'closed bottle method' (Lampert, 1984). Respiration in animals is affected by their feeding activity (Fig. 1). When an animal starts feeding the respiration rate increases from the BMR (here considered synonymous with fasting or standard metabolic rate) to a maximal level and then decreases asymptotically towards BMR again when feeding stops.

In order to determine if and how food quality affects it, the respiration of *B. calyciflorus* was measured under four different treatments (see also Fig. 1;

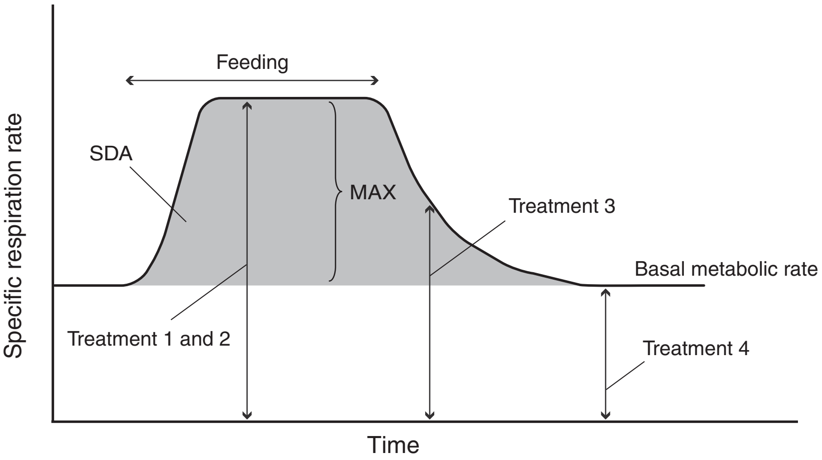

Fig. 1 Schematic representation of how feeding of an animal affects its respiration. The respiration rate increase from the basal metabolic rate (BMR) to a maximal respiration rate when the animal starts feeding. When feeding stops the respiration rate will approach BMR again asymptotically. The feeding event is shown in the figure. The specific dynamic action (SDA; the area shown in grey below the curve above BMR) comprises an obligatory part related to digestion and growth, but it may also include a facultative part related to dissipation of excess energy (Krieger, 1978; Simonson & Defronzo, 1990). Respiration measured in our experiments under different conditions is also shown: Respiration of feeding animals (treatment 1 and 2), respiration of well fed animals without food (treatment 3) and BMR (treatment 4). MAX is maximal weight specific respiration rate above fasting metabolic rate. See text for further details. Modified from Sigsgaard *et al.* (2003).

Table 1). In treatment 1 (Table 1), respiration measurements were conducted on feeding animals after short-term acclimation to nutrient limited food. The animals were grown from birth to the start of the experiment (48 h) on nutrient sufficient algae (F). Animals were then transferred to food suspensions (6 mg C L^{-1}) with either nutrient sufficient or nutrient deficient algae and left to acclimate for a short period (30 min) before being transferred with food suspension to the respiration chambers. In treatment 2 (Table 1), respiration measurements were conducted on feeding animals after a long period of acclimation to the different food qualities. In this treatment animals were grown from birth to the start of the experiment (48 h) on either nutrient sufficient or nutrient deficient algae (F, −P or −N). Animals were then transferred to the food suspensions (6 mg C L^{-1}) with algae of the same quality as with which they were raised, and left to acclimate for 30 min before being transferred with food suspension to the respiration chambers. In treatment 3 (Table 1) animals were also long acclimated to the different food qualities as in treatment 2 but respiration was measured in pure medium without food. Again animals were grown from birth and onwards for 48 h to the start of the experiment on either nutrient sufficient or nutrient deficient algae. Before measurement, the animals were transferred to pure medium and allowed to empty their gut for 30 min, before being rinsed with nutrient-free COMBO medium and transferred to the

Table 1 Treatment definitions and experimental conditions during the growth and respiration measurement phases. Food algae were offered at a concentration of 6 mg C L^{-1} during growth phases in all treatments and when respiration measurements were taken in treatments 1 and 2.

Treatment	Acclimation period	Algae offered during growth	Conditions during respiration measurement	Acclimation time to algae quality
1	Short	F (48 h)	F, −N or −P	0.5 h
2	Long	F, −N or −P (48 h)	F, −N or −P	48 h
3	Long	F, −N or −P (48 h)	No food	48 h
4	Long	F, −N or −P (48 h) followed by 18 h fasting period	No food	48 h

Letters in the table refer to algal culture conditions: nutrient sufficient (F), P-deficient (−P) algae or N-deficient algae (−N).

respiration chamber in pure nutrient-free COMBO medium. In treatment 4 (Table 1) measurements of basal metabolism were performed on animals long acclimated to the different food qualities. In this treatment respiration measurements were conducted after a period of fasting. During fasting the respiration of previously fed *B. calyciflorus* decreases asymptotically to a constant lower level reached after approximately 18 h (Kirk, Ellis & Taylor, 1999). This lower level can be taken as a measure of the basal respiration rate of the animal (Sigsgaard, Petersen & Iversen, 2003). As in treatment 2 and 3 animals were grown for 48 h (from birth) on each of the three food qualities. Then animals were rinsed with nutrient-free COMBO medium, transferred to new medium and allowed to fast for 18 h. After fasting the animals were rinsed again with nutrient free COMBO medium and transferred to the respiration chamber in pure nutrient-free COMBO medium.

Respiration experiments were made using a MRCh System (Unisense A/S, Århus, Denmark). Animals were placed in a 0.370 μL glass respiration chamber in a thermostatically controlled waterbath (19 ± 0.1 °C). The chambers contained either food suspension or pure nutrient-free COMBO medium depending on the experiment. Dissolved oxygen content was measured with a calibrated Clark-type oxygen microsensor (model OX-MRCh; Unisense A/S). Animals were allowed to acclimate for 10 min, after which oxygen consumption rate was taken as the linear slope of the O_2 concentration plotted against time for the next 20 min (Fig. 2). The short incubation times minimised the problem of decreasing oxygen- and food-concentrations. O_2 concentration in the chamber never declined below 85% saturation. The number of animals in the chambers was between 80 and 150. These are high densities but animal densities only have a minimal effect on respiration rate in rotifers (Kirk *et al.*, 1999). In order to correct for the background oxygen consumption, controls were set up in a separate chamber containing only food suspension or pure nutrient-free COMBO medium (Fig. 2). Background respiration was below 10% of animal respiration. After measuring respiration, the animals were removed from the respiration chamber, preserved, measured and body volumes calculated as described above. These were transformed to dry masses from predetermined relations between body volume and dry mass of individuals fed nutrient sufficient algae [$W = (4.3 \times 10^{-7} \times b) - 0.296$ where W is dry mass (μg individual^{-1}) and b is calculated body volume (μm^3); $n = 26$, $R = 0.72$, $MS_{residual} = 0.00166$, $P < 0.0001$].

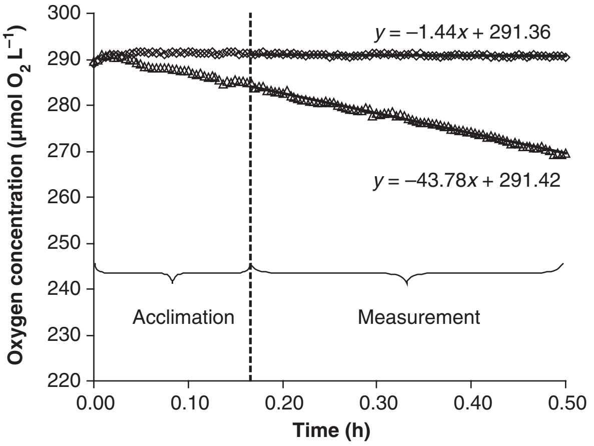

Fig. 2 Example of oxygen consumption curves from the respiration experiments with *Brachionus calyciflorus*. Upper curve (diamonds): background oxygen consumption in respiration chamber with food suspension. Lower curve (triangles): rotifer oxygen consumption in respiration chamber with food suspension. Incubations started with 10 min of acclimation. Then oxygen consumption rate was taken as the linear slope of the curves for the next 20 min (indicated by the lines in the figure).

Respiration rates in *Brachionus* decrease at very low food concentrations. However, as long as concentrations are kept at saturating levels this effect of food concentration is minimal (Hirata & Yamasaki, 1987). Assuming maximum ingestion rates for *B. calyciflorus* (Rothhaupt, 1990a) of 18 ng C individual^{-1} h^{-1}, food concentrations were kept above saturating levels during the incubation.

In the respiration experiments with *B. calyciflorus* only a few individuals were carrying eggs. In order to estimate 'somatic' respiration rates the egg respiration was subtracted from the total respiration assuming that specific respiration in eggs is 50% of that of the animals (Pilarska, 1977; Galkovskaya, 1995). To account for variation in respiration rates because of size, oxygen consumption rates can be adjusted using relations between respiration rates and size (Fu & Xie, 2004). We did this for *B. calyciflorus* using a relation between respiration and size for rotifers (Stemberger & Gilbert, 1987). Mass-specific respiration rates represent an animal size of 0.21 μg dry mass.

Specific dynamic action of animals can be characterised indirectly by calculating the maximal specific respiration rate above the BMR (MAX) (Sigsgaard *et al*., 2003). In our experiments we calculated this for *B. calyciflorus* grown and fed on different food qualities as the difference between specific respiration rate of feeding animals (treatment 2) and specific respiration rate after fasting, i.e. BMR (treatment 4, see also Fig. 1).

Statistics

The effect of food quality treatment on growth rates and specific respiration rates was tested by the Kruskal–Wallis test. For pair-wise comparisons of means of growth rates and specific respiration rates, Tukey's HSD test was applied.

Effects of food quality treatment on elemental ratios and MAX values of *B. calyciflorus* were tested using bootstrap procedures. For each food quality treatment, elemental ratios (C : N, C : P or N : P) were first calculated for all combinations of samples for C, N and P. Similarly, MAX values were calculated for all combinations of measurements of respiration during treatment 2 and 4. Then, we used a between-class variance analysis to test for the overall effect of food quality treatment. Between-class variance was calculated either on three subsamples of the total possible number of combinations for each food quality treatment (1), or on three subsamples of the total possible number of combinations for all food quality treatments pooled (2). If there is a food quality treatment effect, (1) should be greater than (2). Food quality treatment effect was thus evaluated by calculating the proportion of (1) – (2) values greater than zero (one-tailed test). We also performed pairwise comparisons (pairwise tests) of the effects of food quality treatments on elemental ratios and MAX values of *B. calyciflorus*. Considering two food quality treatments (t1 and t2), this was assessed by calculating the difference between means of subsamples of the total possible number of combinations for t1 and t2. We checked that the difference between means was non-null using a two-tailed test. In addition, we used Bonferroni corrections (Sokal & Rohlf, 1997) to deal with the multiplicity of tests. For all tests, we used 10 000 randomisations and subsamples of four values for elemental ratios and five values for respiration (corresponding to the minimal numbers of measures realised in the different experiments). Statistical analyses were carried out with the JMP (SAS Institute Inc., 2002, Cary, NC, U.S.A.) and Splus (Lucent Technologies, Inc., 2002, Seattle, WA, U.S.A.) analytical packages.

Results

Food quality

The degree of nutrient depletion of the algal cultures was visible from the C yields (data not shown). Algal cultures (−P and −N) grown on nutrient-depleted medium always had reduced yields as well as reduced specific N and P content, compared with their nutrient-sufficient (F) counterparts (Table 2). Both nutrient-deplete and nutrient-sufficient algae had a size of 4–5 μm equivalent spherical diameter (ESD).

Stoichiometry of B. calyciflorus

The elemental composition of *B. calyciflorus* was far less flexible than its food (Table 2). Food treatment had no overall effect on the body C : P ratios of *B. calyciflorus* (Table 2; between-class variance analysis, $P = 0.1637$). Pairwise comparisons showed that individuals receiving nitrogen limited algae had significantly higher C : P ratios than animals receiving nutrient sufficient food (Table 2; pairwise test, $P = 0.0000$). Although the change in C : P ratios of *B. calyciflorus* is not significant with the P-limited diet (Table 2; pairwise test, $P = 0.0990$), the ratio is still

Table 2 Elemental composition of the green alga *Selenastrum capricornutum* used as food and of the rotifer *Brachionus calyciflorus* (fed nutrient replete, phosphorus-deplete and nitrogen-deplete algae)

	C : P	C : N	N : P
S. capricornutum			
F	66 (11)	7.6 (0.4)	8.8 (1.8)
−P	484 (99)	12.9 (1.6)	38.1 (8.4)
−N	76 (5)	16.3 (1.2)	4.7 (0.4)
B. calyciflorus			
F	70 (4)	5.6 (0.3)	12.6 (0.8)
−P	105 (18)	7.0 (0.3)	15.1 (2.6)
−N	121 (18)	5.6 (0.2)	21.7 (3.3)

Values given are atomic ratios (mean ± SD). Algal stoichiometry were analysed from six separate samples. Rotifer stoichiometry were analysed from four samples containing 300 individuals per sample. Abbreviations as in Table 1. Mean values and standard deviations for *B. calyciflorus* were calculated using a bootstrap method (10 000 permutations).

about 50% higher compared with individuals fed the nutrient sufficient diet. This lack of significance could be due to large variation in the data. Concerning body C : N ratios of *B. calyciflorus*, food treatment tended to have an effect (Table 2; between-class variance analysis, $P = 0.0595$). The pairwise comparisons showed that individuals receiving P-limited algae had significantly higher C : N ratios than those receiving nutrient sufficient and N-limited food respectively (Table 2; pairwise test $P = 0.0162$ and $P = 0.0000$ respectively). As for the N : P ratios of *B. calyciflorus* food treatment had no overall effect (Table 2; between-class variance analysis, $P = 0.1722$), but animals receiving N-limited algae somewhat surprisingly had significantly higher N : P ratios than animals receiving nutrient sufficient food (Table 2; pairwise test, $P = 0.0000$).

Somatic growth

Nutrient-limited food caused reduced growth of *B. calyciflorus*. Somatic growth rates differed significantly after 24 h (Kruskal–Wallis test, $P < 0.0001$, d.f. = 2, $n = 108$). Individuals fed nutrient sufficient algae had an average growth rate of 0.73 ± 0.04 day^{-1} (mean ± 1 SE). This was significantly higher than those fed P-limited algae (Tukey's HSD test, $P < 0.05$), having a growth rate of 0.60 ± 0.03 day^{-1} (mean ± 1 SE). This again was significantly higher than animals receiving N-limited algae (Tukey's HSD test, $P < 0.05$), they had the lowest rates of 0.43 ± 0.03 day^{-1} (mean ± 1 SE).

Respiration

Specific respiration rates of feeding *B. calyciflorus* were not affected after a short period of acclimation to poor food algae (treatment 1) with high C : nutrient ratios (Fig. 3a; Kruskal–Wallis test, $P = 0.1057$, d.f. = 2, $n = 32$).

In treatment 2, 3, and 4 individuals were acclimated for a long period to the respective foods (i.e. they were fed F, −P or −N algae from birth, 48 h) and thus had a different size because of different growth rates. In treatment 2 the respiration of feeding animals was examined and specific respiration rates of *B. calyci-*

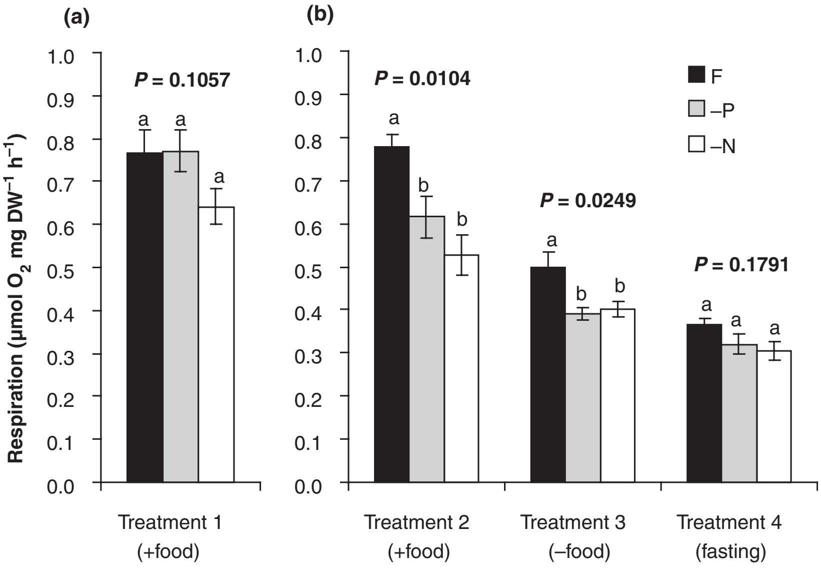

Fig. 3 Respiration rates (mean ± 1 SE) of *Brachionus calyciflorus*. The letters in the figure refer to algal culture conditions: Nutrient sufficient (F, black), P-deficient (−P, grey) or N-deficient algae (−N, white). (a) In treatment 1 respiration measurements were carried out on feeding animals after a short period (30 min) of acclimation to the respective food algae. The animals in treatment 1 had been grown from birth on nutrient sufficient algae (48 h). (b) In treatment 2 respiration measurements were carried out on feeding animals after a long period (48 h, i.e. from birth) of acclimation to the respective food algae. Treatment 3: as in treatment 2 but respiration measurements were measured without food. Treatment 4: as in treatment 2 but respiration measurements were measured after 18 h of starvation. Results from the statistical analysis (*P*-values) are shown in the figure, significant values in bold. Different letters above columns indicate a significant difference between means (Tukey–Kramer HSD test, $P < 0.05$).

florus differed significantly between food qualities (Fig. 3b, Kruskal–Wallis test, $P = 0.0104$, d.f. $= 2$, $n = 15$): individuals fed and grown on nutrient-replete algae had higher respiration rates than individuals grown on P-limited and N-limited algae (Tukey's HSD test, $P < 0.05$). In treatment 3, animal respiration was measured in pure medium without food, i.e. prefed but fasting animals that would rely on energy storage for respiration. Under these conditions respiration rates were significantly affected by food quality (Fig. 3b; Kruskal–Wallis test, $P = 0.0249$, d.f. $= 2$, $n = 25$): individuals fed and grown on nutrient-replete algae had higher respiration rates than individuals grown on nutrient-limited algae (Tukey's HSD test, $P < 0.05$). Treatment 4 examined the effect of food quality on basal metabolism (i.e. respiration of fasting rotifers). Under these conditions respiration rates of *B. calyciflorus* did not differ for the different food qualities (Fig. 3b; Kruskal–Wallis test, $P = 0.1791$, d.f. $= 2$, $n = 30$).

The results from the respiration experiments clearly demonstrate the effect of feeding on the respiration rate. The respiration rates of feeding individuals were always considerably higher than for non-feeding individuals under all food qualities (Fig. 3b). Food quality had no overall significant effect on the maximum respiration above basal metabolic level (MAX) for *B. calyciflorus* (between-class variance analysis, $P = 0.1036$, $n = 10\,000$). However, the pairwise comparisons showed that MAX was significantly higher for animals fed and grown on nutrient sufficient algae (F) as compared with animals receiving nitrogen limited (−N) food (pairwise test, $P = 0.0048$).

The group of rotifers with the highest growth rate (nutrient sufficient, F) also had significantly higher respiration rates (both when considering respiration in feeding animals and MAX as an indirect measure of SDA) than the group of animals with lowest growth rates (nitrogen limited, −N).

Modelling

We used a stoichiometric model (Anderson *et al.*, 2005) to aid in the interpretation of the experimental results for *B. calyciflorus* and in particular to examine whether or not excess C in the diet was respired or disposed of by some other mechanism by the animal. The model includes C, N and P as currencies, assimilation, maintenance and production as sequential steps for substrate use, and subdivides maintenance into separate terms for protein turnover and the associated energetic costs and other basal costs such as osmoregulation. Anderson *et al.* (2005) used their model to study the fate of excess nutrients and carbon in *Daphnia*, the model indicating in that case that regulation of excess C seems to occur primarily postassimilatory by respiration and excretion of DOC.

Comparison of data with the model requires that respiration data be converted into biomass-specific units. This conversion was done using the measured C content of rotifers fed the three different food algae. Rates of oxygen consumption were converted to carbon units by assuming respiratory quotients (RQ) of 1.0 for feeding and non-feeding animals (treatments 2 and 3) and 0.7 for fasting animals (treatment 4) (Richman, 1958).

The model was originally parameterised by Anderson *et al.* (2005) for *Daphnia*. When applying it to *B. calyciflorus*, several adjustments were made: (i) assimilation efficiency (AE) of P, parameter β_P, was increased from its original setting of 0.8 to a new value of 0.95, which was necessary to reconcile the budget of the −P food treatment. Assimilation efficiencies for P as high as 0.95 are not unusual in zooplankton (DeMott *et al.*, 1998); (ii) AE for C was decreased by 10% so that predicted growth in the F food treatment better matched the observed rate of 0.73 day^{-1}. Carbon is divided between protein and non-protein fractions in the model, with new values for the associated assimilation efficiencies, β_N and β_M, being 0.62 and 0.56, respectively. The result is an average C AE of 0.59 for a food item with C : N of 7.6 (algae in the F food treatment; assumes protein C : N of 3.7), nearly equal to an efficiency of 0.6 estimated for *B. calyciflorus* by Verschoor, Boonstra & Meijer (2005) for algae of comparable quality; (iii) Respiration measured under starvation conditions (treatment 3) was 0.22, 0.17 and 0.17 day^{-1} for the F, −P and −N food treatments respectively, averaging 0.18 day^{-1}. Parameter ζ_B, which represents 'other basal costs', was increased from its original setting of 0.052 day^{-1} in Anderson *et al.* (2005) to a new value of 0.09 day^{-1}, thereby giving a total basal respiration rate (but which excludes any assimilation costs, which are absent in starving animals) matching that of the experimental treatments, i.e. 0.18 day^{-1}. The remaining parameters were set to their default settings as in Anderson *et al.* (2005), except that, because *Brachionus* does not moult,

Table 3 Parameter values used in the model (see Anderson *et al.*, 2005)

Parameter	Definition	Value	Unit of measure
$\theta_{CN(H)}$	C : N ratios of consumer	See Table 2	mol C (mol N)$^{-1}$
$\theta_{CP(H)}$	C : P ratios of consumer		mol C (mol P)$^{-1}$
$\theta_{CN(F)}$	C : N ratios of food		mol C (mol N)$^{-1}$
$\theta_{CP(F)}$	C : P ratios of food		mol C (mol P)$^{-1}$
β_N	Assimilation efficiency: protein	0.62	Dimensionless
β_M	Assimilation efficiency: non-protein C	0.56	Dimensionless
β_P	Assimilation efficiency: P	0.95	Dimensionless
ζ_B	Other basal costs	0.09	day^{-1}
θ_v	C : N of protein	3.7	mol C (mol N)$^{-1}$
τ_{CN}	Biomass turnover: C, N	0.094	day^{-1}
τ_P	Biomass turnover: P	0.094	day^{-1}
η_{CN}	Fraction CN turnover reclaimed	0.38	Dimensionless
η_P	Fraction P turnover reclaimed	0.38	Dimensionless
ζ_I	Cost of assimilation	0.06	mol C (mol C)$^{-1}$
φ_C	Cost of biosynthesis	0.75	mol C (mol C)$^{-1}$

the moult parameters were rendered redundant. A complete list of parameter values is provided in Table 3.

We use the model to study the factors underlying the total respiration of *B. calyciflorus* as measured in the animals that were fed a saturating ration and that were long acclimated to different food qualities (treatment 2). Algae were supplied at a concentration of 6 mg C L^{-1}. The first step when using the model is to specify intake, which was estimated from this food concentration as follows. Measured growth and respiration for the F treatment were 0.73 and 0.66 day^{-1} respectively. Using a C AE of 0.59 (see above), intake can then be estimated as $(0.73 + 0.66)/0.59 = 2.36$ day^{-1}. By means of comparison, a functional response curve for *Brachionus rubens* ingesting *Scenedesmus* subjected to three treatments – nutrient sufficient, P-limited or N-limited – is provided by Rothhaupt (1995). Ingestion rate, which was insensitive to algal nutrient status, saturated at approximately 18 ng C individual^{-1} h^{-1}, equivalent to 2.4 day^{-1} (body weight 0.18 μg C), remarkably close to our estimate above. *Brachionus calyciflorus* has a similar saturated ingestion rate of 0.18 ng C individual^{-1} h^{-1} (Rothhaupt, 1990a) and ranged in length between 220 and 285 μm (Rothhaupt, 1990b). Calculated ingestion rates, using a conversion to biovolume according to Ruttner-Kolisko (1977), conversion to dry mass as in this study, and assuming C as 33.4% of dry mass (this study, *B. calyciflorus* fed F algae), are 1.3–4.2 day^{-1}, a range that encompasses the 2.36 day^{-1} used here.

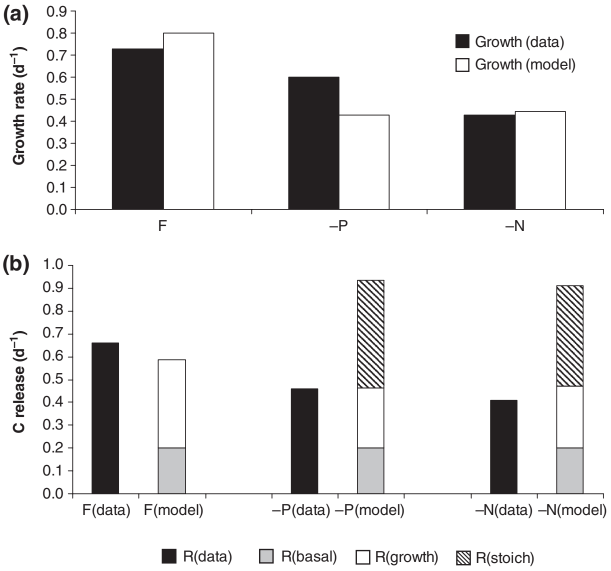

Fig. 4 Effects of food quality on growth and C release in *Brachionus calyciflorus* (a) Growth rates as predicted by the model of Anderson *et al.* (2005) (white bars), compared with data (black bars) from our experiments. (b) Modelled release of C in *Brachionus calyciflorus*; consisting of a standard part [respiration related to maintenance and production, R(basal) and R(growth)] and stoichiometric release of excess C [R(stoich)]. The model is compared with data from our experiments on respiration of feeding animals long term acclimated to different food qualities (treatment 2) at a saturating concentration of 6 mg C L^{-1}.

Model results are shown in Fig. 4. Animals in the F food treatment were predicted to be limited by carbon, the modelled growth rate of 0.82 day^{-1} being slightly higher than the experimentally observed rate of 0.73 day^{-1} (Fig. 4a). As might be expected, predicted growth rates were significantly decreased in the −P and −N food treatments because of limitation of *B. calyciflorus* production by phosphorus and nitrogen respectively. Calculated threshold elemental ratios (TERs) for C : P and C : N in the −P and −N treatments were 280.6 and 9.65 respectively, substantially lower than the food C : P and C : N of 484 and 16.3, thereby indicating strong limitation by nutrient elements rather than carbon in each case. Predicted growth rates of 0.80 and 0.44 day^{-1} for the F and −N food treatments show a close match with the experimental rates. However, the predicted growth rate under P limitation of 0.43 day^{-1} is somewhat lower than the experimental value of 0.60 day^{-1}. This discrepancy suggests that P was in fact used for growth by the animals more effectively than was predicted by the model. It is difficult to increase P use efficiency in the model by increasing assimilation given the already high AE of 0.95 and low usage of P in basal metabolism. Postabsorptive mechanisms that enable a more effective use of P under P-limitation could be hypothesised to explain this discrepancy. One possibility is a higher protein synthesis rate per ribosome under P-limiting conditions, acting to increase growth rate without increasing requirements for P-rich rRNA. Experiments in which *B. calyciflorus* were fed P-limited algae showed a higher production per unit RNA as compared with animals fed nutrient sufficient and N-limited algae (T.C. Jensen and M. Kyle, unpubl. data), indicating that such an increase in protein synthesis rate per ribosome actually does take place. Further evidence for such a mechanism comes from the study of Acharya, Kyle & Elser (2004) showing the same type of response in *Daphnia galeata* under P-limitation. Such a 'P-saving' mechanism is not incorporated in the model.

Limitation of growth in the F food treatment was predicted by the model to be by carbon, in which case one would expect there to be no stoichiometric excess of this element and modelled and observed respiration to show good agreement. The predicted respiration rate for the F food treatment of 0.58 day^{-1} is indeed reasonable close to the observed rate of 0.66 day^{-1} (Fig. 4b). The modelled rate is shown subdivided into its separate components, namely that associated with basal metabolism (31% of the total) and that associated with growth and assimilation over and above basal metabolism, i.e. SDA (69% of the total). One would expect the respiration associated with assimilation and growth to be lower in the −P and −N food treatments compared with the F food treatment because of the lower growth rates involved. Total respiration rates [the sum of the R(basal) and R(growth) terms in Fig. 4b] are indeed lower in the modelled −P and −N food treatments. Agreement is good with data, but only if release of C in stoichiometric excess is not included as part of respiration. If this C was released as respired CO_2 by the animals, then the total respiration predicted would unacceptably exceed the observed respiration rates. Therefore respiration does not appear to be used by *B. calyciflorus* as a postabsorptive regulative mechanism for disposing of excess C in the diet, other mechanisms such as excretion of DOC being used instead to achieve this disposal.

Discussion

Somatic growth of *B. calyciflorus* was clearly reduced when consuming nutrient depleted food. Decreased intake is one potential cause. However, as noted previously, algal nutrient content did not affect ingestion rates in the closely related species *B. rubens* (Rothhaupt, 1995). Furthermore, nutrient-deficient and nutrient-sufficient algae in the present study were of similar size and therefore any influence of size on ingestion (Rothhaupt, 1990b) can be excluded. Nutrient-limited algae of high C : nutrient ratios can have thickened cell walls reducing digestion (Van Donk & Hessen, 1993; Van Donk *et al.*, 1997). However, food algae in our experiments were not so heavily nutrient limited as in these previous studies. The models requirement for very high P AE is also contrary to digestion resistance, which would reduce AE for all elements. Further, rotifers posses a specialised stomach, the mastax, with which they are able to crush ingested food which should thereby minimise problems associated with reduced digestibility. Our results indicate that the observed decreased growth of *B. calyciflorus* was caused by limitation by N and P when consuming nutrient-deplete algae, a result supported by the modelling analysis.

Biomass-specific respiration rates of non-feeding *B. calyciflorus* (receiving F algae) in the present study were similar to previous results reported in the literature (Table 4). The rates from our experiment with animals starved for 18 h were twice as high as those found by Kirk *et al.* (1999). We have no obvious explanation for this difference (Table 4), although the acclimation period in the respiration chamber before measurement was much shorter in our experiments. We found

Table 4 Specific respiration rates of *Brachionus calyciflorus* and *Brachionus plicatilis* at 20 °C from different studies under different conditions (feeding, previously fed non-feeding, starved 18 h). To account for variations in specific respiration rates (represent animal size of 0.21 μg dry mass) because of size, rates from previous studies were adjusted using relations between respiration rates and size (Stemberger & Gilbert, 1987).

	Specific respiration rates at 20 °C (μmol O_2 mg DW^{-1} h^{-1})				
Species	Feeding	No food	Basal/fasting (18 h starvation)	Experimental temperature (°C)	References
B. calyciflorus	–	0.67	–	20	Doohan (1973)
	–	0.47	–	20	Galkovskaya (1995)
	–	0.52	0.20	20	Kirk *et al.* (1999)
	0.86	0.55	0.40	19	Present study*
B. plicatilis	0.67	0.55	0.28	20	Hirata & Yamasaki (1987)†

*Only results from the F food treatment included, results were temperature corrected from 19 to 20 °C assuming a Q_{10} for *B. calyciflorus* of 2.66 (Galkovskaya, 1995).

†Specific respiration rates not allometrically scaled, because animal size was not reported.

respiration rates of feeding *B. calyciflorus* to be considerably higher than those of individuals starved for 18 h. For the F algae this increase was approximately twofold (Table 4), comparable with the results of Hirata & Yamasaki (1987) for *Brachionus plicatilis*.

The results from treatment 1 (short acclimation to respective food quality) in the respiration experiments suggest that *B. calyciflorus* does not transiently increase respiration to dispose of excess C, as suggested for *D. magna* (Darchambeau *et al.*, 2003). However, respiration could still play a role when animals are long-term acclimated to a diet with excess C; i.e. feeding or fasting metabolic rate could increase after long-term exposure to a high C : P or C : N diet. The results from treatments 2, 3 and 4 (long acclimation to respective food quality) show that this is not the case. Instead, feeding metabolic rate or MAX (i.e. SDA) decrease with growth rates of animals fed nutrient-limited diets. SDA consists of an obligatory part related to digestion and protein synthesis (growth). It may also, however, comprise a facultative component related to the dissipation of excess C or energy (Krieger, 1978; Simonson & Defronzo, 1990). Respiration should increase with growth and intake where SDA is primarily related to growth and digestion (increased metabolic activity). If, however, respiration is also involved in the stoichiometric regulation of homeostasis then it could remain high or even be inversely related to growth when animals feed on excess C diets. Thus our experimental results, in which respiration decreased sharply when animals were fed on nutrient deficient diets, indicate that SDA in *B. calyciflorus* comprises components for digestion and growth only, with no stoichiometric component. The modelling work further emphasised this finding, with predicted and observed respiration rates showing good agreement only if excess dietary C was not respired as CO_2. Even altering the total predicted respiration by ±10% makes little difference to the main conclusion, namely that there is a large stoichiometric excess of C under nutrient limitation that is not respired. Similarly, possible errors in the respiration measurements, because of the applied RQ in converting oxygen consumption rates to C units, will not alter this conclusion.

The model is not dynamic in nature and therefore does not predict acclimation of animals to changing circumstances, such as occurred in treatment 3. In this instance we measured the metabolic rate of well-fed animals subjected to long-term acclimation to different food qualities and then deprived of food for a short period. Although growth in mass cease immediately when the food is removed, some growth related processes such as protein synthesis would probably continue for a while during a period of fasting. As respiration associated with growth is a significant fraction of the total, the metabolic rates from this treatment are probably intermediates between the feeding metabolic rate and the BMR. Thus the starvation treatment (treatment 4) appears to be the best indicator of basal metabolism, although down regulation (metabolic depression) under starvation is possible as observed for *Daphnia* (Glazier & Calow, 1992).

The respiration measurements together with the model predictions strongly suggest that excess C in *B. calyciflorus* is not respired. In the model, food intake and assimilation efficiencies were constant, and the release of excess assimilated C was postabsorptive. An alternative to respiration as a disposal mechanism is excretion of surplus C as DOC. Two previous investigations both demonstrated high release of DOC in *B. plicatilis* (Olsen *et al.*, 2002; Vadstein *et al.*, 2003). The origin of this grazer-derived DOC was not clear, but may have been excretion. The study of Darchambeau *et al.* (2003) suggested that *Daphnia* can release excess C by excretion of DOC as well as CO_2. Yet another alternative regulatory mechanism to achieve homeostatic balance that could be used by *B. calyciflorus* is reduced C AE for nutrient deficient food. This could be incorporated in the model, and thus excess C would be released as faecal material (POC and DOC). The study of DeMott *et al.* (1998) showed that *Daphnia* can use such a decrease in AE of C coupled to a high P AE to cope with P-deficient high C diets. Thus, further studies are clearly needed to reveal how *B. calyciflorus* cope with high dietary C.

In conclusion, P- and N-limited food had a strong effect on growth of *B. calyciflorus*, indicating the need for stoichiometric regulation of excess ingested C. Furthermore, our study shows that this rotifer does not use respiration as a physiological mechanism in coping with high dietary C, in which case excess C must be released in other ways, either as faecal material or excreted as DOC. Different zooplankton taxa may regulate the release of ingested excess C in different ways (defecation, excretion or respiration). This has potentially important implications for the sequestration of C in ecosystems. If regulation occurs by adjusting assimilation within the gut one would expect an increased vertical flux of POC of faecal origin, to the sediments of lakes. Excretion of excess C as DOC would lead to recycling of organic C, a potential source for bacterial production. The composition of the zooplankton community and the physiology of dominating species could therefore be important factors in determining the C-flux in nutrient limited systems.

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[4] **Daufresne M.** and O. Renault (2006). Population fluctuations, regulation and limitation in stream-living brown trout. *Oikos.* **113**, 459–468. (13)

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Population fluctuations, regulation and limitation in stream-living brown trout

Martin Daufresne and Olivier Renault

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Determining causes of variation in population size and identifying factors responsible for fluctuations in species abundance are crucial questions both in theoretical and applied ecology. Based on the analysis of abundance time series, many studies have concluded that population dynamics of the stream-living brown trout (*Salmo trutta* L.) are mainly driven by year-to-year variation in the discharge level during emergence. Endogenous regulatory processes have often been considered as weak explanations for these fluctuations. This led some authors to consider that brown trout was able to persist in time with no operation of density-dependent processes. Using a model of population dynamics, we studied the influence of both discharge level during emergence and density-dependent regulatory processes on population limitation and fluctuations. We show that density-dependent and density-independent processes can act together on population density and stability at equilibrium (limitation process). We also show that the effects of internal feedbacks regulating population may often be invisible when analyzing abundance fluctuations at the interannual scale. Our results question the accuracy of studies based on the analysis of interannual fluctuations in abundance to identify processes driving population density at equilibrium.

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Determining sources of variation in the size of populations and identifying factors causing fluctuations in species' abundance are crucial questions in ecology (Begon et al. 1987). The relative merits of density-dependent and density-independent processes in explaining population fluctuations have been widely debated (Nicholson 1933, Haldane 1953, Andrewartha and Birch 1954). Today, these patterns are still not fully understood and retain a great deal of interest (Sinclair and Pech 1996, Ranta et al. 2000, Ricklefs and Miller 2000, Paradis et al. 2002, Kammenga et al. 2003, Lobón-Cervià and Rincón 2004). Part of the debate arises from confusion in terminology and in the questions studied. For example, the main argument between Andrewartha and Birch (1954) and Nicholson (1958) resulted from the former being interested in population limitation, whereas the latter investigated population regulation (Sinclair and Pech 1996). Limitation is the process that sets equilibrium points; regulation is the process whereby population demographic rates decrease when population size increases (and vice versa), relative to some equilibrium. From this viewpoint, both environmental constraints and regulatory processes are likely to generate population fluctuations.

In the particular context of fish populations, recruitment has long been known to be a key factor in population fluctuations (Victor 1983, Doherty and

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Fowler 1994, Elliott 1994, Myers 2001) and it is said to be strongly influenced by environmental factors (Cushing 1995). However, internal factors also play a role. For instance, Sinclair (1989) showed that the earlier in the life cycle both density-dependent and density-independent factors affect a population, the greater the fluctuations in population abundance are. Fish populations are thus relevant subjects to study the relative influence of density-dependent and density-independent processes on population dynamics.

Salmonid recruitment has received substantial attention with respect to fisheries management. The anadromous brown trout (*Salmo trutta* L.) have been widely studied, showing that recruitment of stream-living brown trout was shrunk by winter and spring high flows (Allen 1951, Spina 2001, Cattanéo et al. 2002), especially if high flows occurred when recruits emerged from gravel nests (Allen 1951, Nehring and Anderson 1993, Nuhfer et al. 1994, Latterell et al. 1998, Liebig et al. 1999, Lobón-Cervià and Rincón 2004). In particular, Cattanéo et al. (2002) showed that this was the only hydrological constraint consistently influencing brown trout populations across 30 French streams. On the other hand, Elliott (1994) suggested that populations were regulated by a density-dependent recruit mortality. Such regulation is usually called stock-recruitment regulation because it describes the relationship between the number of recruits entering the population and the parent stock of fish (Ricker 1954). However, some authors failed to find stock-recruitment regulation in stream-living brown trout populations (Elliott and Hurley 1998, Cattanéo et al. 2002, Lobón-Cervià and Rincón 2004), which led Lobón-Cervià and Rincón (2004) to conclude that brown trout is the example of an environmentally driven animal species able to persist in time with little or no operation of density-dependent processes. These studies were well designed and greatly helped understand the factors causing population fluctuations. Nevertheless, they attempted to detect density dependence by using time series of abundance. In fact, the effectiveness of this approach is hindered by short temporal data sets (Woiwod and Hanski 1992) and measurement errors (Shenk et al. 1998), and it depends on the spatial scale of the study (Ray and Hastings 1996). In addition, studying population fluctuations alone should not be adequate to conclude on species' persistence (as was done by Lobón-Cervià and Rincón 2004), since persistence depends on both limitation and regulation.

We suggest that both external (environmental) and internal (density-dependent) processes are actually at play in driving brown trout population dynamics even if their respective effects are not easily detected by studying abundance time series. To test this hypothesis, we theoretically studied the cumulative influence of density-dependence and high-flow discharge at emergence on brown trout population dynamics by building a matrix population model based upon its life cycle. First, we analyzed the influence of density-dependent and density-independent factors on population limitation. Then we used a stochastic version of our model to study the visibility of regulatory processes modulating interannual population fluctuations.

Methods

The model

The brown trout (*Salmo trutta* L.) life cycle is displayed in Fig. 1. Adults spawn during December (Elliott 1994, Charles 1998, Gouraud et al. 1998). We considered a prebreeding census (October) and time is discrete (Caswell 2001). The population was subdivided into three age classes (Charles et al. 1998, Chaumot et al. 2002): the young of the year (YOY), the juveniles (two summers old), and the adults (more than two summers old).

Recruitment is a function of winter/spring (January–May) discharge and is density-dependent (Fig. 1B). Elliott (1994) showed that density-dependent regulation occurred during a critical period ranging from emergence to 33–70 days after emergence. Both field and experimental works revealed that emerging trout were sensitive to the discharge up to 12 days after emergence

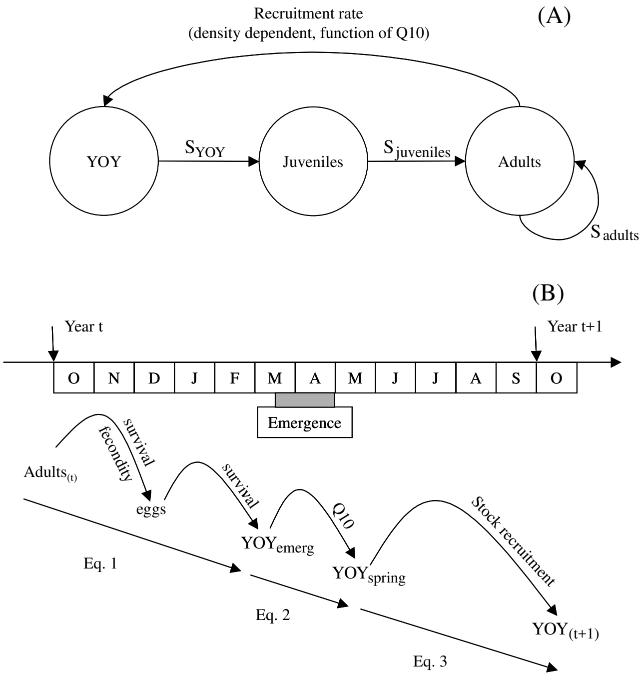

Fig. 1. (A) The brown trout life cycle was used for the construction of the matrix population model. S_{YOY}, $S_{juveniles}$, S_{adults} are survival transitions. The recruitment rate is density-dependent (the Ricker model) and depends on S_{adults}, sex ratio, female fecundity (number of eggs), and a synthetic variable of high winter–spring discharge (Q10). The transition matrix of this model is provided in Appendix 1. (B) Details of the adults-YOY transition.

(Ottaway and Forrest 1983, Jensen and Johnsen 1999, Daufresne et al. 2005), suggesting that discharge constraints occur before density-dependent regulation. For each year studied, the YOY density at emergence (YOY_{emerg}) was provided by:

$$YOY_{emerg} = n_{adults,t}(S_{adults})^{2/12}\sigma\ f\ (0.9) \quad (1)$$

where $n_{adults,t}$, $(S_{adults})^{2/12}$, σ, f, and (0.9) were, respectively, adult density in October (year t), adult survival between October and spawning (hence the exponent 2/12 since spawning occurs in December), sex ratio, fecundity, and under-gravel survival of eggs (Bardonnet and Prévost 1994). Then the effect of emergence discharge was taken into account by calculating the YOY density (YOY_{spring}) as:

$$YOY_{spring} = YOY_{emerg}\ S_w \quad (2)$$

where S_w was the extra mortality due to high discharges during the January–May period, calculated as $S_w = \exp((-0.73)\cdot Q10)$. Following Strange et al. (1992), we derived S_w from the negative slope of the relationship (consistent across 30 French streams) between the high discharge levels during January–May (hereafter referred to as Q10) and the ln-transformed abundance of YOY in early autumn established by Cattanéo et al. (2002). For a stream × year combination, Q10 was the ln transformed ratio between the 10th percentile of the daily discharges (during January–May) and the interannual median daily discharge. Finally, the density-dependent regulation was modeled by a Ricker stock-recruitment function (Ricker 1954, Caswell 2001), expressed as:

$$n_{yoy,t+1} = a\cdot YOY_{spring}\exp(-\gamma\ YOY_{spring}) \quad (3)$$

where $n_{yoy,t+1}$ was the YOY density in October (year t+1); a represented survival between spring and October at low density. The Ricker model has already been shown to describe salmonid recruitment well (Ricker 1954, 1975, 1989, Gardiner and Shackley 1991), especially for brown trout (Elliott 1994).

Finally, we considered constant survival probabilities between two consecutive censuses for all age classes (they were labeled S_{YOY}, $S_{juveniles}$ and S_{adults} for YOY, juveniles, and adults, respectively). The transition matrix (A_n) of the model is presented in Appendix 1.

Parameter values

Parameter values were chosen in order to fit those of resident brown trout living in headwater streams to avoid interspecific competition and reproductive migratory behavior.

Survival

$S_{YOY} = 0.51$ and $S_{juveniles} = 0.50$ were derived from Elliott and Hurley (1998) (assuming a constant mortality rate across time). These rates were consistent with values observed in other brown trout populations (Needham et al. 1945, McFadden and Cooper 1962, Hunt 1969, Mortensen 1977, Brynildson and Brynildson 1984, Olsen and Vollestad 2001, Baglinière and Maisse 2002). S_{adults} was fixed at 0.50 (Needham et al. 1945, McFadden and Cooper 1962, Hunt 1969, Baglinière and Maisse 2002).

Recruitment

Half the adults were considered female individuals (sex ratio $\sigma = 0.5$). All the adult females spawned. Fecundity (f) was of 208 eggs per female (average value for a 180-mm individual (mean adult size in three French headwater streams, H. Capra pers. comm.); Euzenat and Fournel 1976, Maisse et al. 1987, Gouraud et al. 1998, Olofosson and Mosegaard 1999).

We considered a 0.092 mean survival for the YOY between emergence and census at low density (no density dependence) and less than average discharge conditions during emergence (Brynildson and Brynildson 1984, Elliott and Hurley 1998, Baglinière and Maisse 2002). Parameter a was thus calculated as $a = (0.092)/\exp((-0.73)\cdot \bar{Q}10_{mean})$, where $\bar{Q}10_{mean}$ is the average of 30 (yearly) mean $\bar{Q}10$ provided by Cattanéo et al. (2002).

Gamma (γ) was set at 0.000279, in agreement with Elliott et al. (1997), who estimated this value from the stock recruitment relationship between egg density (number per 60 m^2) and YOY density in September (number per 60 m^2) in a sea trout population. This parameter is probably somewhat inaccurate to describe resident brown trout population dynamics, but to our knowledge, it has never been estimated for resident brown trout populations. We check below that population dynamics are not at all sensitive to this parameter.

Population density at equilibrium

The impact of the internal (limitation-related) factors was analyzed by computing the elasticity (i.e. relative sensitivity; Caswell 2001) of the equilibrium population density ($\hat{N}$) to the demographic parameters (p_i). The role of external (environment-related) factors was evaluated by studying the relative effects of Q10 and p_i on both level and local stability of $\hat{N}$. We paid particular attention to the range of $\bar{Q}10$ observed in the 30 sites studied by Cattanéo et al. (2002): 1.1–2.5.

$\hat{N}$ was calculated solving:

$$\hat{n} = A_{\hat{n}}\ \hat{n} \quad (4)$$

where $A_{\hat{n}}$ is the transition matrix containing the demographic parameters and $\hat{n}$ is the vector of population densities at equilibrium. $\hat{N}$ was calculated as $\hat{N} = \hat{n}_{YOY} + \hat{n}_{juveniles} + \hat{n}_{adults}$, $\hat{n}_{YOY}$, $\hat{n}_{juveniles}$ and $\hat{n}_{adults}$ being the densities in the different age classes at equilibrium.

The local stability of $\hat{N}$ was assessed calculating the greatest eigenvalue ($\lambda_1^{(B)}$) of the matrix $B = A_{\hat{n}} + \begin{pmatrix} 0 & 0 & \hat{n} \cdot \frac{\partial A}{\partial n_{adultes}} |_{\hat{n}} \end{pmatrix}$. The equilibrium is stable if $|\lambda_1^{(B)}| < 1$ (Caswell 2001).

The elasticities of $\hat{N}$ to the demographic parameters (p_i) are proportional to the corresponding elasticities of the greatest real eigenvalue (λ) of $A_{\hat{n}}$ (Caswell 2001). The elasticities of $\hat{N}$ to p_i are thus given by $\frac{p_i}{\hat{N}} \times \frac{\partial \lambda}{\partial p_i} |_{\hat{N}}$.

Stochasticity

Environmental variation from one year to another is likely to impede the detection of population regulatory processes. This may be particularly true in studies searching to detect density-dependent processes by looking for interannual correlations in population time series. In order to investigate this question, we generated a virtual 20-year time series with a stochastic version of the model described above. To keep analysis as simple as possible, we only added stochasticity in survival of the two main components of the stock-recruitment relationship (i.e. n_{YOY} and n_{adults}) by drawing Q10 and S_{adults} in beta distributions. Densities at $t = 0$ were densities at equilibrium calculated for mean Q10 and S_{adults}. The duration of 20 years was chosen to fit the maximum length of the actual time series.

Then we analyzed the influence of the mean and standard deviation of Q10 and S_{adults} on population density mean and variance, and on the residual sum of squares (RSS) of the linearized Ricker model $\ln\left(\frac{n_{YOY,t}}{n_{adults,t\text{-}1}}\right) = \ln(a) - b\ n_{adults,t\text{-}1}$ (Elliott 1994), where $n_{YOY,t}$ is the YOY density in October year t, and $n_{adults,t-1}$ is the adults density in October year $(t-1)$. We used RSS as a synthetic variable enlightening the ability of the Ricker model to describe trout recruitment. However, since the density-dependence could also be masked by low variation in the density of adults, we additionally analyzed the range (as a difference between maximum and minimum values) of density of adults in the 20 years simulated time series. Note that we did not aim here to discuss statistical methods for detecting density-dependence.

Results

Elasticity analysis of equilibrium

The elasticities of $\hat{N}$ (see Appendix 2 for literal expression) to the survival probabilities S_{YOY}, $S_{juveniles}$, S_{adults} were equal and greater than the elasticity of $\hat{N}$ to fecundity f (Table 1). The elasticity of $\hat{N}$ to γ was null. Actually, $1/\gamma$ was a multiplicative factor of $\hat{N}$ and thus only influenced the level of the population density but not its relative variability.

Relative effects of discharge and demographic parameters on equilibria

Population density

For parameter values classically measured in natura, the population density at equilibrium ($\hat{N}$) tended to be maximized by $\bar{Q}10_{mean}$. Low and high Q10 values yielded small population densities $\hat{N}$ (Fig. 2). Indeed, at low levels of discharge, density-dependent processes induced a strong decrease in population densities. At high levels of discharge, environmental constraints drive a population to extinction.

For $\bar{Q}10_{mean}$, $\hat{N}$ was not absolutely maximized by survival probabilities classically measured in natura (i.e. a, S_{YOY}, $S_{juveniles}$, S_{adults}) (Fig. 2). However, it is worth noting that on the range of $\bar{Q}10$ observed on 30 French streams (i.e. 1.1–2.5), $\hat{N}$ was not very sensitive to an increase in survival probabilities.

Conversely, for $\bar{Q}10_{mean}$, the classically observed fecundity value (f) led to a maximized population density at equilibrium (Fig. 2, bottom-right panel). Moreover, we found that on the observed range of $\bar{Q}10$ (i.e. 1.1–2.5), $\hat{N}$ was sensitive to fecundity.

Local stability

Areas of instability associated with high Q10 and low demographic parameter values correspond to extinction zones (Fig. 3). For high S_{YOY}, $S_{juveniles}$, f, a values and low Q10, we observed quasi-periodic equilibrium.

Table 1. Elasticities (i.e. relative sensitivities, Caswell 2001) of the population density at equilibrium ($\hat{N}$) to demographic parameters. S_{YOY}, $S_{juveniles}$, S_{adults} are the survival of the YOY, juveniles, and adults, respectively. f is the fecundity of adult females. γ and a are parameters of the stock-recruitment Ricker model. The parameters' actual values are in parentheses.

	S_{YOY} (0.5077)	$S_{juveniles}$ (0.5030)	S_{adults} (0.5000)	f (208)	γ (0.000279)	a (0.2950)
Elasticity of $\hat{N}$	0.0013	0.0013	0.0012	–0.0005	0	0.0013

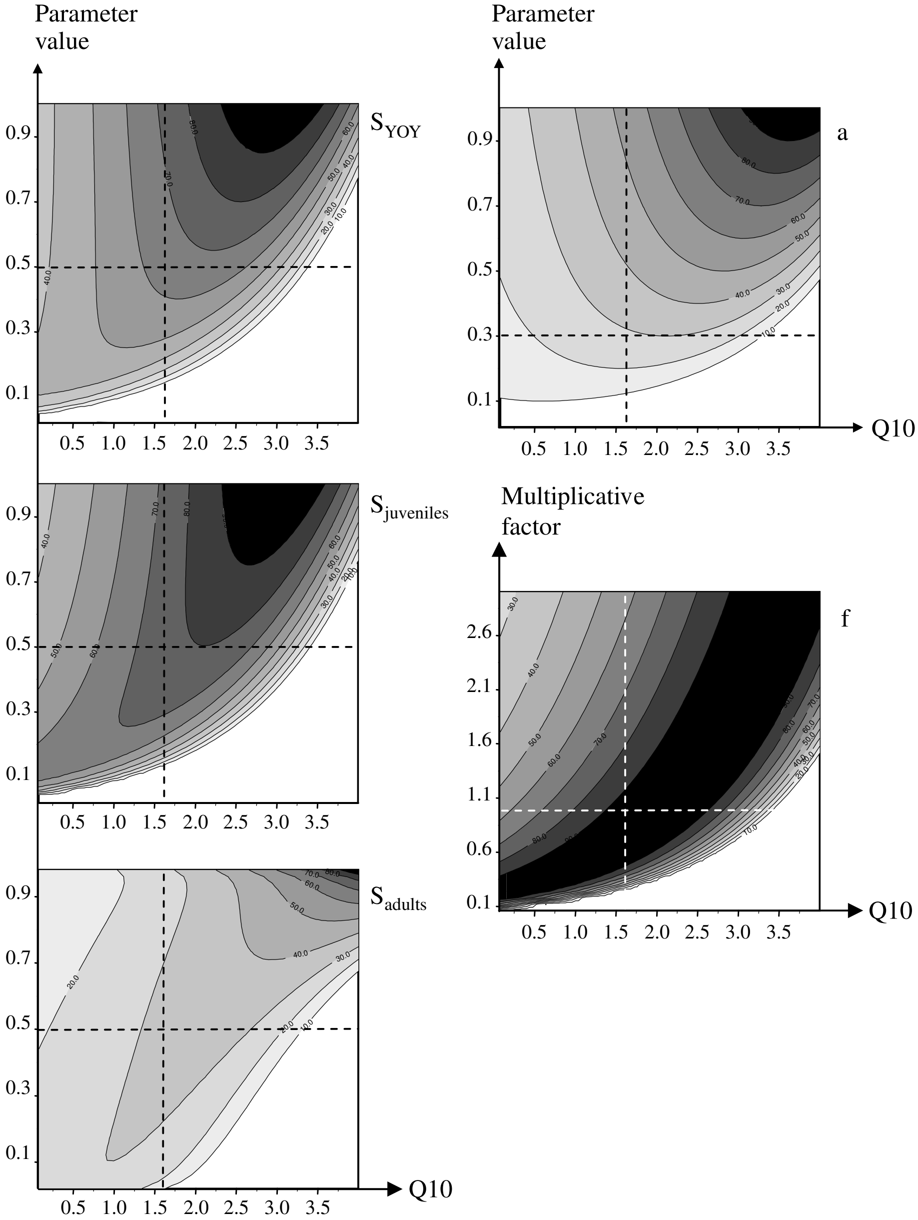

Fig. 2. Contour plots of the population densities at equilibrium as a function of demographic parameter values and a synthetic variable of high winter–spring discharge (Q10). For each graph, densities are expressed as percentages of the maximum density obtained over the Q10 and demographic parameter range. Densities increase from white (0–10%) to dark zones (>90%) by 10% intervals. The dashed line represents observed demographic parameter values and the average value of the 30 average (from yearly values) $\bar{Q}10$ by site ($\bar{Q}10_{mean}$) studied by Cattanéo et al. (2002). Parameter descriptions are given in the legend to Table 1.

Effects of interannual stochasticity of adult survival and level of discharge

Mean and standard deviation of population density

Interannual stochasticity of S_{adults} and Q10 slightly decreased the population density as compared to $\hat{N}$ predicted by the deterministic model (for instance, mean population density ranged from 80% to 100% of $\hat{N}$ for $\bar{Q}10 = 1.6$ and mean S_{adults} ($\bar{S}_{adults}$) $= 0.5$). This effect was most pronounced for low and highly variable S_{adults} (e.g. mean population density could drop to 15% of $\hat{N}$ at $\bar{S}_{adults} = 0.2$ and S_{adults} standard deviation $= 0.3$).

The standard deviation of population density increased when both S_{adults} and Q10 standard deviations increased. Again, this effect was more pronounced for low and highly variable S_{adults} (e.g. $\bar{S}_{adults} = 0.2$ and S_{adults} standard deviation $= 0.3$).

The ability to detect density-dependent recruitment

For $\bar{S}_{adults} = 0.5$ and $\bar{Q}10 = 1.6$ (which are values observed in the field) and for high S_{adults} standard deviations and low Q10 standard deviations, RSS were low (Fig. 4A) and ranges of density of adults were high (Fig. 4B). The Ricker model thus described the recruitment of brown trout rather well (Fig. 4C). Conversely, the model was quite inaccurate for high Q10 standard deviations (low RSS, Fig. 4A), especially for high S_{adults} standard deviations (Fig. 4D, black diamonds). Finally, for low S_{adults} standard deviations and low Q10 standard deviations, both RSS and ranges of density of adults were low (Fig. 4D, grey

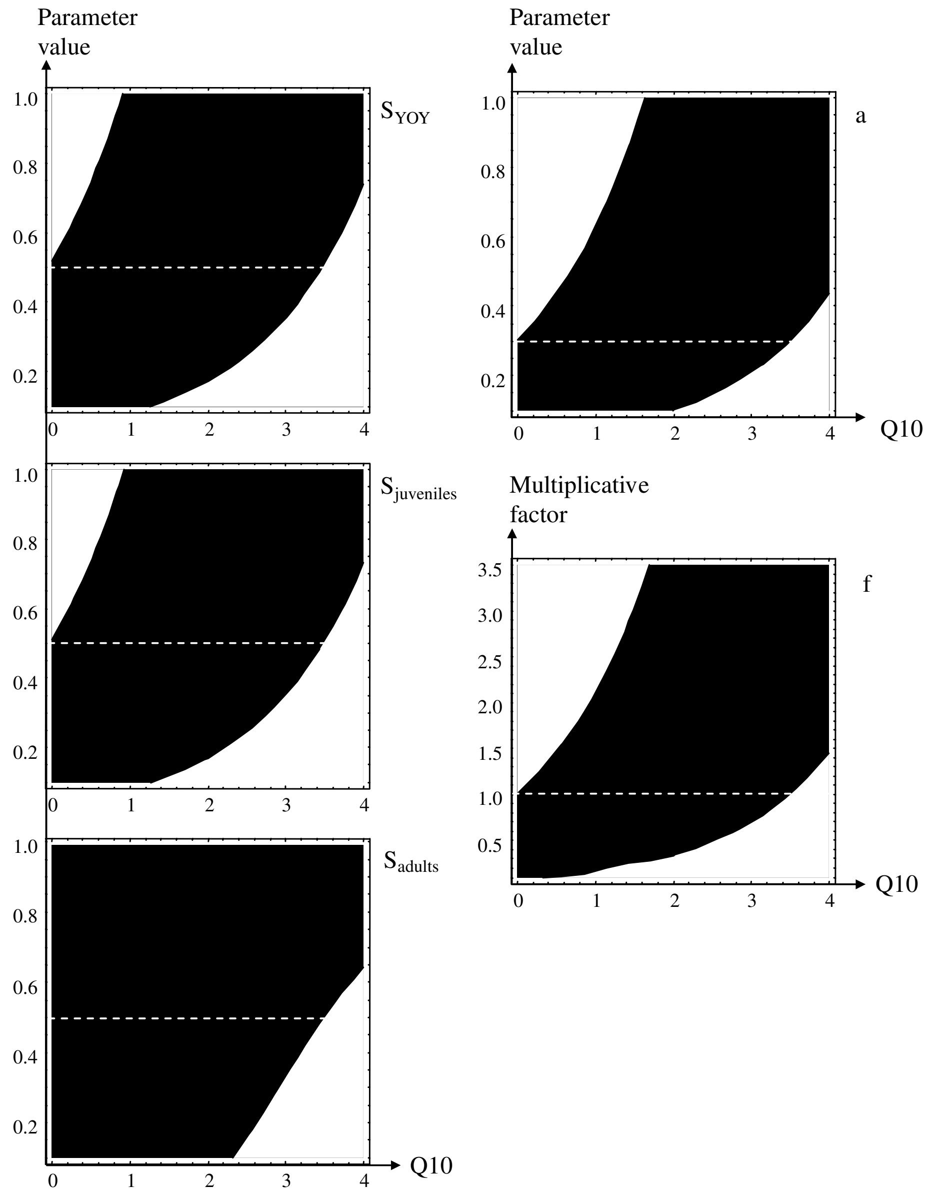

Fig. 3. Contour plots of the local stability of equilibrium as a function of demographic parameter values and a synthetic variable of high winter–spring discharge (Q10). Dark zones correspond to stable equilibrium. The dashed line represents observed demographic parameter values. Parameter descriptions are given in the legend to Table 1.

squares). Despite a good fit of the Ricker model, it was impossible to conclude about the density-dependence of the recruitment because of the low variation in the density of adults (and recruitment).

A proportional increase or decrease of $\bar{Q}10$ and $\bar{S}_{adults}$ simultaneously provided results closer to those observed at $\bar{Q}10 = 1.6$ and $\bar{S}_{adults} = 0.5$.

For fixed $\bar{S}_{adults}$, the area for which the Ricker model accurately described the recruitment (low RSS) decreased when the $\bar{Q}10$ increased, especially for high standard deviations of S_{adults} (Fig. 4A). Area for which it was possible to detect density-dependent processes thus tended to decrease.

Similar results were found for fixed $\bar{Q}10$ when $\bar{S}_{adults}$ decreased. Overlapping of low RSS and high ranges of density of adults conditions was almost inexistent (Fig. 4A, 4B). It was thus nearly impossible to detect density-dependent recruitment.

Discussion

The detection of density-dependent and/or density-independent factors acting on brown trout population dynamics usually relies on the analysis of time series fluctuations performed at the interannual scale. Using such methods, Lobón-Cervià and Rincón (2004) reached the conclusion that the brown trout was mainly an environmentally driven species able to persist with no or few internal biological feedbacks. Our results provide evidence that endogenous mechanisms are also likely to play an important role in this species dynamics. Actually, density-dependent and density-independent processes probably act together to determine population density and stability at equilibrium.

A simple model fitted with demographic data from the literature showed that population density and

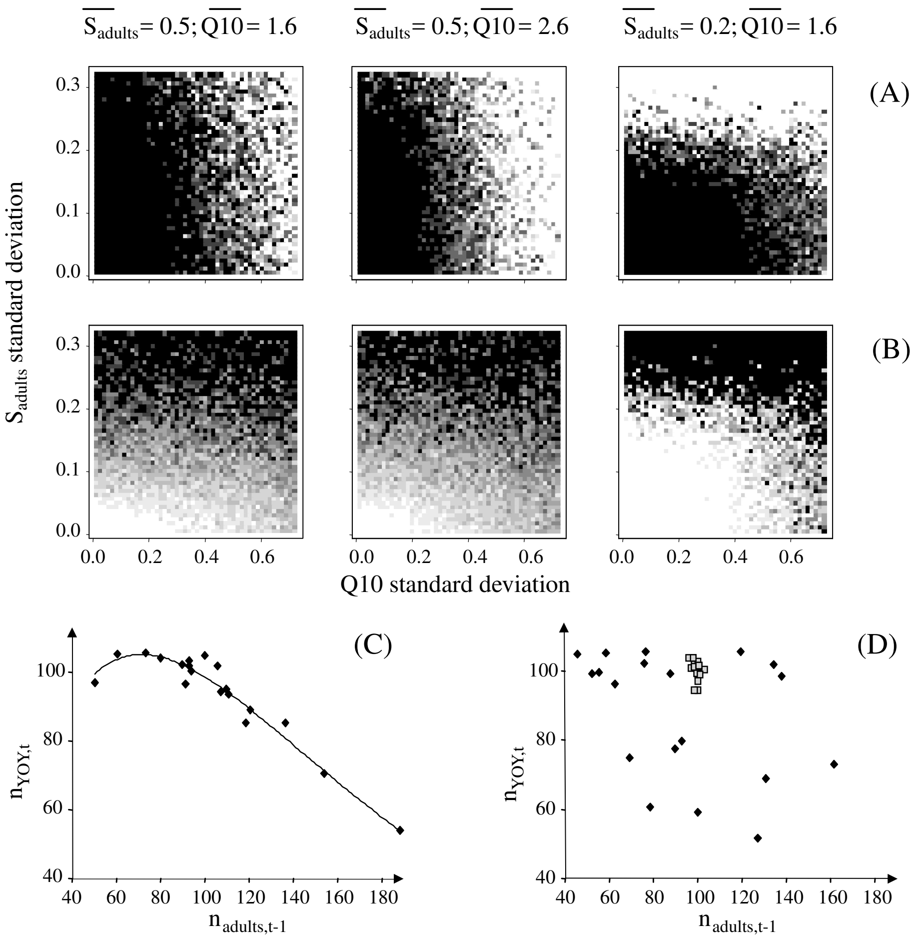

Fig. 4. (A) Levels plots of the residual sum of square (RSS) of the linearized Ricker model as a function of mean (μ) and standard deviation (SD) of a synthetic variable of high winter–spring discharge (Q10) and adult survival (S_{adults}). All statistics were calculated for 20-year densities simulated time series. To homogenize the plots, RSS were expressed as percentages of the 10th percentile of the RSS values calculated for mean Q10 and S_{adults} observed in nature (i.e. 1.6 and 0.5 respectively; upper-left panel values). The RSS decrease from white (0–10%) to dark zones (>90%) by 10% intervals. (B) Ranges of $n_{adults,t-1}$ (expressed as percentages of densities at equilibrium of adults) in simulated time series used to calculated the RSS. Ranges increase from white (0–10%) to dark zones (>90%) by 10% intervals. (C) Example of the relationship between $n_{YOY,t}$ and $n_{adults,t-1}$ (expressed as percentages of densities at equilibrium of YOY and adults, respectively) for low RSS and high range of $n_{adults,t-1}$ [Q10 (μ =1.6; SD =0.1); S_{adults} (μ = 0.5; SD =0.3)]. The curve fitted is estimated from the linearized Ricker model. (D) Examples of the relationship between $n_{YOY,t}$ and $n_{adults,t-1}$ for low RSS and low range of $n_{adults,t-1}$ [squares; Q10 (μ =1.6; SD =0.1); S_{adults} (μ =0.5; SD =0.01)] and for high RSS and high range of $n_{adults,t-1}$ [diamonds; Q10 (μ =1.6; SD = 0.7); S_{adults} (μ =0.5; SD =0.3)]. Note that for a 0.32 SD and 0.5 mean S_{adults}, the beta distribution used is close to a uniform distribution between 0 and 1. For a 0.72 SD and 1.6 mean Q10, the beta distribution used is close to the observed Q10 distribution (Cattanéo et al. 2002) but with a higher variance and roughly a uniform distribution between 1 and 2.

stability are optimal over ranges of stream discharge (our variable Q10 above) commonly observed in the field (Cattanéo et al. 2002). A level of discharge that is too high would induce substantial mortality within the YOY class that used to emerge from under-gravel nests during winter–spring floods. If these high levels of discharge were maintained for a long period of several years, they would substantially lower the population growth rate (hence the population density at equilibrium) and probably lead the population to extinction (Fig. 2 and 3, bottom-right-hand corner of all panels). Discharge levels that are too low also do not seem suitable for this species. Emerging YOY survivorship would then be too high, driving the populations into smaller and highly variable population densities due to density-dependent regulation (Fig. 2, 3).

Our results support the hypothesis that density-dependent processes predominate in benign environments, whereas density-independent processes predominate in harsh environments (Haldane 1953). The introduction of stochasticity on two parameters of the model (the adult survival probability and a synthetic measure of winter–spring discharges) did not change the results above, except that mean population sizes were slightly smaller than predicted at equilibrium because populations became extinct. This effect seemed important only for highly variable and low mean adult survival, but this type of situation is probably rare in natural headwater streams. The studies conducted by Elliott and Hurley (1998) and Lobón-Cervià (2003) indicated that variability in adult survival was low. Moreover, mean survival after the first year is generally high, ranging from 27% to 55% (Needham et al. 1945, McFadden and Cooper 1962, Hunt 1969, Mortensen 1977, Elliott and Hurley 1998, Olsen and Vollestad 2001, Baglinière and Maisse 2002). The lowest estimates are probably underestimated because of technical difficulties (catchability, tag loss, migration) or interspecific competition (Olsen and Vollestad 2001).

Finally, we found that brown trout fecundity optimized population size at equilibrium and that elasticity of the population density at equilibrium to fecundity was low. However, assuming no density-dependent recruitment regulation, the elasticity of the population growth rate (dominant eigenvalue λ of the transition matrix) to fecundity increased and equal positive elasticity of the population growth rate to survivals. Density-dependent recruitment thus probably made it possible to cope with recruit mortality due to harsh environmental conditions during emergence without an intuitive increase in fecundity. This could explain the low fecundity of the brown trout compared to other European fish species (Bruslé and Quignard 2001) despite harsh conditions during early stages of development, which generally refer more to r-environments than to K-environments (Reznick et al. 2002).

Maximization of the population density at equilibrium under mean discharge conditions during emergence is probably helpful to prevent population extinction. Thus, contrary to the conclusions reached by Lobón-Cervià and Rincón (2004), internal regulatory mechanisms might play a significant role in persistence of brown trout populations. We showed that the visibility of density-dependent and density-independent processes at the interannual scale was context-dependent. This could explain the paradoxical conclusions on the main factors that drive population fluctuations in the brown trout (e.g. Elliott 1994 compared to Lobón-Cervià and Rincón 2004). We found that the Ricker model was quite inaccurate in describing brown trout recruitment under highly variable discharge conditions. The observed standard deviation of the variable Q10 was 0.47 (Cattanéo et al. 2002). Considering also that interannual variability of adult survival is often low, it is not surprising that many authors have failed to find stock-recruitment relationships by studying the fluctuation in abundance of the YOY and adult brown trout (Fig. 4). With this level of average discharge variability, the fluctuations in population size are mainly driven by environmental constraints, as revealed by numerous studies (Allen 1951, Solomon et al. 1980, Nehring and Anderson 1993, Nuhfer et al. 1994, Liebig et al. 1999, Spina 2001, Cattanéo et al. 2002, Lobón-Cervià and Rincón 2004).

Our results add weight to those of Ranta et al. (2000), who symmetrically showed that regulatory processes could mask the effect of environmental noise on population dynamics. Clearly, these findings suggest that studies based on analysis of interannual abundance fluctuations could result in biased conclusions on the relative influence of density-dependent and density-independent mechanisms on population limitation. Consequently, this also questions the relevance of the classical suggestions made concerning population management. Regarding the brown trout, such suggestions are usually based upon analyses highlighting interannual hydrological constraints on abundance fluctuations, and often recommend limitations of the flow downstream from dams during the emergence period in regulated streams (Cattanéo et al. 2002). This proposal could be valuable at the interannual scale to increase the strength of a single cohort. However, our results suggest that, at least for fixed demographic parameters, a decrease in the mean discharge level during emergence could induce a decrease in the population size at the long-term scale (i.e. at equilibrium) due to the effects of endogenous regulatory mechanisms (Fig. 2).

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Appendix 1

Transition matrix of the model. S_{YOY}, $S_{juveniles}$, S_{adults} are the survival of the YOY, juveniles, and adults, respectively; f is the fecundity of adult females; σ is the sex ratio; S_w is the extramortality due to high discharges in January–May; γ and a are parameters of the stock-recruitment Ricker model.

$$A_n = \begin{pmatrix} 0 & 0 & a(0.9)(S_{adults})^{2/12}\sigma\ f\ S_w \exp(-\gamma(0.9)(S_{adults})^{2/12}\sigma\ f\ S_w n_{adults}) \\ S_{YOY} & 0 & 0 \\ 0 & S_{juveniles} & S_{adults} \end{pmatrix}$$

Appendix 2

Literal expression of the population density at equilibrium ($\hat{N}$). S_{YOY}, $S_{juveniles}$, S_{adults} are the survival of the YOY, juveniles, and adults, respectively; f is the fecundity of adult females; σ is the sex ratio; Q10 is a synthetic variable of high discharges in January–May; γ and a are parameters of the stock-recruitment Ricker model. $\hat{n}_{adults}$ is the adults density at equilibrium.

Equation 1 gives:

$$\hat{n}_{adults} = 0,\ \text{or, for } S_{adults} \neq 0 \text{ and } S_{adults} \neq 1{:}\ \hat{n}_{adults} = \frac{\exp(0.73\ Q10)}{-\gamma\ S_{adults}^{1/6}\sigma}\left(0.73\ Q10 - \ln\left(\frac{a\ f\ S_{YOY} S_{juveniles} S_{adults}^{1/6}\sigma}{1 - S_{adults}}\right)\right),$$

and

$$\hat{N} = 0,\ \text{or, for non-null age-class survivals and } S_{adults} \neq 1{:}\hat{N} = \hat{n}_{adults}\left(1 + \frac{1 - S_{adults}}{S_{juveniles}} + \frac{1 - S_{adults}}{S_{YOY}\ S_{juveniles}}\right).$$

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Downstream displacement of post-emergent brown trout: effects of development stage and water velocity

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Brown trout *Salmo trutta* were introduced at hatching into distinct sections of two parallel artificial channels, one with a constant low velocity (control) and one with velocity changes (experimental), at such times as to produce 12, 3 and 0 day old fish (age after emergences) when the velocity was changed in the experimental channel. This experimental design was repeated in 2002 and 2003 at comparable dates. Young brown trout were sensitive to an increased water velocity for 5 to 6 days after emergence. Water velocity modified the displacement patterns qualitatively but not quantitatively. Eighty per cent of fish moved downstream at all water velocities. Velocity changes, however, advanced the time by which 80% of the fish had displaced downstream.

Key words: brown trout; downstream displacement; emergence; stage of development; water velocity.

INTRODUCTION

As early stages of development are particularly sensitive to biotic and abiotic constraints, studying these stages in terms of survival is essential in population ecology. In the particular context of stream ecosystems, salmonids, and especially brown trout *Salmo trutta* L., have received considerable attention relative to other fish groups. Several studies showed that high flows in winter and spring reduce young-of-the-year (YOY) brown trout density (Allen, 1951; Spina, 2001; Cattanéo *et al.*, 2002), especially if high flows occurred during emergence (Allen, 1951; Nehring & Anderson, 1993; Nuhfer *et al.*, 1994; Latterell *et al.*, 1998; Liebig *et al.*, 1999). The mechanisms by which high flows during emergence influence YOY losses, however, are still unclear.

In brown trout, a first phase of downstream displacement occurs immediately after emergence (Huet, 1961; Elliott, 1966; Timmermans, 1966). As a first step in the understanding of YOY losses under high discharge conditions, some authors studied the influence of water velocity (hereafter called velocity) on such displacements. Ottaway & Clarke (1981) and Crisp & Hurley (1991*a*)

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experimentally submitted emerging brown trout to velocity changes. They observed a positive relationship between displacement rate and velocity. The strength of the relation seemed to depend on the stage of fish development. Fish were introduced, however, directly into the experimental channels without emerging naturally from the gravel and some experiments were performed pooling fish at different stages of development (Crisp & Hurley, 1991*a*). As a consequence, the critical stage of development affected by velocity in terms of the number of days since emergence could not be defined.

In further experiments, Ottaway & Forrest (1983) and Crisp (1991) dealt with this difficulty by introducing fish long before emergence in different channels. In each channel, the velocity was set at different but constant values. Displacement mainly occurred when young brown trout entered the free-feeding stage and the rate of displacement was higher at high than at low velocities. The final density was similar at all velocities (Crisp, 1991) but was approached more and more rapidly as velocity increased.

These experiments were useful contributions in determining the stage of development that was most sensitive to velocity. Fish, however, were submitted to constant velocity conditions. In a final experiment, Crisp & Hurley (1991*b*) showed that changes in velocity (from high to low or low to high) were associated with a higher displacement rate than under constant velocity conditions. Therefore, further experiments seemed essential to determine the stage of development most sensitive to changes in velocity conditions. In this present study, the simultaneous influence of velocity changes on the downstream displacement of brown trout groups at different stages (0, 3 and 12 days after emergence) was studied in an artificial flume. The experimental design included controls, involved fish emerging *in situ* and was repeated in comparable periods in 2002 and 2003. The latter consideration meant experiments could be repeated under comparable night duration, which is known to influence salmonid downstream displacement (Crisp, 1991; Bardonnet *et al.*, 1993).

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The simultaneous influence of water velocity changes on downstream displacement of 12, 3 and 0 day-old brown trout (age after emergence) was investigated. Fish at each stage of development were introduced at hatching into distinct sections of two parallel artificial channels (experimental and control). Initially, the velocity was similar in both channels but in the experimental channel it was increased and then decreased between days $d = -1$ and $d = 2$, where $d = 0$ indicates the day on which the velocity reached its maximum value (Fig. 1). Channels were separated into four successive sections by traps. Three sections were used in each channel (those with the most homogeneous hydraulics). In the three sections, newly hatched brown trout were introduced at dates *c.* $d = -30$, $d = -20$ and $d = -16$. The dates were chosen as a function of temperature, with the objective that the three sections would contain 12, 3 and 0 day-old groups of brown trout (age after emergence) at $d = 0$. This experimental design was repeated in 2002 and 2003 at comparable dates, with $d = 0$ on 2 April 2002 and $d = 0$ on 29 March 2003 (see Table I for full details).

Artificial channels

Both channels had a length of 40 m and a slope of 0·5% (Fig. 2; Gaudin & Caillère, 1985). They were filled with gravel (1 to 5 cm size range). Natural food was available in

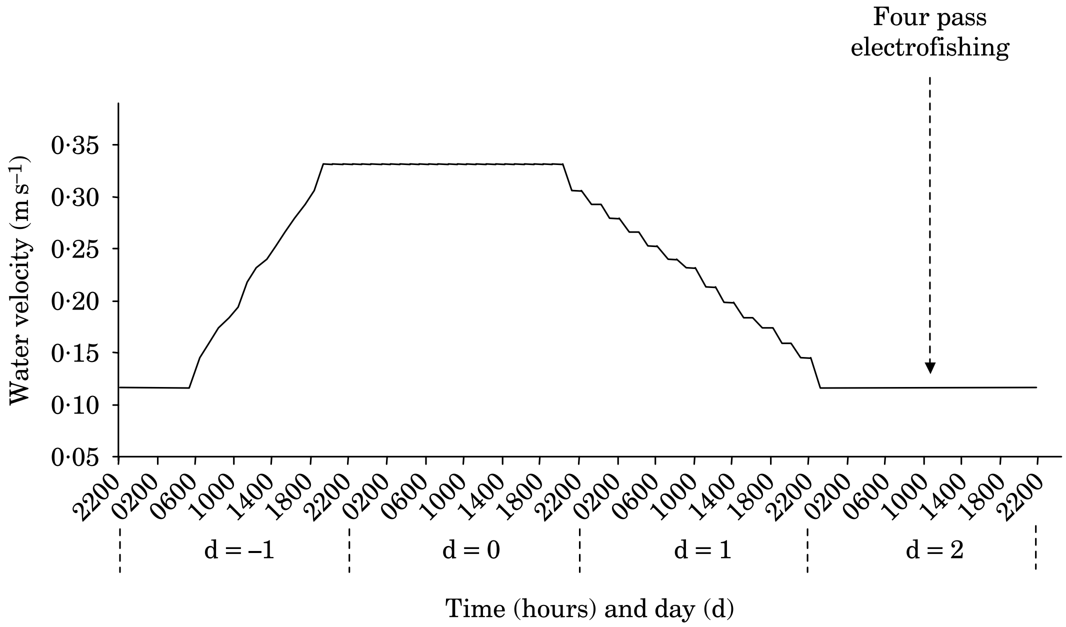

FIG. 1. Average water velocity (measured at 40% of total depth above the bed, every 10 cm, along three transects regularly spaced along each section) of the experimental channel from day d = −1 to day d = 2, where d = 0 is the day on which the velocity reached its maximum value. The date of the four-pass electrofishing of the channel (*i.e.* the end of the experiment) is given.

the channels. Ground water re-circulated by pumps, with a constant fresh supply of 1 l s^{-1}, was used. Water velocity was controlled by adjusting the discharge rate (pumps) into each channel.

For both 2002 and 2003, the mean ± 95% CL width of sections was 0·94 ± 0·01 m (calculated from $n = 60$ regularly spaced measurements in the sections). During low-velocity periods, the mean ± 95% CL velocity (at 40% of total depth above the bed and measured every 10 cm along three transects regularly spaced along each section) was 12·0 ± 0·3 cm s^{-1} ($n = 320$ measuring points) and mean ± 95% CL depth (same measuring points) was 7·0 ± 0·1 cm. For maximum velocity in the experimental channel, the mean ± 95% CL velocity was 33·0 ± 1·2 cm s^{-1} ($n = 162$ measuring points) and the mean ± 95% CL depth was 19·5 ± 0·1 cm. Point velocities varied spatially across sections, especially in upstream parts of the sections but were well contrasted between low

TABLE I. Main characteristics of the experiments. d, number of days. Velocity was increased then decreased between d = −1 and d = 2 in the experimental channel. d = 0, the day on which the velocity reached its maximum value. The 0 d, 3 d and 12 d groups represented individuals that were 0, 3 and 12 days old (age after emergence) at d = 0

	First experiment	Second experiment
Date of introduction		
12 d group	d = −29	d = −31
3 d group	d = −19	d = −21
20 d group	d = −15	d = −17
Mean ± 95% CL egg mass at eyeing (g)	0·0780 ± 0·0002	0·1020 ± 0·0004
Mean ± 95% CL water temperature between d = −1 and d = 2 (° C)	16·4 ± 0·1	16·2 ± 0·1

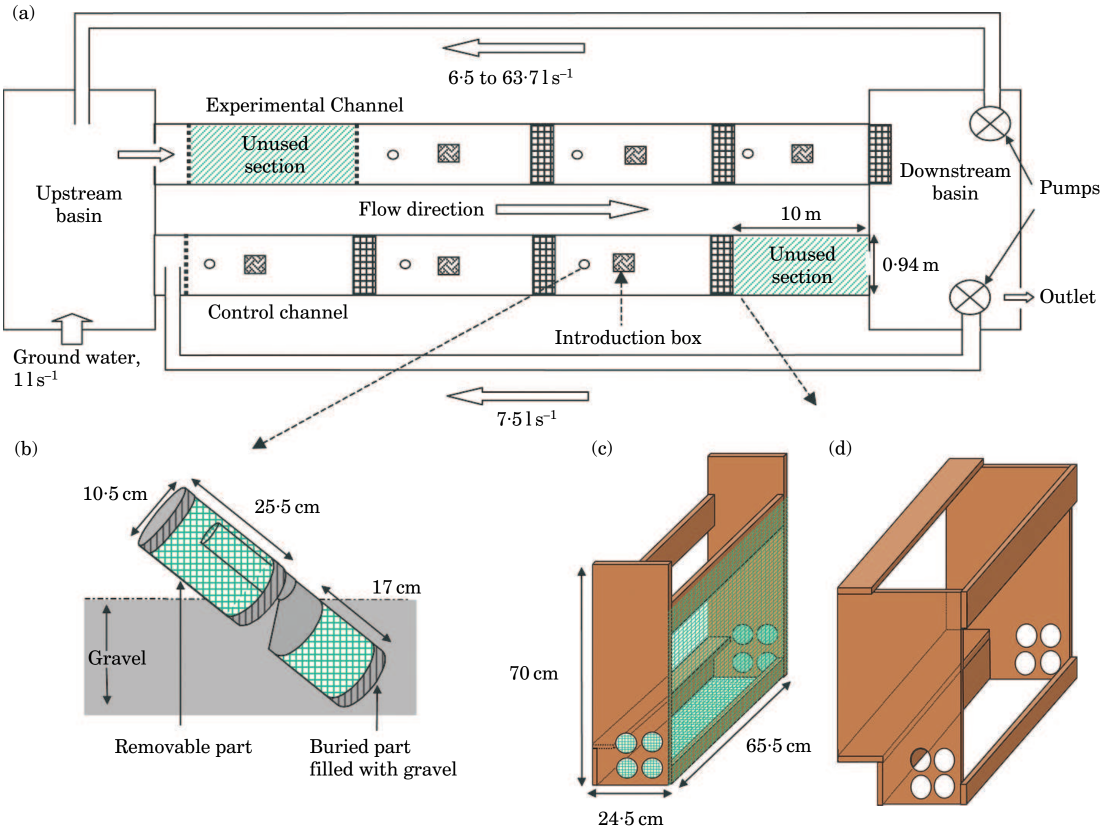

FIG. 2. (a) Schematic representation of the two artificial channels. Introduction box is the buried box (30 cm wide × 30 cm long × 15 cm high, wire netting mesh = 2 mm) in which 250 fry were introduced at hatching. (b) Details of the emergence box (used to check for the timing of emergence of the fish) are given. (c) Removal trap and (d) fixed part of the dispersal trap are also shown.

and high velocity and comparable between years. The low velocity value corresponded to a low fish displacement rate (Ottaway & Clarke, 1981). The high velocity value matched observed values on spawning sites (Haury *et al.*, 1991), but such velocities were generally avoided by newly emerged fishes (Gaudin *et al.*, 1995; Heggenes *et al.*, 2002).

To control velocity change, before each experiment, additional measurements were taken in the experimental channel at velocities ranging from low to high. From d = −1, 0500 hours (solar time) to d = −1, 1900 hours, average velocity was increased every hour ($+1{\cdot}5$ cm s^{-1} h^{-1}; Fig. 1). From d = 0, 2000 hours to d = 2, 2300 hours, average velocity was decreased every 2 hours [$-1{\cdot}5$ cm s^{-1} $(2\ h)^{-1}$; Fig. 1].

Fish

Each of the groups of newly hatched brown trout introduced in the three sections of each channel (*c.* d = −30, d = −20 and d = −16, Table I) contained 250 individuals. They were all obtained from eggs of hatchery-reared wild fish. Progenitors were from the Furans River (69 km east of Lyon, France). All eggs were fertilized simultaneously following traditional methods and reared at 9° C until they became eyed. Eggs were then separated into three equal groups and incubated at 3° C. The incubation temperature of each group was then gradually increased to 12° C until hatching. The dates of the gradual warming of the incubation temperature of each group were adjusted to obtain the expected developmental stage at d = 0. Dates of emergence were predicted according to Crisp's (1992) equations (which provided an assessment of the brown trout development stage using mean daily temperatures) and from the fish farmer's experience for the Furans River stock. In each channel, the association between egg group and section was random. Fish were introduced into the middle of the sections in a buried box (30 cm wide × 30 cm long × 15 cm high, wire netting mesh = 2 mm) filled with cleaned gravel.

Measuring the timing of emergence

In each section, 100 additional newly hatched fish (reared together with the other eggs) were introduced in closed emergence boxes, in order to check for the emergence dates of all groups [Fig. 2(b)]. The boxes, adapted from Gaudin & Persat (1985) were made of two connected cylinders: a buried part (10 cm in diameter × 17 cm long, wire netting mesh = 2 mm) filled with cleaned gravel where the fish were placed; and an upper removable part (10 cm in diameter × 25·5 cm long, wire netting mesh = 2 mm) in which fish were trapped as they emerged from the gravel. After the fish were introduced, the emergence boxes were checked each day at 1100 hours (solar time). At the end of the experiment (d = 2, 11 h), emergence boxes were emptied to evaluate fish loss.

Measuring the downstream displacement of fish

Experimental sections were separated by displacement traps [Fig. 2(c), (d)], used to collect fish moving downstream. They consisted of a fixed part receiving a removable trap. Two removal traps were made for each fixed part so that they could be replaced immediately when checked. Displacement traps were cleaned regularly to avoid blockage. From the first introduction on d = −2, displacement traps were checked every day at 1100 hours. Between d = −1, 0500 hours and d = 2, 1100 hours (solar time), the displacement traps were checked every 6 h (2300, 0500, 1100 and 1700 hours). This provided information on the influence of night on displacement rates. At the end of the experiment (d = 2, 1100 hours), all sections were electrofished with four passes to estimate the residual population in each section. Estimated efficiency of electrofishing ranged from 0·73 to 1 (Carle & Strube, 1978). At this efficiency rate, a four-pass electrofishing operation removes 99–100% of the population. In all cases, no fish were caught during the fourth pass. All fish trapped or electrofished were weighed.

STATISTICAL ANALYSIS

Total loss and emergence in boxes

The total loss in each emergence box was estimated as the difference between the initial number of fish introduced and the total number of fish emerged and recovered at d = 2.

The cumulative proportion of emerged fish in a box at sample i, E_i, was calculated as the ratio between the total number of emerged fish at sample i and the number of fish not lost before sample i. The number of fish not lost before sample i was obtained by summing the total number of emerged fish at sample i with the number of fish, not lost, remaining in the buried part of the box. This involved partitioning the total loss into instantaneous losses, as done by Crisp & Hurley (1991*a*): a constant loss rate was estimated that provided the observed total loss knowing the number of emerged fish at each sample; this estimation was obtained numerically using a simplex method.

Total loss and dispersion estimates in sections

The total loss in each section was estimated as the difference between the initial number of fish introduced and the total number of fish trapped and electrofished.

A cumulative displacement rate Cd_i was estimated as the ratio between the total number of fish displaced before sample i and the total number of fish not lost during the entire experiment.

For each section, the displacement rate at sample j, Γ_j, was calculated as the ratio between the number of fish trapped at sample j (n_j) and the number of emerged fish remaining in the section (not trapped before, not lost) at sample j. This again involved partitioning the total loss into instantaneous losses as done for the boxes. Finally, $\Gamma_j = n_j\,(S_j E_j)^{-1}$, where S_j is the potential number of fish remaining in the section at sample j (accounting for lost and trapped fish) and E_j is the cumulative proportion of emerged fish at sample j. E_j is derived from the relevant E_i value given by box samples, knowing that fish mainly emerge at night (Crisp, 1991; Bardonnet *et al.*, 1993).

Analysis of Γ_j values between $d = -1$ and $d = 2$

For each group, the effects of velocity (average from hourly values between sample j–1 and j; continuous variable) and night-time (2300 to 0500 hours; categorical variable) on Γ_j differences between the two channels (control and experimental) were tested using generalized linear models (GLM) (with an identity link function and a Gaussian dependent variable; McCullagh & Nelder, 1983). The dependent variable for these tests was the extra displacement rate R_j defined as:

$$R_j = (\Gamma_{experimental,j} - \Gamma_{control,j})\left\{\sqrt{p_j(1-p_j)[(S_{experimental,j}E_{experimental,j})^{-1} + (S_{control,j}E_{control,j})^{-1}]}\right\}^{-1},$$

with

$$p_j = (S_{experimental,j}\,E_{experimental,j}\,\Gamma_{experimental,j} + S_{control,j}\,E_{control,j}\,\Gamma_{control,j})(S_{experimental,j}\,E_{experimental,j} + S_{control,j}\,E_{control,j})^{-1}.$$

R_j is the difference between Γ_j in the two channels, weighted by the number of emerged fish remaining in the sections. $R_j = 0$ when $\Gamma_{experimental,j} = \Gamma_{control,j}$. It is used in proportion comparison tests (Saporta, 1978) and accounts for non-equality in error rates.

To test for the accuracy of performing one model per group, a global model incorporating the velocity and night time as explanatory variables (model 1) was used. The fish group and the interactions between fish group and velocity and fish group and night-time as explanatory variables (model 2) were then added. The two models were compared using an analysis of deviance (McCullagh & Nelder, 1983). The difference between the residual deviances of two models is a χ^2 variable with n d.f. where n is the difference between the residual d.f. of the two models. This test checked whether the additional explanatory variables significantly decreased the residual deviance of the model.

Finally, to test the effect of the year (2002 or 2003; categorical variable) over all the experiments, a third model (model 3) was constructed, adding the year and the interactions between fish group and year to the explanatory variables of model 2. Model 3 and model 2 were compared using an analysis of deviance.

The influence of a particular sample (j) on Γ_j differences was tested using χ^2 tests. Estimators of the number of emerged fish remaining in the sections, however, are not integer numbers. For a conservative solution, the estimators of the number of emerged fish remaining in the sections were rounded to the nearest higher integer for the experimental channel and to the lower integer for the control channel.

To check for the influence of the estimation method of S_j and E_j on the results, an analysis was also performed in which it was considered that all the fish were emerged at any sample j ($E_j = 1$) and that all the observed loss occurred when fish were introduced into the channels. S_j were thus calculated as the difference between the total number of fish recovered at the end of the experiment (trapped or electrofished) and the total number of fish displaced before sample j, as was done by Ottaway & Clarke (1981).

Finally, for each section, the mean masses of trapped fish were compared with the masses of fish electrofished at d = 2 using a standard *t*-test. All tests were performed using S-plus software (Mathsoft, 2000).

RESULTS

TOTAL LOSS (EMERGENCE BOXES AND SECTIONS)

The total loss was similar between emergence boxes and sections in 2002 (1·2–15·6%) (Table II). The losses were more variable and higher in 2003 (Table II). In particular, losses were 57·6% of the 0d-group fish in the control

TABLE II. Total loss rate observed in the emergence boxes and in the different channel sections for each group of brown trout (see Table I). Loss rates were the proportion of fry which were not recovered (*i.e.* not trapped and not electrofished). These proportions were calculated for each section as a proportion of the total number of fish introduced (*i.e.* 100 for the emergence boxes and 250 for the sections). The number of fish electrofished at the end of the experiment in each section are in parentheses. High loss rates are in bold

Year	Group		Emergence box (%)	Section (%)
2002	0d	Control channel	6·0	15·6 (106)
		Experimental channel	8·0	12·4 (82)
	3d	Control channel	4·0	6·7 (45)
		Experimental channel	5·0	15·2 (41)
	12d	Control channel	2·0	4·0 (45)
		Experimental channel	11·0	1·2 (41)
2003	0d	Control channel	**99·0**	**57·6** (60)
		Experimental channel	**21·0**	5·2 (92)
	3d	Control channel	3·0	0·4 (53)
		Experimental channel	2·0	4·0 (48)
	12d	Control channel	2·0	5·9 (38)
		Experimental channel	0·0	**26·3** (26)

section, 26·3% of the 12d-group fish in the experimental section, 21·0% of the 0d-group fish in the control emergence box and 99% of the 0d-group fish in the experimental emergence box (only one fish was recovered).

EMERGENCE IN BOXES

The E_i values revealed that the synchronization between emergence of the different fish groups and increased velocity periods was well controlled and similar for both experiments (Fig. 3).

In 2002, most of the 12d-group fish (55%) emerged in both channels at $d = -13$. Most of the 3d-group fish (62%) emerged in the experimental channel at $d = -3$. Half of the 3d-group fish (48%) emerged in the control channel at $d = -3$. Most of the 0d-group fish (64%) emerged in both channels at $d = 0$.

In 2003, most of the 12d-group fish (63%) emerged in both channels at $d = -12$. Half of the 3d-group fish (*c.* 50%) emerged in both channels at $d = -3$. Most of the 0d-group fish (58%) emerged in the experimental channel at $d = 0$.

CUMULATIVE DISPLACEMENT RATE IN SECTIONS

About 80% of the 12d-group fish were already trapped 5 to 6 days after median emergence ($d = -7$, Fig. 4) in both channels. The velocity increase did not modify the global displacement dynamics of this fish group.

Similarly, *c.* 80% of the 3d-group fish were trapped at the end of the experiment (5 days after median emergence) in both channels. The velocity increase in the experimental channel, however, slightly accelerated downstream displacement patterns (see Fig. 4).

In contrast, displacement of the 0d-group fish differed between channels, with an immediate, stronger downstream displacement in the experimental channel during velocity increase (Fig. 4).

ANALYSIS OF Γ_J VALUES BETWEEN D = −1 AND D = 2

Because of an exceptionally high loss rate of the 0d-group fish in the control emergence box in 2003, E_i values from the 0d-group in the experimental emergence box were used to calculate Γ_j values of the 0d-group in the control section.

The GLMs per group highlighted a positive effect of high water velocity and night-time on R_j values of the 0d-group and a positive effect of night-time on R_j values of the 3d-group (Table III and Fig. 5). Sample-specific tests indicated that the latter effect was due to a high displacement rate of the 3d-group in the experimental channel during the first night (Fig. 5) in 2002 (χ^2 test, d.f. = 1, $P < 0{\cdot}01$ for both the 2300 and 0500 hour samples) and 2003 (χ^2 test, d.f. = 1, $P < 0{\cdot}01$ for the 2300 hours sample). Nothing influenced the 12d-group displacement. The displacement rates of the 12d-group were low (close to 5%; Fig. 5) except in the experimental channel on $d = 0$ at 0500 hours in 2003 (χ^2 test, d.f. = 1, $P < 0{\cdot}001$, $\Gamma_j = 14{\cdot}7\%$). Additional explanatory variables of model 2 significantly decreased the deviance of the model compared to model 1 (χ^2 test, d.f. = 6, $P < 0{\cdot}01$). Comparison of models 2 and 3 indicated that the year did not significantly influence the result (χ^2 test, d.f. = 3, $P > 0{\cdot}05$).

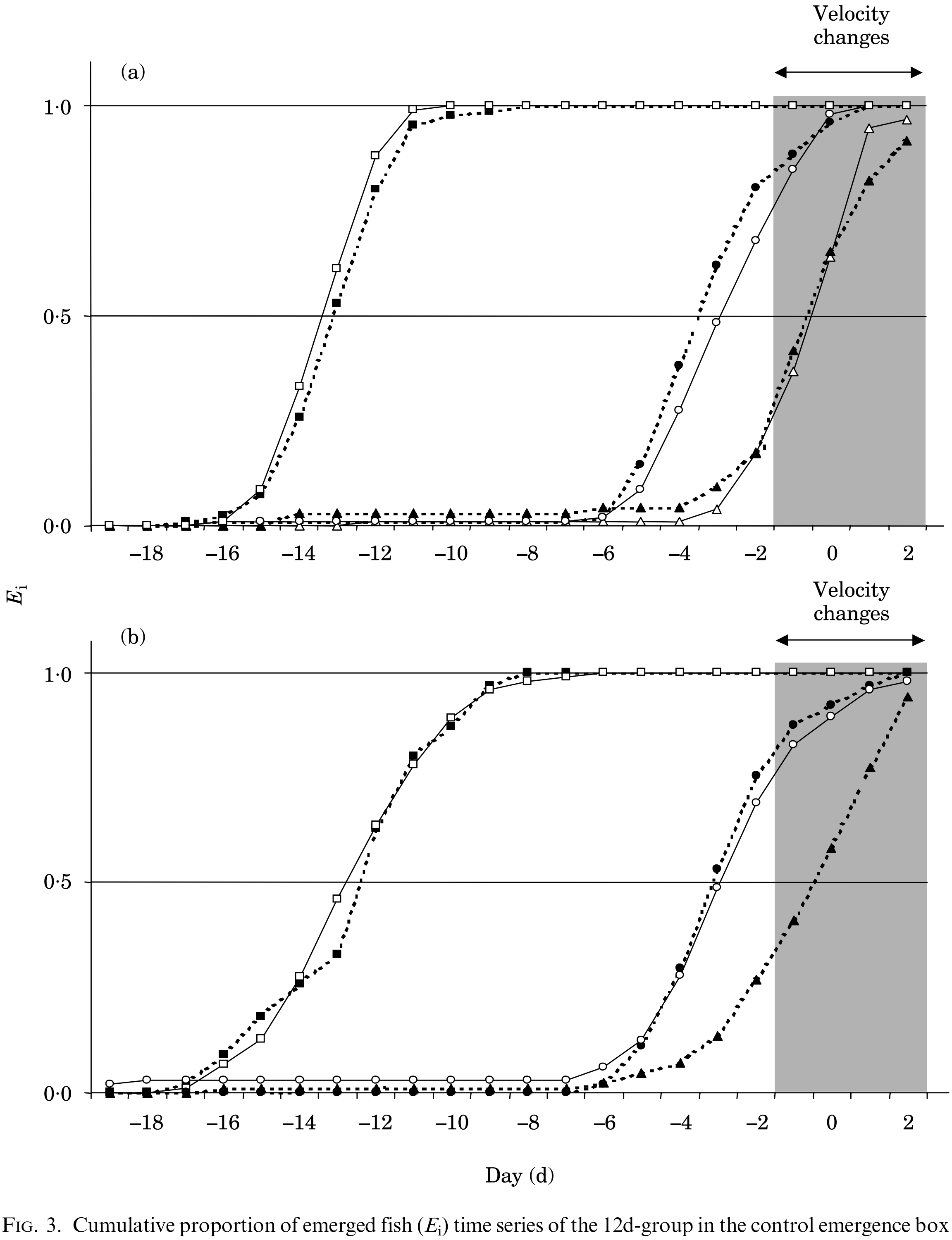

FIG. 3. Cumulative proportion of emerged fish (E_i) time series of the 12d-group in the control emergence box (–□–), the 12d-group in the experimental emergence box (--■--), the 3d-group in the control emergence box (—○—), the 3d-group in the experimental emergence box (--○--), the 0d-group in the control emergence box (—△—) and the 0d-group in the experimental emergence box (--▲--) in (a) 2002 and (b) 2003. ■, the period during which the velocity was increased then decreased in the experimental channel. Because of a high loss rate, the time series for the cumulative proportion of emerged fish of the 0d-group in the control emergence box in 2003 is not represented. (See Table I for group definitions.)

All these results were similar considering that $E_j = 1$ for any sample j and calculating S_j, as was done by Ottaway & Clarke (1981).

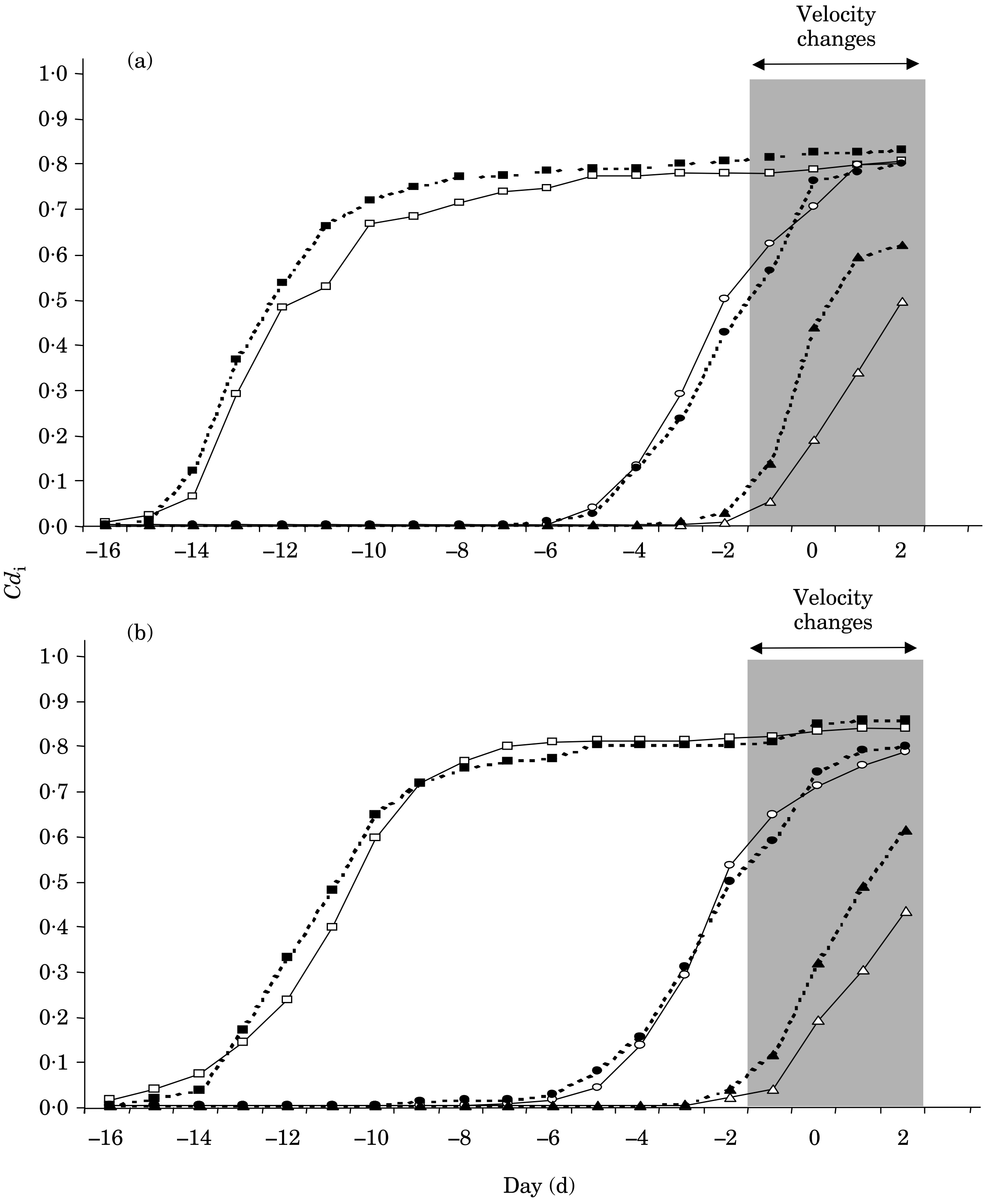

FIG. 4. Cumulative dispersal rate (Cd_i) of the 12d-group in the control channel (–□–), the 12d-group in the experimental channel (--■--), the 3d-group in the control channel (—○—), the 3d-group in the experimental channel (--●--), the 0d-group in the control channel (—△—), the 0d-group in the experimental channel (--▲--) in (a) 2002 and (b) 2003. (See Table I for group definitions.)

In 2002 and 2003, the non-displaced fish (*i.e.* electrofished at $d = 2$) were significantly larger than the fish trapped between $d = -1$ and $d = 2$ in all sections (*t*-tests, d.f. = 34 to 210, $P < 0{\cdot}01$). The mass difference appeared to be particularly pronounced for the 12d-group.

TABLE III. Results of the GLMs used on the extra dispersal rate R_j observed in the experimental channel between d = −1 and d = 2. (See Table I for group definitions)

Group		Value	s.e.	*t*	*P*
0d	Intercept	−1·86	0·76	−2·45	<**0·05**
	Velocity	10·33	2·92	3·54	<**0·01**
	Night-time	0·50	0·24	2·10	<**0·05**
			(Residual deviance = 32·64 with 23 d.f.)		
3d	Intercept	−1·21	1·04	−1·16	>0·05
	Velocity	4·94	4·01	1·23	>0·05
	Night-time	0·87	0·33	2·69	<**0·05**
			(Residual deviance = 61·62 with 23 d.f.)		
12d	Intercept	−0·40	0·53	−0·76	>0·05
	Velocity	1·73	2·05	0·85	>0·05
	Night-time	0·17	0·17	1·01	>0·05
			(Residual deviance = 16·10 with 23 d.f.)		

DISCUSSION

The results suggest that young brown trout were highly sensitive to velocity changes during the first few days after emergence, especially during night-time. Occurring during emergence, velocity changes induced strong downstream displacement. The displacement patterns of the 3d-group indicated that this effect lasted until 80% of young brown trout had moved downstream. Once these thresholds had been reached, very few individuals were displaced downstream, independent of all water velocity treatment. Fish from the 3d-and 12d-groups reached the same thresholds under constant, low velocity conditions but the period of displacement was much more extended (5 to 6 days after emergence compared to 3 days after emergence under velocity changes). In this way, velocity changes advanced the time by which 80% of the fish moved downstream. The first days after emergence correspond to the acquisition of swimming capacity (Héland, 1991). Then fish establish territories up to about 12 days after emergence (Héland, 1991). The important mass differences between displaced and non-displaced 12d-group fish revealed that the displacement pattern was probably driven more by competition for space 12 days after emergence.

The results were highly consistent in 2002 and 2003. This showed that the variable loss rate observed between sections and between years did not influence the downstream displacement patterns. In addition, a large maternal size could be considered as a competitive advantage for salmonids because it is negatively related to the young fish mortality rate (Einum & Fleming, 2000). Larger egg size in 2003 (*i.e.* larger maternal sizes; Elliott, 1994) (Table I), however, did not influence displacement patterns. Finally, for both years, displacement patterns were highly consistent in the control channel for all three groups (especially for the 3d-and 12d-groups), indicating that the different incubation conditions did not influence the results.

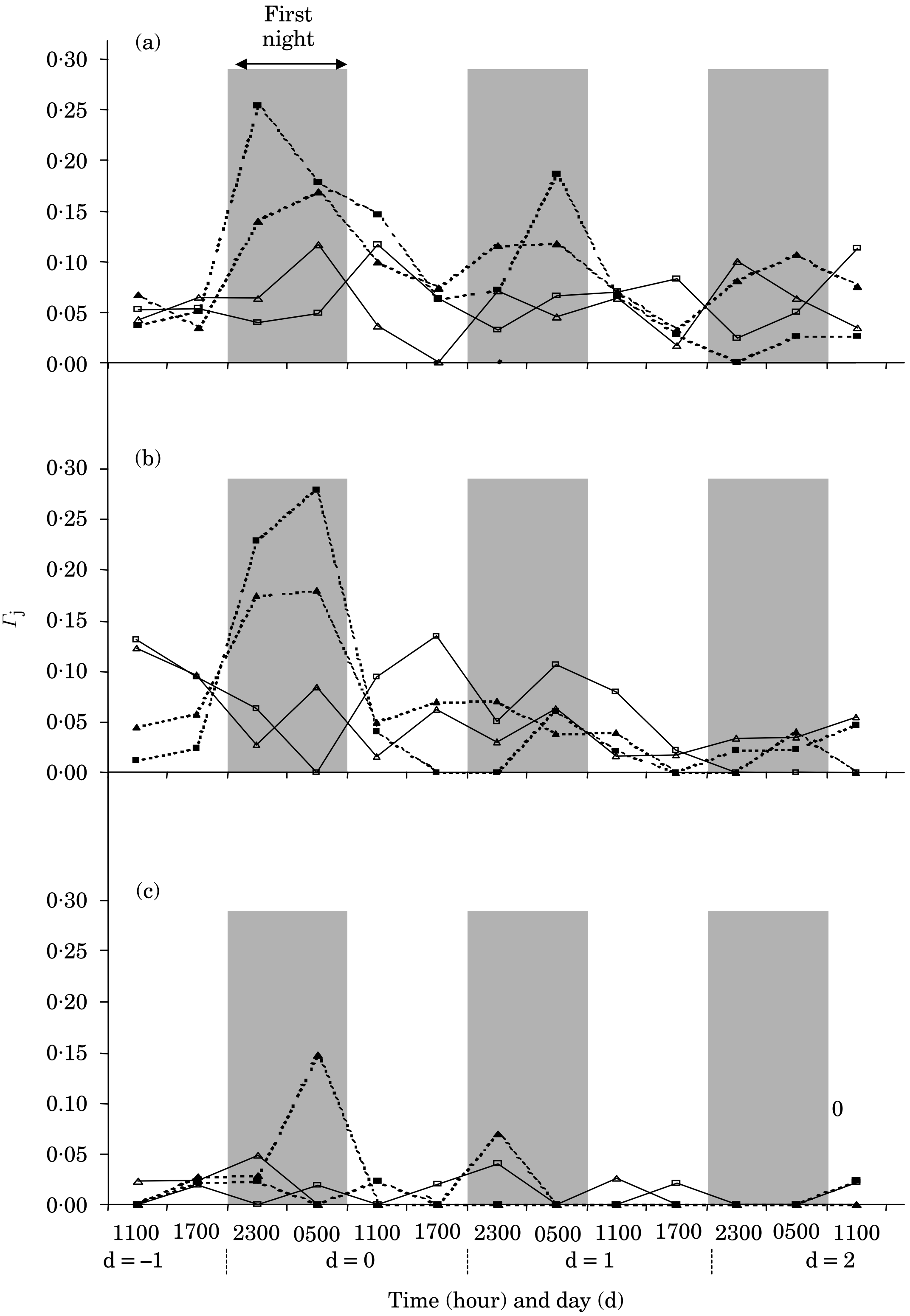

FIG. 5. Dispersal rate (Γ_j) of (a) 0d-, (b) 3d- and (c) 12d-group fish in the experimental channel in 2002 (--■--), the control channel in 2002 (—□—), the experimental channel in 2003 (--▲--) and the control channel in 2003 (—△—). ■, night-time. (See Table I for group definitions.)

Equal and high total rates of displacement (80%) for the 3d-and 12d-groups in all water velocity treatments were observed. This could mean that the low velocity (12·0 cm s^{-1}) was already too fast to be withstood by the fish. Newly

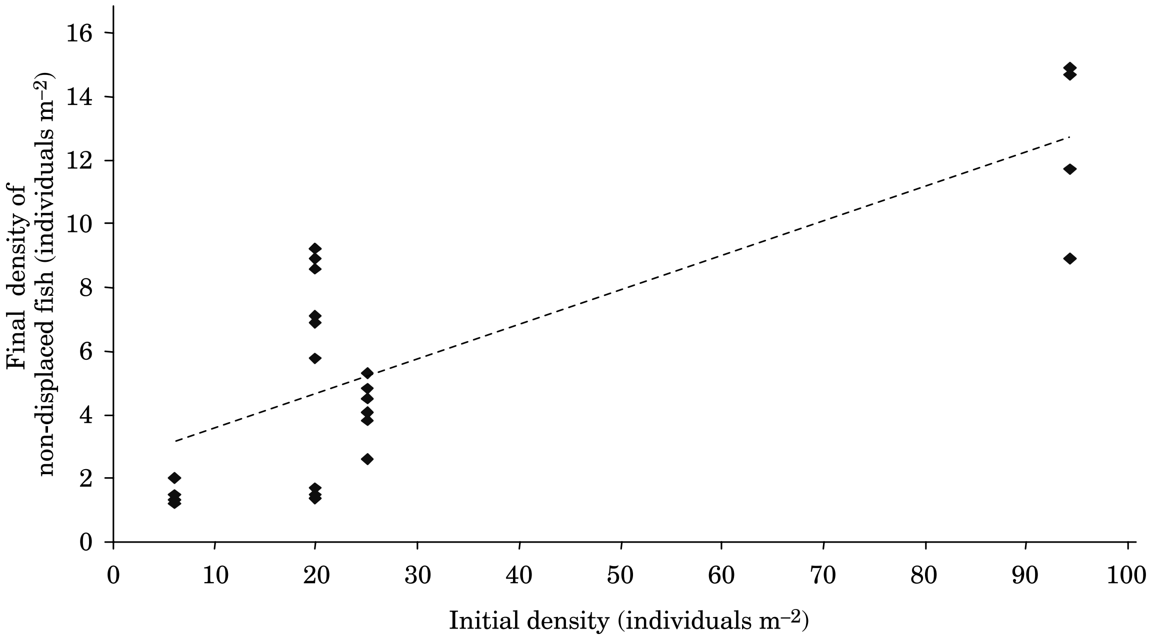

FIG. 6. Relationship between the initial density and the final density of non-dispersed fish in the present experiments (initial density = 25 individuals m^{-2}) and the experiments of Crisp (1991) and Crisp & Hurley (1991*a*, *b*). The trend curve (linear regression line) was fitted by $y = 0{\cdot}11x + 2{\cdot}5$. Final densities of non-dispersed 0d-group fish were not used to calculate the mean final density in the experiments.

emerged fish, however, naturally experience comparable velocities (Gaudin *et al.*, 1995) and roughness of the substratum probably allowed fish to shelter in 'dead' water zones (Crisp & Hurley, 1991*a*). In addition, greater displacement rates were observed at both lower and higher velocities (Ottaway & Clarke, 1981; Crisp & Hurley, 1991*a*), indicating that displacement could not be only attributed to swimming abilities (Crisp & Hurley, 1991*a*). Temperature could also influence fish swimming performance. Experiments were conducted, however, at water temperatures (16·4° C in 2002 and 16·2° C in 2003) close to the optimal temperature for the swimming performance of young brown trout (*i.e.* optimal temperature >12·4° C for fish just after resorption of the yolk sac; Heggenes & Traaen, 1988; optimal temperature equal to 16·1° C *c.* 4 months after the resorption of the yolk sac; Ojanguren & Brana, 2000). Finally, although the proportion of displaced fish seemed high, final densities (ranging from 2·6 to 5·3 individuals m^{-2} for the 3d-and 12d-groups) corresponded to densities observed in other experiments and in the field (Mortensen, 1977; Héland, 1980; Ottaway & Clarke, 1981; Ottaway & Forrest, 1983; Crisp, 1991; Crisp & Hurley, 1991*a*, *b*; Elliott, 1994).

In Crisp's (1991) experiments, similar final fish density was observed in channels submitted to different but constant velocities. This final density was approached more rapidly at high velocity. The present results, involving velocity changes, were consistent with these results. A constant final proportion of displaced fish (80%) but not a constant final density, however, was observed in the present experiments. For example, the final density of the 12d-group in the experimental channel was 4·1 and 2·6 individuals m^{-2} in 2002 and 2003, respectively, because of different total loss rates. It is thus probably more accurate to consider that a velocity change advanced the attainment of the proportion of 80% of displaced fish rather than the attainment of a constant final density. This hypothesis is also supported by the relationship between initial and final densities observed in both the present and Crisp's (1991) experiments (Fig. 6). Despite very different durations and velocity treatments, initial and final densities seem positively linked.

The present results have shown that there is a short critical period during the early life history of brown trout. This period corresponds to the time it takes to reach a total rate of displaced fish close to 80%, that is to say the 5 to 6 day period after median emergence at constant low velocity (12 cm s^{-1}). During this period, it was surprising to observe a qualitative effect of velocity changes on the fry displacement pattern (velocity change advanced the attainment of a constant final proportion of displaced fish) consistent with Crisp's (1991) experiments. This makes it difficult to easily explain the link between displacement patterns and extra-mortality of YOY observed *in natura* under the high discharge condition during emergence (Allen, 1951; Nehring & Anderson, 1993; Nuhfer *et al.*, 1994; Latterell *et al.*, 1998; Liebig *et al.*, 1999). Environmental constraints associated with flood other than velocity (*e.g.* an increase in suspended matter) may hindered YOY survival. Since Héland (1980) showed that displaced fish are not necessarily moribund, studies focusing on the fate of displaced fish could also be of major interest for a full understanding of salmonid recruitment processes. The advance of fish downstream displacement under high water velocity could, for example, negatively influence their survival in downstream zones.

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Fin

– La rivière s'écoule dans une lagune avant de rejoindre la mer. Maugio, Mai 2014. –