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par

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Influences de facteurs environnementaux sur le comportement natatoire de copépodes calanoïdes

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Résumé

Les processus comportementaux forment une composante majeure de l'écologie des copépodes calanoïdes. Ils sont affectés par une large gamme de facteurs environnementaux et se sont révélés extrêmement sensibles à la présence de polluants chimiques à des concentrations non létales, représentant une métrique de choix en écotoxicologie et justifiant de nombreuses études visant à quantifier la dynamique des copépodes sous l'influence de diverses conditions environnementales.

L'étude du comportement de nage des copépodes requièrt l'acquisition d'un nombre conséquent de longues trajectoires. Suivre en trois dimensions le mouvement d'organismes de petite taille se déplaçant simultanément est difficile. En écologie zooplanctonique la caractérisation d'un nombre important de trajectoires tridimensionnelles reste rare. La vélocimétrie tridimensionnelle par suivi de particules est une méthode de mesure développée en mécanique des fluides et utilisée pour étudier les écoulements turbulents. La technique est basée sur la visualisation et le suivi de traceurs inertes. Elle fournit une description lagrangienne de leurs déplacements en trois dimensions.

La technique a été utilisée pour étudier le comportement de nage de *Pseudodiaptomus annandalei* et d'*Eurytemora affinis*, deux espèces de copépodes calanoïdes provenant d'écosystèmes contrastés. *Eurytemora affinis* domine souvent la communauté zooplanctonique des estuaires de l'hémisphère nord. *Pseudodiaptomus annandalei* vit dans les eaux tropicales et sub-tropicales d'Asie du Sud-Est. Un système d'enregistrement en temps réel a été mis en place dans les deux laboratoires pour étudier leur comportement et les effets de différents facteurs environnementaux. Un ensemble de métriques a été utilisé pour décrire à la fois la cinématique du mouvement et sa complexité. Ces métriques se sont révélées efficaces et peuvent être utilisées dans des analyses standardisées.

La salinité est un facteur majeur de l'écologie des copépodes estuariens, mais ses effets sur le comportement sont peu connus. Nous avons observé des changements marqués dans le comportement natatoire de *P. annandalei* provenant de l'estuaire du fleuve Danshuei (nord de Taiwan) en réponse à une augmentation réaliste de salinité. Les trois stades adultes (mâles, femelles non ovigères et femelles ovigères) ont nagé activement dans toute la gamme de salinité testée. Néanmoins, une augmentation de salinité de 5 à 25 a causé une diminution de la vitesse de nage et de l'activé des femelles ovigères. La vitesse des mâles et femelles a été supérieure dans des conditions de salinité moyenne et plus faible aux salinités 5 et 25. Les mâles ont été plus fortement affectés par la variation de salinité que les femelles non ovigères. Les vitesses faibles et élevées ont été expliquées par des variations dans les proportions de nage lente et rapide et dans la fréquence des épisodes de repos. Le degré de convolution des trajectoires a été estimé par leur dimension fractale et n'a pas été affecté par la salinité. Une forte intermittence dans le comportement de nage a été mise en évidence par l'importance relative des valeurs élevées de vitesse et d'accélération. De manière générale, nos résultats montrent une salinité optimale pour le comportement et mettent en évidence des différences entre les stades adultes. Ils donnent un meilleur aperçu des effets de la salinité sur le comportement de nage des copépodes. Une redirection de la dépense énergétique du mouvement à l'osmorégulation quand la salinité diverge de la zone optimale est une explication possible mais qui reste à confirmer.

Les estuaires sont pollués par une large gamme de composés organiques et inorganiques. Ces polluants affectent à des degrés divers le comportement des organismes aquatiques. La réponse comportementale des copépodes, pourtant, reste peu connue. Nous avons étudié les effets immédiats de concentrations non létales de trois polluants communs sur le comportement de nage d'*E. affinis* provenant de cultures de laboratoire. Nonylphénol à $2\,\mu g\,L^{-1}$, cadmium à $45\,ng\,L^{-1}$ et un mélange d'hydrocarbures aromatiques polycycliques à $40\,ng\,L^{-1}$ ont provoqué une augmentation de la vitesse de nage chez les trois stades adultes. Dans la plupart des cas, les effets ont été observés pendant la période d'exposition de 30 minutes et ont persisté ou diminué pendant une période de décontamination d'une durée similaire. Chez les femelles ovigères exposées au cadmium et aux hydrocarbures aromatiques polycycliques, les effets ont été plus prononcés durant la période de dépuration, indiquant que la présence de sacs d'oeufs peut nuire au rétablissement après une exposition à la pollution. Les fréquences relatives des périodes de repos, de nage lente et de nage rapide ont confirmé des différences notables dans la distribution des vitesses de nage. Le degré d'occupation de l'espace n'a pas été affecté par la présence des polluants. Puisque même des composés narcotiques ont induit une hyper-activité, nos résultats tendent à montrer que la réponse immédiate à la présence de polluants est indépendante de leurs propriétés toxiques. La réponse comportementale observée ressemble à une réaction de fuite visant à échapper à des conditions stressantes. De manière générale, ces résultats indiquent que des concentrations environnementales de polluants peuvent induire des changements significatifs dans le comportement de nage libre des copépodes. La réponse de ce composant important de la communauté planctonique à la pollution mérite d'être étudiée de manière plus approfondie, tant au niveau des interactions entre individus que des effets combinés de différents polluants.

Les diatomées sont des algues unicellulaires communes aux habitats marins et qui dominent de façon récurrente les épisodes de prolifération de micro-phytoplancton. Certaines espèces de diatomées produisent des toxines, dont des aldéhydes poly-insaturés, en réponse au broutage par le méso-zooplankton. Les effets négatifs de ces aldéhydes sur le succès reproductif des copépodes ont été largement démontrés, mais peu d'informations sont disponibles quant à une possible influence sur leur comportement de nage. Les trois stades adultes du copépode *P. annandalei* ont été exposés à trois concentrations de 2-*trans*, 4-*trans* decadienal, une toxine de diatomées souvent utilisée comme aldéhyde modèle. Une exposition brève à la toxine à $3\,\mu mol\,L^{-1}$, $6\,\mu mol\,L^{-1}$ et $12\,\mu mol\,L^{-1}$ a provoqué une hyper-activité dose-dépendante pour les trois stades adultes, mise en évidence par une augmentation très marquée du nombre de trajectoires. Chez les mâles et femelles ovigères, cette hyper-activité s'est accompagnée d'une diminution de la vitesse de nage dose-dépendante à $3\,\mu mol\,L^{-1}$ et $6\,\mu mol\,L^{-1}$. L'augmentation de la vitesse de nage des mâles et femelles ovigères en présence de decadienal à $12\,\mu mol\,L^{-1}$ suggère un mode d'action complexe. L'exposition à la toxine a eu un effet moindre sur la vitesse des femelles non ovigères. L'analyse multifractale de la norme du déplacement a montré des différences dans le comportement des trois stades adultes. L'éthanol utilisé comme solvant de dilution à $200\,ppm$ a provoqué une augmentation de la vitesse de nage et un comportement plus linéaire chez les mâles et femelles ovigères. L'exposition à la toxine a réduit ou annulé cet effet, rapprochant le comportement d'un mouvement brownien et réduisant l'importance des déplacements de grande amplitude. Aucun effet de la toxine sur le type de mouvement n'a été observé chez les femelles non ovigères sauf à la concentration la plus élevée, supportant l'hypothèse de leur moindre sensibilité à certains facteurs environnementaux. Nos résultats démontrent que les aldéhydes produits par les diatomées peuvent altérer le comportement des copépodes de façon immédiate.

Abstract

Zooplanktonic organisms have limited swimming abilities and follow water mass movements. At small scale however, many of them show a variety of relatively complex behaviors. In calanoid copepods, these behaviors have received a great deal of attention, as they form a fundamental component of their ecology. They are affected by a variety of environmental factors, motivating studies to better quantify the dynamics of copepods under different conditions. They are also extremely sensitive to the presence of chemical contaminants and represent an endpoint of particular interest in ecotoxicologial studies.

The study of copepod swimming behavior requires a sufficient number of long trajectories to form a reliable basis for statistical analysis. To track the movement of copepods in the real three-dimensional space is complex. In copepod ecology, quantitative characterization of a large number of three dimensional trajectories is uncommon, primarily because there is no standard technique to recover the position information of many animals moving simultaneously. Three-dimensional particle tracking velocimetry (3D-PTV) is a flow measurement technique which identifies and follows individual particles in time and provides a Lagrangian description of their displacements in the three-dimensional space.

The swimming behavior of *Eurytemora affinis* and *Pseudodiaptomus annandalei*, two widespread species of estuarine calanoid copepods, was investigated by means of 3D-PTV. A measurement system was implemented in each laboratory and the technique was used to measure the dynamics of the three adult states under the influence of several environmental stressors. Scale-dependent and scale-independent metrics were used for a complete description of the movements of copepods. They have proved effective in describing both the complexity and the kinematic properties of copepod trajectories and can be used in standardized routine analyses.

Salinity is a major parameter in the ecology of estuarine copepods, affecting their reproduction, development and *in situ* distribution. However, its effects on swimming behavior are relatively unclear. We observed changes in the individual swimming behavior of *P. annandalei* from the Danshuei Estuary (Northern Taiwan) in response to a realistic salinity variation. The three adult states i.e. males, non-ovigerous females and ovigerous females were actively swimming over the experimental range of salinity. However, an increase in salinity from 5 to 25 caused a decrease in ovigerous female speed and activity. In males and females, swimming speeds were higher at medium salinities and lower at salinities 5 and 25. Males were much more sensitive to salinity variation than non-ovigerous females. Slower or faster swimming speeds were explained by variations in frequencies of periods of break, slow and fast swimming. The degree of space occupation of trajectories was estimated through their fractal dimension and was unaffected by salinity. The relative importance of extreme events i.e. large amplitudes of velocity and acceleration indicated intermittency in behavior. Overall, our results show an optimal salinity for swimming activity and highlight differences between adult states. Results add insight into how salinity may influence copepod swimming behavior. Redirection of energy from swimming to osmoregulation when salinity diverges from the optimal range of the species is a possible explanation. Further tests on total metabolism are warranted to confirm this hypothesis.

Estuarine waters contain a variety of chemicals which affect to various extents the behavior of aquatic organisms. Little is known, however, on the behavioral response of copepods. We investigated the immediate effects of sub-lethal concentrations of three commonly found contaminants on the swimming behavior of *E. affinis* from laboratory cultures. Nonylphenol at $2\,\mu g\,L^{-1}$, cadmium at $45\,ng\,L^{-1}$ and a mixture of low to medium molecular weight polycyclic aromatic hydrocarbons at $40\,ng\,L^{-1}$ all affected the swimming behavior of *E. affinis* adults, increasing both swimming speed and activity. In most cases, effects were observable within 30 minutes of exposure and persisted or faded during a period of depuration in uncontaminated water of similar duration. In ovigerous females exposed to cadmium and hydrocarbons, effects appeared to be more pronounced during the depuration period, suggesting that carrying ovisacs may impair recovery. We quantified differences in the distribution of swimming speed values by considering the relative frequencies of periods of break, slow and fast swimming and we observed a trend toward faster movements in the presence of pollutants. The degree of trajectory complexity was unaffected by pollutants. Since both narcotic and non-narcotic pollutants induced hyperactivity, our results suggest that changes in behavior after a short-term exposure may be independent of the general mode of action of the chemicals. The increase in speed and activity resembles an escape reaction permitting copepods to evade stressful conditions. Overall, these results indicate that environment-relevant concentrations of pollutants can induce rapid changes in copepod behavior. Since behavioral processes represent a fundamental element in the ecology of copepods, our results raise concern about the effects of background levels of pollution on a major component of the plankton community. The long-term response of copepods to waterborne pollutants, their synergistic effects and their interactions with other environmental factors need further investigation.

Diatoms are broadly present in marine habitats and often dominate seasonal phytoplankton blooms. Certain species produce polyunsaturated aldehydes upon mechanical wounding caused by mesozooplankton grazing. Ample evidence is available on toxin-induced reproductive failure in copepods, yet their behavioral effects remain unclear. Here we present results of laboratory experiments in which we investigated the effects of the diatom-derived aldehyde 2-*trans*, 4-*trans* decadienal on the swimming behavior of *P. annandalei*. Short-term exposure to the toxin at $3\,\mu mol\,L^{-1}$, $6\,\mu mol\,L^{-1}$ and $12\,\mu mol\,L^{-1}$ induced hyperactivity in the three adult states, as evidenced by a marked and dose-dependent increase in the number of trajectories. In males and ovigerous females exposed to the toxin at $3\,\mu mol\,L^{-1}$ and $6\,\mu mol\,L^{-1}$, hyperactivity came with an equally specific dose-dependent decrease in swimming speed. Males and ovigerous females swam faster at $12\,\mu mol\,L^{-1}$ than at $6\,\mu mol\,L^{-1}$, suggesting a complex mode of action of the toxin. In non-ovigerous females, decadienal caused less alteration in swimming speed, supporting the assumption that female copepods are less affected by certain environmental stressors. Multifractal analysis revealed differences in the statistical properties of the swimming behavior between experimental conditions. The moment structure function of the displacement appeared to be moderately multifractal in the three adult states swimming in control water. Ethanol as carrier solvent at 200 ppm caused an increase in swimming speed and a switch toward a more ballistic motion in males and ovigerous females. On the opposite, exposure to the toxin reduced or cancelled the effects of ethanol and resulted in a more Brownian motion for high moment values. Decadienal had little effects on non-ovigerous females except at the highest concentration. Our results demonstrate that decadienal, a model diatom aldehyde, can affect the behavior of adult copepods. They provide further information on the interaction between diatoms and their main predator.

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Notation

$\mathbf{v}$ and $\mathbf{v}^\top$	a vector in $\mathbb{R}$ and its transpose
$\mathbf{A}$	a matrix
M_w	a point of coordinates $\mathrm{M}_w = [X\ Y\ Z]^\top$ in the world coordinate system $\mathcal{R}_w$
M_c	a point of coordinates $\mathrm{M}_c = [x\ y\ z]^\top$in the camera coordinate system $\mathcal{R}_c$
M_i	a point of coordinates $\mathrm{M}_i = [u\ v]^\top$in the image plane coordinate system $\mathcal{R}_i$
M_p	a point of coordinates $\mathrm{M}_p = [a\ b]^\top$ in the pixel coordinate system $\mathcal{R}_p$
$\tilde{\mathrm{M}}$	a point in homogeneous coordinates
C	the camera optical center of coordinates $\mathrm{C} = [x_0\ y_0\ z_0]^\top$
I	the camera principal point of coordinates $\mathrm{I} = [a_0\ b_0]^\top$

Chapter 1

Particle tracking in three dimensional space

Three Dimensional Particle Tracking Velocimetry is a non-intrusive technique used in the field of hydrodynamics for the determination of velocity fields in flows. The method is based on the visualization of a flow seeded with small and neutrally buoyant particles and the acquisition of sequences of stereoscopic images from which the position of the particles, velocity vectors and trajectories in the three dimensional space can be recovered. The work presented here has been conducted using the software developed at the Institute of Geodesy and Photogrammetry and the Institute of Environmental Engineering, ETH Zürich. This chapter briefly describes the very basis of the technique, from the photogrammetric determination of three-dimensional particle coordinates to the reconstruction of trajectories.

1.1 Homogeneous space

Rotations in three dimensions can be expressed in matrix form. In contrast, translations cannot be expressed in terms of multiplicative 3 × 3 matrices. However, it is often useful to express general transformations in three dimensions as sequences of basic rotations and translations relative to individual coordinate axes, using a notation which unifies the mathematical treatment so that a generalized displacement can be expressed as a product of matrices. This is possible if homogeneous coordinates are used. Homogeneous coordinates allow the easy combination of transformations in a linear form. To achieve this, the matrices must be augmented to 4 × 4. The generalized displacement linking a point of coordinates $[X\ Y\ Z]^\top$ to a point of coordinates $[X'\ Y'\ Z']^\top$ and resulting from a rotation and a translation by a vector **t**=$[t_x\ t_y\ t_z]^\top$ can for instance be written as:

$$\begin{bmatrix} X' \\ Y' \\ Z' \\ 1 \end{bmatrix} = \begin{bmatrix} r_{11} & r_{12} & r_{13} & t_x \\ r_{21} & r_{22} & r_{23} & t_y \\ r_{31} & r_{32} & r_{33} & t_z \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} X \\ Y \\ Z \\ 1 \end{bmatrix}$$

where the terms r_{ij} are the coefficients of the rotation matrix. The above transformation is described in the homogeneous space, which is an embedding of the n dimensional physical space into the n_{+1} dimensional space by introducing a scaling factor w such as:

$$x : \{(x_1, x_2, ..., x_n)^\top \in \mathbb{R}^n\} \longmapsto \tilde{x} : \{w.(x_1, x_2, ..., x_n, 1)^\top \in \mathbb{R}^{n+1}\}$$

where $\tilde{x}$ are called homogeneous coordinates. The Cartesian point M = $[x\ y]^\top$ corresponds to the homogeneous point $\tilde{\text{M}}$ = $[wx\ wy\ w]^\top$ where w is an arbitrary constant. Using homogeneous coordinates, several successive affine transformations can be combined and represented as matrix multiplication. Homogeneous coordinates also form a basis for the projective geometry and are used extensively to project a three dimensional scene onto a two dimensional image plane, allowing projective transformation to be expressed in a matrix form and providing a method for doing calculations in the projective space. Homogeneous coordinates also have the advantage that any points, including points at infinity, can be represented using finite coordinates by their *ideal points*. Let us consider a line going through the origin and the point (x, y). The points (kx, ky) $\forall k \in \mathbb{R}$ all lie on this line and may be represented by (x/α, y/α) for $\alpha = 1/k$. The coordinates of the ideal point in the direction determined by this line are (x/α, y/α) for $\alpha = 0$. However, such division is undefined. An alternative approach is to use homogeneous coordinates ($\tilde{x}$, $\tilde{y}$, w). For $w \neq 0$ the corresponding ordinary point has non-homogeneous coordinates (x, y) were $x = \tilde{x}/w$ and $y = \tilde{y}/w$. For $w = 0$, we have the ideal point in the direction of the line. This special point receives, by definition, ($\tilde{x}$, $\tilde{y}$, 0) and each real multiple of ($\tilde{x}$, $\tilde{y}$, 0) as homogeneous coordinates.

For any given point, the homogeneous coordinates are not uniquely determined: ($\tilde{x}$, $\tilde{y}$, w) and ($k\tilde{x}$, $k\tilde{y}$, kw) are coordinates of the same point for all $k \neq 0$. But every triad ($\tilde{x}$, $\tilde{y}$, w)$\backslash\{(0, 0, 0)\}$ correspond to a unique point, ordinary if $w \neq 0$ and ideal if $w = 0$. Similarly, we define homogeneous coordinates for points in three dimensions as quadruples of real numbers, not all zeros[1].

In the Cartesian space, parallel lines have no common point. Ideal points are added to the set of Cartesian points[2] and represent the intersection of two parallel lines and, by extension, of all parallel lines having the same direction. In other words, every line in space contains one ideal point and all lines in a given direction have the same ideal point in common. From the above formulation it can be seen that two non-parallel line will intersect in an ordinary point, and two parallel lines in an ideal point. Both points will be represented in the two dimensional Cartesian plane. The ideal points of a plane constitute a straight line called the *ideal line* of the plane. Similarly, the ideal points of three dimensional space constitute a plane called the *ideal plane*. The points common to the ideal plane and an ordinary plane Π constitute the ideal line of Π.

1.2 Camera model

The pinhole camera model gives an approximation of the relationship between the coordinates of a point in the three-dimensional space and its projection onto the image plane. In this model the aperture is described as a point and no geometric distortion induced by lenses is considered.

A pinhole camera is modeled by its optical center C (also called center of projection) and its image plane $\mathcal{R}_i$. We assume that the center of projection coincides with the origin of the camera coordinate system so that the optical axis is aligned with the z-axis of the camera reference frame and that the image plane is in front of the center of projection so that image inversion is avoided. A point M$_w$ in the world reference frame $\mathcal{R}_w$ is projected onto an image point M$_i$ defined by the intersection of $\mathcal{R}_i$ with the line containing C and M$_w$. The line containing C and orthogonal to $\mathcal{R}_i$ is called the optical axis and its intersection with $\mathcal{R}_i$ is the principal point I. The principal point is usually not located at the center of the image plane. The origin of the pixel coordinate system is by convention located in the upper left corner of the image. The distance between C and the image plane (i.e. the camera chip) is the focal length f.

1.2.1 Camera parameters

The world and pixel coordinate systems are related by a set of physical parameters such as the focal length of the lens, the pixel size, the coordinates of the principal point and the position and orientation

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[1] For a line with direction numbers a, b and c, the ideal point in the direction of the line has coordinates $[a, b, c, 0]^\top$.

[2] Ordinary points and lines in the Cartesian plane plus ideal points and lines form the extended plane.

of the camera. These parameters need to be recovered in order to reconstruct the geometry of the scene. They can be classified as:

- **Extrinsic camera parameters** They define the location and orientation of the camera reference frame with respect to a known world reference frame.
- **Intrinsic camera parameters** They are required to link the pixel coordinates of a point M_p in the final image with the coordinates of its corresponding point M_c in the camera reference frame.

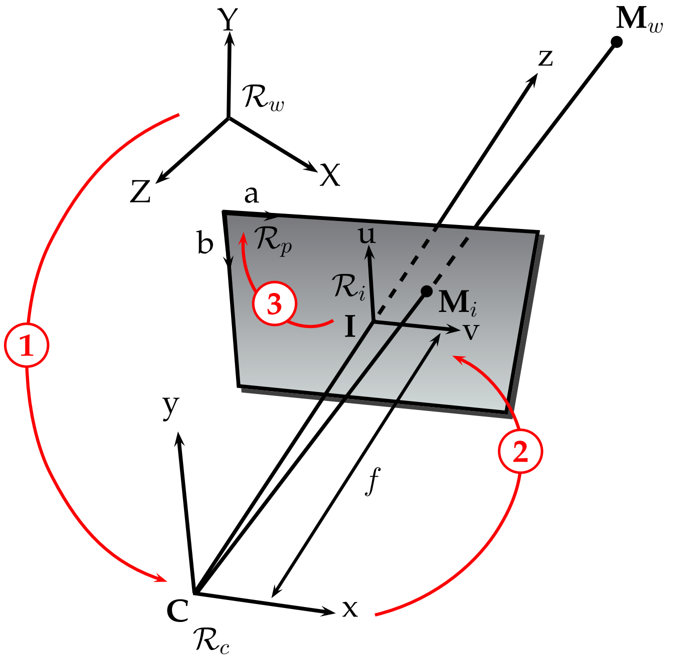

Figure 1.1: Transformations between a three-dimensional point and its image projection. The first and second transformations link the position of a point M_w in the world reference frame $\mathcal{R}_w$ to its position in the camera reference frame $\mathcal{R}_c$ and to its projection M_i into the focal plane of the camera. The third transformation links the point M_i expressed in the image reference frame $\mathcal{R}_i$ to its corresponding point in the pixel coordinate system $\mathcal{R}_p$. The origin of $\mathcal{R}_c$ is defined by the projective center C. The intersection of the optical axis and the focal plane defines the principal point I and the origin of $\mathcal{R}_i$. The focal plane is located at a distance f from the optical center corresponding to the focal length of the lens. The focal plane is represented in front of the optical center to avoid image inversion.

Extrinsic parameters

Determining these parameters means finding the translation vector $\mathbf{t} = [t_x\ t_y\ t_z]^{\mathrm{T}}$ between the relative positions of the origins of the world and camera reference frames and finding the rotation matrix **R** which brings the corresponding axes of the two frames into alignment (Fig. 1.1). Assume rotation matrix **R** results from successive Euler rotations of the camera reference frame around its x axis by ω (*pan angle*), its y axis by ϕ (*tilt angle*) and its z axis by κ (*swing angle*).

$$\mathbf{R} = \begin{bmatrix} \cos\omega\cos\kappa & -\cos\phi\sin\kappa & \sin\phi \\ \cos\omega\sin\kappa + \sin\omega\sin\phi\cos\kappa & \cos\omega\cos\kappa - \sin\omega\sin\phi\sin\kappa & -\sin\omega\cos\phi \\ \sin\omega\sin\kappa - \cos\omega\sin\phi\cos\kappa & \sin\omega\cos\kappa + \cos\omega\sin\phi\sin\kappa & \cos\omega\cos\phi \end{bmatrix} = \begin{bmatrix} r_{11} & r_{12} & r_{13} \\ r_{21} & r_{22} & r_{23} \\ r_{31} & r_{32} & r_{33} \end{bmatrix}$$

Extrinsic parameters link a point M_w of coordinates $\mathrm{M}_w = [X\ Y\ Z]^{\mathrm{T}}$ in the world coordinate system to the point M_c in the camera coordinate system (Fig. 1.1). Using homogeneous coordinates, the relation can be written as a rigid transformation combining a translation and a rotation and expressed as a matrix **T** called the extrinsic parameter matrix.

$$\tilde{\mathrm{M}}_c \cong \underbrace{\begin{bmatrix} r_{11} & r_{12} & r_{13} & t_x \\ r_{21} & r_{22} & r_{23} & t_y \\ r_{31} & r_{32} & r_{33} & t_z \\ 0 & 0 & 0 & 1 \end{bmatrix}}_{\mathbf{T}} \tilde{\mathrm{M}}_w$$

Intrinsic parameters

They link the point M_c in the camera coordinate system to the point M_p of coordinates $[a\ b]^{\mathrm{T}}$ in the pixel coordinate system (Fig. 1.1). Their are the focal length f, the pixel size and the position of the principal point at the intersection of the optical axis and the image plane. First, a perspective projection **P**, depending on the focal distance f, links the coordinates in the camera reference frame to the coordinates in the image plane.

$$\tilde{\mathrm{M}}_i \cong \begin{bmatrix} f & 0 & 0 & 0 \\ 0 & f & 0 & 0 \\ 0 & 0 & 1 & 0 \end{bmatrix} \tilde{\mathrm{M}}_c$$

A second transformation describes the sampling of $\mathcal{R}_i$ by the camera sensor and links the coordinates in the image plane to the coordinates in the pixel reference frame. This transformation depends on the vertical and horizontal scale factors k_a and k_b relating pixels to distance and on a_0 and b_0, coordinates of the principal point I[3]. An additional term, the *skew factor* θ corrects for the non-orthogonality of the pixels (El-Hakim, 1986). Geometric distortions induced by the optics are non-linear intrinsic parameters and cannot be included in the linear camera model. Most of the time, the skew factor is equal or very close to $\pi/2$ and the relation can be written as:

$$\tilde{\mathrm{M}}_p \cong \begin{bmatrix} k_a & k_a \cos\theta & a_0 + b_0 \cos\theta \\ 0 & k_b/\sin\theta & b_0/\sin\theta \\ 0 & 0 & 1 \end{bmatrix} \tilde{\mathrm{M}}_i \cong \begin{bmatrix} k_a & 0 & a_0 \\ 0 & k_b & b_0 \\ 0 & 0 & 1 \end{bmatrix} \tilde{\mathrm{M}}_i$$

1.2.2 Collinearity equations

Let $k_a f = \alpha_a$ and $k_b f = \alpha_b$. The combination of these three transformations can be written as the product of the matrix **T** with a matrix denoted by **K** and containing the intrinsic parameters:

$$\tilde{\mathrm{M}}_p \cong \begin{bmatrix} k_a & 0 & a_0 \\ 0 & k_b & b_0 \\ 0 & 0 & 1 \end{bmatrix} \mathbf{P}\mathbf{T}\tilde{\mathrm{M}}_w \cong \begin{bmatrix} k_a f & 0 & a_0 \\ 0 & k_b f & b_0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \end{bmatrix} \mathbf{T}\tilde{\mathrm{M}}_w \cong \underbrace{\begin{bmatrix} \alpha_a & 0 & a_0 & 0 \\ 0 & \alpha_b & b_0 & 0 \\ 0 & 0 & 1 & 0 \end{bmatrix}}_{\mathbf{K}} \mathbf{T}\tilde{\mathrm{M}}_w$$

The equation above gives the collinearity equations linking a point $\mathrm{M}_w = [X\,Y\,Z]^{\mathrm{T}}$ in the world coordinate system to its corresponding point $\mathrm{M}_p = [a\ b]^{\mathrm{T}}$ in the pixel reference frame.

$$\begin{aligned} a &= a_0 + \alpha_a \frac{r_{11}X + r_{12}Y + r_{13}Z + t_x}{r_{31}X + r_{32}Y + r_{33}Z + t_z} \\ b &= b_0 + \alpha_b \frac{r_{21}X + r_{22}Y + r_{23}Z + t_y}{r_{31}X + r_{32}Y + r_{33}Z + t_z} \end{aligned}$$

---

[3] The center of the sensor is usually not on the optical axis.

1.2.3 Distortion

The basic pinhole model is extended with some corrections for the distorted image coordinates. In optical systems made of spherical lenses whose centers are imperfectly aligned along the optical axis and which are not perfectly parallel, geometric distortions occur. A point is imaged at a position which is different from predicted under linear Gaussian approximations. Distortion is smaller for directions near parallel to the optical axis, increases with distance from the center and tends to be more noticeable with wide-angle lenses. For an unobservable point M_{iG} = [*u* *v*]$^{\mathrm{T}}$ imaged under Gaussian approximation, its distorted coordinates M_{iD} = [*u'* *v'*]$^{\mathrm{T}}$ are given by the relation M_{iD}= $\mathrm{M}_{iG} + \Delta$ where Δ (known as distortion vector) is the sum of the two components δ_r and δ_d. In other words, the position of M_{iD} is given by $u' = u + \Delta_u(u, v)$ and $v' = v + \Delta_v(u, v)$.

Radial distortion

δ_r=(δ_{ru} δ_{rv}) corresponds to the **radial distortion** which causes the actual image point to be displaced radially in the image plane (Brown, 1966, 1971). This type of distortion is caused by an imperfect radial curvature curve of the lenses and is symmetric about the optical axis. A point is imaged at a distance from the principle point which is larger (*pin-cushion distortion*) or smaller (*barrel distortion*) than predicted by the perspective projection equations. Assuming that the center of distortion is at the principal point and for a lens focused at infinity, the displacement due to radial distortion can be approximated by:

$$\begin{aligned}\delta_{ru}(u,v) &= u(k_1r^2 + k_2r^4 + ...)\\ \delta_{rv}(u,v) &= v(k_1r^2 + k_2r^4 + ...)\end{aligned}$$

where r is the distance from the principal point of the image plane and k_n are the coefficients of radial distortion.

Decentering distortion

Centers of curvature of lens surfaces are not always strictly collinear. This introduces another common distortion type, **decentering distortion** δ_d=(δ_{du} δ_{dv}) which has both a radial and tangential component (Brown, 1966; Fryer and Brown, 1986). For a lens focused at infinity, decentering distortion can be modeled by its coefficients p_n as follow:

$$\begin{aligned}\delta_{du}(u,v) &= [p_1(r^2 + 2u^2) + 2p_2uv][1 + p_3r^2 + ...]\\ \delta_{dv}(u,v) &= [p_2(r^2 + 2v^2) + 2p_1uv][1 + p_3r^2 + ...]\end{aligned}$$

Total distortion

During the calibration process the coefficients k_n and p_n of these power series are recovered. A proper camera model for accurate calibration can be derived by combining the pinhole model with the correction for these two distortion components. Several methods for correcting lens distortion have been developed. In the most commonly used approach only the first, second and third terms of the power series are retained for the radial distortion and only the first and second for the tangential distortion, as follow:

$$\begin{aligned}\Delta_u &= u(k_1r^2 + k_2r^4 + k_3r^6) + p_1(r^2 + 2u^2) + 2p_2uv\\ \Delta_v &= v(k_1r^2 + k_2r^4 + k_3r^6) + p_2(r^2 + 2v^2) + 2p_1uv\end{aligned}$$

The coordinates of $\mathrm{M}_{iD} = [u'\ v']^{\mathrm{T}}$ in terms of M_{iG} are given by the relation M_{iD}=$\mathrm{M}_{iG} + \Delta$:

$$\begin{aligned} u' &= u + u(k_1r^2 + k_2r^4 + k_3r^6) + p_1(r^2 + 2u^2) + 2p_2uv \\ v' &= v + v(k_1r^2 + k_2r^4 + k_3r^6) + p_2(r^2 + 2v^2) + 2p_1uv \end{aligned}$$

Camera model with correction of distortion

Letting **k**=$(u_0\ v_0\ \alpha_u\ \alpha_v)^{\mathrm{T}}$ a vector containing the camera intrinsic parameters defined by the matrix **K** and **d**=$(r_1\ r_2\ r_3\ p_1\ p_2)^{\mathrm{T}}$ a vector containing the intrinsic distortion coefficients, the camera model becomes non-linear and can be expressed as a vectorial function f which is suited to be linearized as observation equations in a non-linear least squares adjustment:

$$\mathrm{M}_{iD}=\mathrm{f}(\mathbf{k}, \mathbf{d}, \mathbf{R}, \mathbf{t}, \mathrm{M}_w)$$

In the calibration procedure implemented in the 3D-PTV software, this model is being applied for a spatial resection using control points with known coordinates and introducing the parameters of exterior and interior orientation, lens distortion and affine transformation as unknowns. After the calibration the same model can be used for a spatial intersection, introducing the coordinates of a particle as unknowns (Maas et al., 1993).

1.2.4 Multimedia geometry

Particles are observed through the aquarium wall. The optical ray passes through different media i.e. water, glass and air and is broken twice due to changes in the refractive indices. Assuming homogeneity and isotropy of the different media and considering a plane glass plate perpendicular to the optical ray, the geometry can be modeled and a radial shift calculated and added into the collinearity equations (Maas et al., 1993). The radial shift is parallel to the aquarium wall and function of the distance between the object point and the nadir point of the camera, the thickness of the aquarium wall, the depth of the object point and the refractive indices of the three media. In the 3D-PTV software, discrete lookup tables with the radial shifts over the observed object volume are calculated and used to compensate for the influence of the multimedia geometry.

1.3 Stereovision

The use of two cameras allows the reconstruction of the geometry of the environment. Knowing the camera orientation and parameters, it is possible to establish correspondences between particle image coordinates and to recover the position of their corresponding points in the three-dimensional space.

1.3.1 Epipolar geometry

Let us consider a simple stereo rig composed of two pinhole cameras. Let C_l and C_r be the optical centers of the left and right cameras and $\mathcal{R}_c^l$ and $\mathcal{R}_c^r$ be their respective coordinate systems (Fig. 1.2). A point M_w in the world reference frame is projected onto both image planes to the points M_i^l and M_i^r which constitute a conjugate pair. For a pair of conjugate points there is only one corresponding point in the world coordinate system. For a point M_i^l in the left image plane, its conjugate M_i^r in the right image plane lies on a line called the epipolar line of M_i. The epipolar line is the intersection of the epipolar plane containing M_w, C_l and C_r with the image plane of the camera. All the epipolar lines in an image pass through a common point called the epipole (e_l and e_r, respectively) which is the projection of the optical

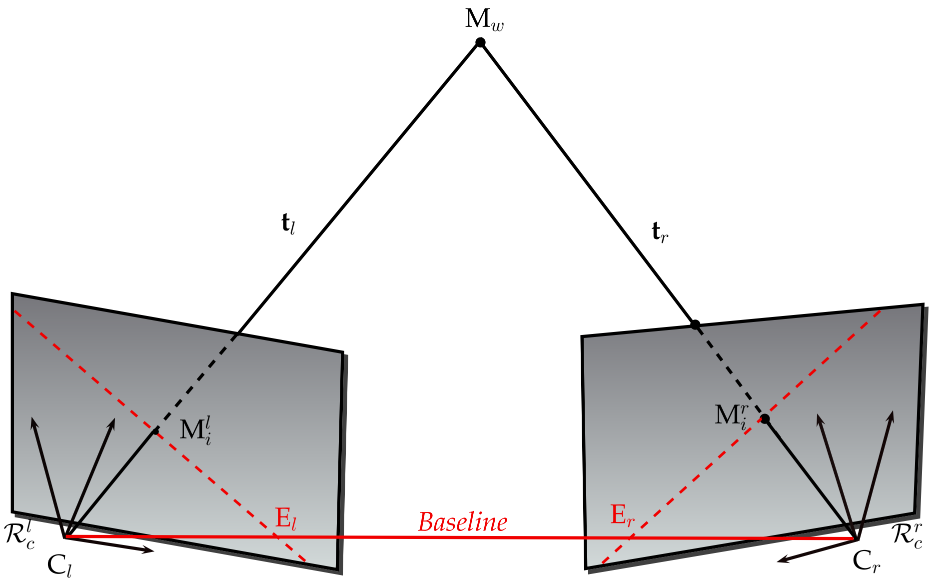

Figure 1.2: Epipolar geometry and conjugate pairs. For each point observed in one image, the same point must be observed in the corresponding epipolar line on the other image.

center of the other camera and which is also the intersection between the baseline (the line containing C_l and C_r) and the image plane.

1.3.2 Correspondences

If the cameras are calibrated for internal parameters, an essential matrix provides the relationship between the position of the same particle in the images of the cameras. The more general case i.e. without camera calibration is represented by the fundamental matrix.

Essential matrix

Let $\mathbf{t}_l$ and $\mathbf{t}_r$ be the vectors from the two camera optical centers C_l and C_r to a point M_w in the object space and let $\mathbf{t}_s$ be the vector between C_l and C_r. The vector $\mathbf{t}_r$ can be expressed in the reference frame of the right camera, as follow:

$$\mathbf{t}_r = \mathbf{t}_l - \mathbf{t}_s$$

The two camera frames are related by a transformation including a rotation $\mathbf{R}$ and the vector $\mathbf{t}_s$. The vector $\mathbf{t}_r$ can be expressed in its own frame of reference as follow:

$$\mathbf{t}'_r = \mathbf{R}\mathbf{t}_r = \mathbf{R}(\mathbf{t}_l - \mathbf{t}_s)$$

The rotation matrix $\mathbf{R}$ being orthogonal, the relation can be written as:

$$\mathbf{t}_r = \mathbf{R}^{-1}\mathbf{t}'_r = \mathbf{R}^\top\mathbf{t}'_r$$

The epipolar plane contains M_w and the projection centers of the two cameras. The points M_w, C and C' are thus coplanar:

$$\mathbf{t}_r \cdot \mathbf{t}_s \times \mathbf{t}_l = 0$$

Substituting in the coplanarity condition gives:

$$(\mathbf{R}^\top \mathbf{t}'_r) \cdot \mathbf{t}_s \times \mathbf{t}_l = 0$$

The vector product notation can be replaced by a skew-symmetrical matrix noted $\mathbf{C}_\times$ and expressed as:

$$\mathbf{C}_\times = \begin{bmatrix} 0 & -t_z & t_y \\ t_z & 0 & -t_x \\ -t_y & t_x & 0 \end{bmatrix}$$

After appropriate transposition, the relation can be written as:

$$\begin{aligned} (\mathbf{R}^\top \mathbf{t}'_r)\mathbf{C}_\times \mathbf{t}_l &= 0 \\ (\mathbf{t}'_r)^\top \mathbf{R}\mathbf{C}_\times \mathbf{t}_l &= 0 \\ (\mathbf{t}'_r)^\top \mathbf{E}\mathbf{t}_l &= 0 \end{aligned}$$

With $\mathbf{E} = \mathbf{R}\mathbf{C}_\times$ known as the **essential matrix** and which expresses the relation between the observed positions of the same point in the two camera reference frames. The essential matrix depends on the extrinsic parameters only and leads to formulas for the epipolar lines. Given a point in one image plane, multiplying by the essential matrix will determine which epipolar line to search along in the second image plane. Let p_l and p_r be the points on the image planes of the left and right cameras, expressed in the reference frame of their own camera. The usual equation of perspective projection relates a point M_w of coordinate $[x\ y\ z]^\top$ expressed in the camera reference frame to its projection on the image plane:

$$\mathrm{p}_l = (f_l/z_l)\mathbf{t}_l \qquad \mathrm{p}_r = (f_r/z_r)\mathbf{t}_r$$

In the camera reference frame, the image plane is located at the focal distance from the center of projection so the relation becomes:

$$\mathrm{p}_r^\top \mathbf{E}\mathrm{p}_l = 0$$

Writing $\mathrm{p}_r^\top \mathbf{E} = \mathbf{l}_1^\top$ and $\mathbf{E}\mathrm{p}_l = \mathbf{l}_2$ leads to the following relations:

$$\mathrm{p}_l^\top \mathbf{l}_1 = 0 \qquad \mathrm{p}_r^\top \mathbf{l}_2 = 0$$

This means that $\mathbf{l}_1$ and $\mathbf{l}_2$ are the epipolar lines corresponding to the points p_r and p_l, respectively[4]. The essential matrix is a rank-deficient 3×3 matrix defining the equation of a line. Finally, the position of the epipoles can be determined from the above formulation. The epipole e lies on every epipolar lines within the same image, which can be expressed as $\mathrm{e}_r^\top \mathbf{E}\mathrm{p}_l = 0$ for all p_l. This means that $\mathrm{e}_r^\top \mathbf{E} = 0$ or $\mathbf{E}^\top \mathrm{e}_r$=0. Similarly, $\mathbf{E}e_l = 0$.

Fundamental matrix

In the previous section, the camera were calibrated, with p_l and p_r the coordinates in the camera reference frames. However, there is often a need to work with pixel measurements and the fundamental matrix is used instead of the essential matrix. The **fundamental matrix F** relates a particle in the first image (i.e. in the pixel reference frame) to the corresponding epipolar line in the image of the second camera (Luong and Faugeras, 1996). The fundamental matrix contains information on the relative orientation of the cameras as well as on their intrinsic parameters. As defined in section 1.2.1, the points q_l and q_r in the

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[4] Let **l** be a line. Its equation in the projective space using homogeneous coordinates is given by $\mathbf{l}^\top \mathbf{p} = \mathbf{p}^\top \mathbf{l} = 0$, with $\tilde{\mathbf{p}} = [\mathrm{u\ v\ 1}]^\top$ being a point on the line and $\tilde{\mathbf{l}} = [\mathrm{a\ b\ c}]^\top$ the coefficients of the line.

pixel reference frames of the left and right cameras are related to the point p_l and p_r in the image planes as follow:

$$\mathrm{q}_l = \mathbf{K}_l \mathrm{p}_l \qquad \mathrm{q}_r = \mathbf{K}_r \mathrm{p}_r$$

where $\mathbf{K}_l$ and $\mathbf{K}_r$ are the matrices of intrinsic parameters for the left and righ camera, respectively. From these relations one can write:

$$\mathrm{p}_l = \mathbf{K}_l^{-1} \mathrm{q}_l \qquad \mathrm{p}_r = \mathbf{K}_r^{-1} \mathrm{q}_r$$

The equation linking the pixel coordinates is then given by:

$$\mathrm{q}_r^{\top} (\mathbf{K}_r^{-1})^{\top} \mathbf{E} \mathbf{K}_l^{-1} \mathrm{q}_l = 0$$

which can be expressed as:

$$\mathrm{q}_2^{\top} \mathbf{F} \mathrm{q}_l = 0$$

where

$$\mathbf{F} = (\mathbf{K}_r^{-1})^{\top} \mathbf{E} \mathbf{K}_l^{-1}$$

Because the fundamental matrix contains all the information that would be needed to calibrate the cameras, it contains more free parameters than the essential matrix. However the two matrices are intended to convey the same basic information. Finally, the epipoles (in pixel coordinates) are given by $\mathbf{F} e_l = 0$ and $\mathbf{F}^{\top} e_r = 0$. It is worth noting that the epipolar constraint can be established with no prior knowledge of the stereo parameters and the fundamental matrix can be recovered from $n \geq 8$ corresponding points. For a description of the method one can refer to Longuet-Higgins (1981), Luong and Faugeras (1996) and Hartley (1997).

1.3.3 Determination of three-dimensional coordinates

The establishment of stereo correspondences is, in the case of two cameras, reduced to a one dimension problem. In the case of more than two cameras, correspondences are established by epipolar line intersection technique and the position of the particle in the image is given by the intersection of the epipolar line segments.

Knowing the camera orientation parameters from the calibration, proceeding from a point in one image, an epipolar line in the other image can be computed, on which the corresponding point has to be found. In the strict mathematical formulation this line is straight, but in the more general case with lens distortion and multimedia geometry, this line will be slightly bent and can be approximated by a polygon. The length of the line can be restricted by the range of depth of the observed particle in the object space i.e. by the depth of the investigation volume. Adding a certain tolerance width to this epipolar line segment due to measurement noise, the search area for the corresponding particle image becomes a narrow two-dimensional band-shaped window in image space (Maas et al., 1993). If a large number of particles is imaged, a problem of ambiguity occurs, as often two or more particle images will be found in the search area. After corresponding particles on the two images are found, the three-dimensional coordinates are derived by forward intersection, introducing their coordinates as unknowns in the augmented projection model (Papantoniou and Dracos, 1989; Maas et al., 1993; Malik et al., 1993; Dold and Maas, 1994).

1.4 Calibration

Camera calibration consists of the recovery of (i) the interior parameters (ii) the positions and relative orientation of the cameras (iii) the parameters modeling geometric distortions induced by lenses and sensors. The overall performance of the system strongly depends on the accuracy of the calibration procedure. For each camera, interior orientation has three degrees of freedom (focal length and coordinates of the principal point). Exterior orientation has six degrees of freedom (three for rotation and three for translation). Five parameters account for lens distortion and two extra parameters correct for image sensor errors. Theses parameters are introduced as unknowns in a calibration procedure. After their determination it is possible to establish correspondences between the particle images from the different cameras.

Several methods for camera calibration are presented in the literature (e.g. Tsai, 1987; Heikkila and Silven, 1997). They can be regrouped in two main categories: the *photogrammetric calibration* uses reference objects with known geometry and the *self calibration* assumes static scene and the structure is given by camera motion. The 3D-PTV software implements a **bundle adjustment** with reference object. Bundle adjustment is a technique for simultaneously refining the structure of the scene and the camera parameters (Triggs et al., 2000). In this procedure, the camera relative orientation and their optical characteristics can be determined from a set of images of a three-dimensional reference object with precisely-known geometry taken at different viewpoints. Bundle adjustment minimizes the projection error between the image locations of observed and predicted image points, expressed as the sum of squares of a large number of nonlinear functions. Measurements and parameter estimates are usually considered to be normally distributed and the minimization is achieved using nonlinear least-squares optimization routines such as the Levenberg-Marquardt or the Gauss-Newton methods. A description of the method is given in Hartley and Zisserman (2004).

1.5 Particle tracking

During the particle detection, a high-pass filtering[5] removes non-uniformity in the background intensity. Particles are detected within images by the application of a peakfitting routine and based on additional features such as the number of pixels and their sum of grey-values. Several methods exist to determine the particle center (e.g. Ouellette et al., 2006). In the 3D-PTV software, the center of gravity of a particle is calculated with a weighted gray value operator. The image is first segmented into groups of pixels representing single particles. The center of a particle is determined by averaging the positions of its component pixels weighted by their intensity gray values (Maas et al., 1993). The corresponding particle coordinates in the object space are derived by forward intersection and form the initial point could at one time instant before the tracking is performed (Willneff, 2003). The tracking procedure then selects correct links for the same particle from one time step to the next.

1.5.1 Particle tracking in image and object space

There are several methods for particle tracking (Ouellette et al., 2006). This section briefly describes the algorithm currently implemented in the 3D-PTV software for the prediction of the next particle position (Dracos, 1996; Willneff, 2003). The tracking algorithm uses the information extracted from image and object spaces simultaneously to establish temporal correspondences. Basically, if a particle can be tracked in the object space over several consecutive time steps, its position in the next time step can be predicted and back-projected onto the images of all cameras. The search in the images either confirms

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[5] High-pass filtering retains the high frequency information and attenuates signals with frequencies lower than a cutoff value. High-pass filters enhance image details and edges.

the predicted location or leads to unmatched detections. The magnitude of velocity and acceleration are limited. This defines a search volume around a particle for the search of the next position in the object space. Within this volume, the prediction of the next particle position is done assuming constant velocity if the particle position in the previous frame is known, or assuming constant acceleration if the positions of the particle in two previous frames are known.

1.5.2 Three or four-frame algorithm

Let f_0 be the current frame, $\mathrm{f}_{(-1)}$ the previous frame (which does not exist for the first step of the tracking procedure) and f_1 and f_2 the frame at t_{+1} and t_{+2}. The location of the particle i in frame f_j is denoted by x_i^j. The procedure defines a search volume centered on the predicted position in f_1 for the search of the most suitable candidate and determines if possible the link between the position x_i^j of a particle in the frame f_j and its position x_i^{j+1} in the frame f_{j+1}. The estimation for the position in frame f_1 is based on the assumption that the particle moves during the time step δ_t with a constant velocity u_i^0 and without acceleration. The velocity u_i^0 is determined from the position of the particle in frame $\mathrm{f}_{(-1)}$ if a link was previously established or, if no link exists to the previous frame, the algorithm uses the position of the particle in f_0 as the search position. The search of candidates is then performed in the image space after projection of the search volume from the object space and leads to a list of possible candidates in the object space. Based on this list, the particle location x_i^2 is then predicted for the time step t_{+2} based on constant acceleration a_i^1 if the position of the particle is known in frame $\mathrm{f}_{(-1)}$ or based on constant velocity if no such link exists.

$$x_i^2 = x_i^1 + u_i^1 \cdot \delta_t + \frac{1}{2} a_i^1 \cdot (\delta_t)^2$$

The search volume is once again reprojected onto the images at t_{+2} where the search of suitable candidates is performed. If the search leads to a list of 3D candidates, the procedure uses the minimum acceleration to establish link to the current time step (Willneff and Gruen, 2002). If the particle positions do not meet the criterion, no link is made. If there is no candidate, the software searches for unmatched detection in all views and calculates additional particle positions for the reconstruction of new trajectories. The particle three-dimensional positions in adjacent time steps are then linked together to reconstruct the particle trajectory.

Chapter 2

Ecological model

Ecological processes occur at different temporal and spatial scales. Their complexity often requires their separation into components which can be studied separately in the laboratory. The behavior of zooplankters for instance is of first importance in the maintenance of stability at the basis of trophic networks, but cannot be studied as a whole. It can however be separated into a variety of elements. Their study offers the hope to better understand fundamental processes within the zooplankton community.

2.1 Model organisms

Copepods are the largest and most diversified group of crustaceans and the most numerous metazoans in aquatic environments. They also form the largest part of the total zooplankton. They have a crucial role at the basis of the trophic network both as primary and secondary consumers and secondary producers and represent a major food source for organisms from higher trophic levels. Their key position prompts efforts to better understand their ecology.

Swimming behavior is a fundamental component of copepod ecology and is the underlying mechanism for population-level behavior. However, field experiments on copepod behavior are constrained by logistics. Behavioral studies in the field are limited by the lack of suitable device for small-scale observations. Changing and hardly measurable environmental conditions make comparisons impossible. In this context, laboratory experiments represent a suitable approach for the analysis of copepod behavior.

In this study we selected the calanoid copepods *Pseudodiaptomus annandalei* (Sewell, 1919) and *Eurytemora affinis* (Popee, 1880) as model species. Both species belong to the *Centropagoidae* superfamily and have similar life cycles. They have a cosmopolitan distribution and dominate the zooplankton in their respective distribution areas in the oligo- to mesohaline part of the estuary. Copepods, especially calanoids, are appropriate test organisms because of their small size, short generation time and ease of culturing in the laboratory at high population densities in relatively small volumes.

2.1.1 *Eurytemora affinis*

Eurytemora affinis is a widespread estuarine copepod of the Northern Hemisphere (Fig. 2.1). Its ecology, physiology and behavior have been intensively studied (Ban, 1994; Andersen and Nielsen, 1997; Appeltans et al., 2003; Devreker et al., 2004; Forget-Leray et al., 2005; Devreker et al., 2007; Cailleaud et al., 2007a,b,c; Winkler et al., 2008; Devreker et al., 2009; Cailleaud et al., 2009, 2011a; Devreker et al., 2010; Mialet et al., 2010; Michalec et al., 2010; Cailleaud et al., 2011b). This egg-carrying species is found in freshwater to hyper-saline waters and is considered as invasive, with its capability to survive in environments of different salinities coming from natural selection of an appropriate genotype rather than a strict eurytolerance (Lee, 1999; Lee and Petersen, 2002; Lee et al., 2003). However, laboratory studies have confirmed its great osmoregulation capabilities (Roddie et al., 1984) and up and down-regulation of specific protein synthesis have been observed under osmotic stress (Gonzalez and Bradley, 1994; Kimmel and Bradley, 2001). In estuaries *E. affinis* is often the dominant copepod species, representing the vast majority of the mesozooplankton diversity in the oligohaline zone and an element of first importance in the ecological equilibrium of the local food web (Mouny and Dauvin, 2002; Maes et al., 2003; Winkler and Greve, 2004; David et al., 2006; Devreker et al., 2008). It should be considered as a complex of cryptic species, with morphologically indistinguishable but genetically different populations resulting from divergent evolutionary histories (Lee, 2000). *Eurytemora affinis* is planktonic to epibenthic, inhabits both the surface of the sediments and the water column and is often associated to the maximum turbidity zone where it finds abundant food (Castel and Viega, 1990; Morgan et al., 1997) but encounters high levels of pollution (Cailleaud et al., 2009). It is able to select its food (Tackx et al., 2003) yet has an omnivorous diet, feeding on phytoplankton, protozoans, detritus and bacteria with a preference for nanoplankton species and smaller microzooplankters (Heinle and Flemer, 1975; Merell and Stoecker, 1998).

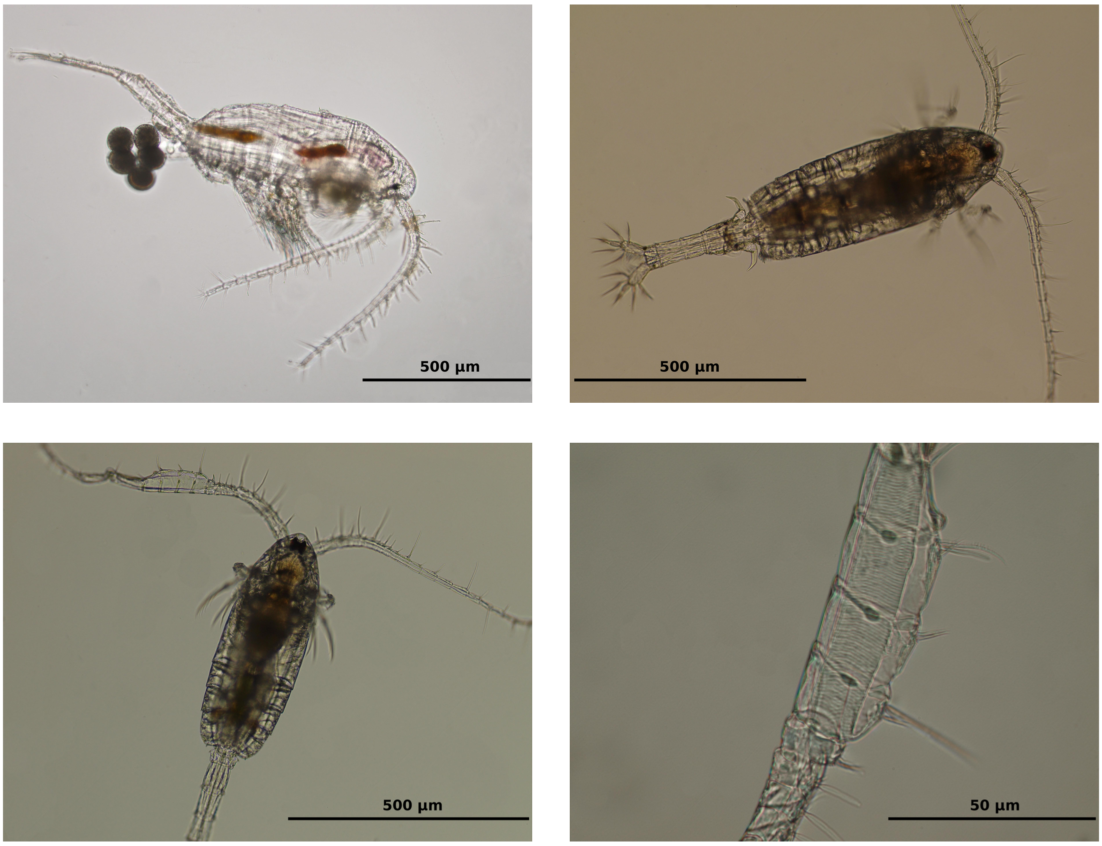

Figure 2.1: *Eurytemora affinis* ovigerous female (top left), *Pseudodiaptomus annandalei* female (top right), *Pseudodiaptomus annandalei* male (bottom left). The modified antenna is visible in male and serves to grip the female during mating (bottom right). *E. affinis* carries a single egg sac while *P. annandalei* bears a pair of sacs. Adult females exhibit lateral processes, one on each side of the genital region.

2.1.2 *Pseudodiaptomus annandalei*

The egg-carrying copepod *Pseudodiaptomus annandalei* has a similar morphology (Fig. 2.1), life cycle and ecology but has been the subject of fewer studies (Chen et al., 2006; Dur et al., 2010, 2011). This species is found in shallow coastal and estuarine waters in the Indo-Pacific region (Walter, 1987) over a wide range of salinity from freshwater to marine. It seems to be well adapted to salinity variations, as suggested by the maintenance of its population in many tropical estuaries. In Taiwan for instance, *P. annandalei* dominates the zooplankton community in the Danshuei Estuary round-year despite high levels of pollution (Hwang et al., 2010). This species shows clear substratum preference, maintaining its position and resisting to relatively strong currents (Shang et al., 2008).

2.2 Study sites

Estuaries are complex transition zones characterized by multiple linear or abrupt ecological discontinuities. They are aquatic-terrestrial and marine-freshwater ecotones and areas of first importance in the maintenance and shaping of ecological diversity at both local and large scales. Estuaries are zones of high productivity mediating exchanges and interactions between systems. They offer a wide variety of habitats and support a vast diversity of species organized in complex trophic networks. Estuaries are characterized by important and incessant variations in environmental parameters such as salinity, temperature, turbidity and concentration in inorganic and organic suspended matters. Despite their first ecological importance, they suffer from high anthropogenic pressure consequence of their high value for human society (Costanza et al., 1997). Estuaries have been traditionally used as convenient disposal sites for urban sewage and industrial effluents and receive contaminated water through their tributaries. They are now highly polluted by organic and inorganic contaminants present in both sediments and water column (Power et al., 1999; Buggy and Tobin, 2008; Matthiessen and Law, 2002), motivating studies on their ecological fate. In this work, two sites of interest were considered: the Danshuei Estuary for the sub-tropical region and the Scheldt Estuary for the temperate region.

2.2.1 Danshuei Estuary

The Danshuei river is formed by the confluence of its three major tributaries: the Tahan Stream, Hsintien Stream, and Keelung River. Its drainage area encompasses more than $2700\,\mathrm{km}^2$ and includes the capital city of Taipei and its metropolitan area. The Danshuei Estuary is the largest estuarine system in Taiwan (Fig. 2.2). Its tidal influence spans a total length of more than $80\,\mathrm{km}$, encompassing the whole Danshuei river and extending to the downstream reaches of its three tributaries. The estuary and especially its upper zone is highly polluted consequence of the direct release of untreated domestic discharges, fallout from atmospheric pollution and both treated and untreated industrial discharges (Jiann et al., 2005; Hung et al., 2007; Jiann and Wen, 2009). Hypoxic and anoxic conditions were reported during the summer months in the upper and middle parts of the estuary (Jiann et al., 2005; Wang et al., 2007). The relatively short residence time (Wang et al., 2004) seems to be one of the limiting factors resulting in low phytoplankton biomass in spite of extremely high nutrient concentrations (Wen et al., 2008).

2.2.2 Scheldt Estuary

The Scheldt river takes its rise in Northern France and flows through west Belgium then into the North Sea in the south-west part of the Netherlands. The river and its tributaries drain an area of over $21\,800\,\mathrm{km}^2$ characterized by a high density of urban population and industrial activity. They are used as a major drain for industrial and domestic wastes. The Scheldt Estury is a strong tidal estuary and one of the largest of the European northwest continental shelf, measuring about $160\,\mathrm{km}$ long with a tidal range of $4\,\mathrm{m}$ at the mouth (Fig. 2.2). It represents an important shipping route to Antwerp industrial area and has been profoundly affected by building of quays and wharfs and is at some places almost completely canalized (Meire et al., 2005). The estuary includes wetlands and marshes and is an important area for ecological diversity. It is fairly well mixed vertically but the existence of a turbidity maximum at low salinity and upward residual currents along the bottom in the brackish zone result in the accumulation of sediments and in the trapping of particulate contaminants in its upper part. The upper estuary receives large amounts of organic matter inducing anoxic conditions in the water column during summer (Baeyens et al., 1998). The residence time of fresh water is high and pollution, especially by toxic metals, is one of the major threats to its ecosystem (Baeyens et al., 1998).

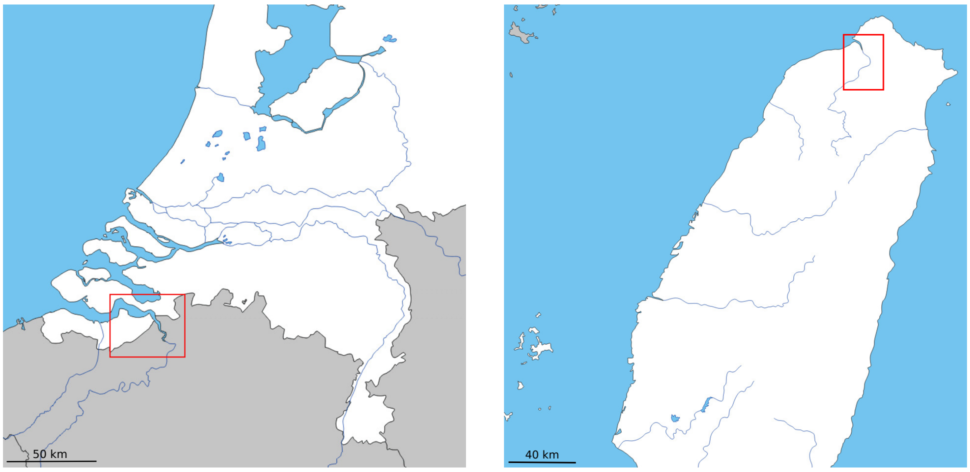

Figure 2.2: Locations of the two sampling sites: the Scheldt Estuary in South West Netherlands (left) and Danshuei Estuary in Northern Taiwan (right).

2.3 Zooplankon behavior in response to environmental parameters

Because behavior links the cellular and biochemical level to the organism level, behavioral indicators of toxicity appear ideal for assessing the effects of waterborne pollutants on aquatic organisms. By considering behavior, we also gain a better ability to quantify anthropogenic perturbations in the aquatic community and to predict their effects. Moreover, copepod swimming behavior is affected by a variety of natural environmental variables. There is therefore a need to quantify the effects that environmental factors, whether natural or anthropogenic, exert upon them. This section gives an overview of the effects of natural parameters and chemical toxicants on the behavior of zooplankters, and more especially copepods.

2.3.1 Perception in copepods

Copepods have the ability to remotely detect food particles, mates and potential predators. A combination of mechano- and chemosensors on their cephalic appendages serves copepods to perceive dissolved substances and hydrodynamic disturbances.

Chemoreception

Calanoid copepods live in a nutritionally dilute environment. Their feeding behavior relies on chemoreception. A copepod hovering or slowly swimming can use chemoreception to remotely detect individual algae entrained by the flow field around itself and uses active coordinated movements of the cephalic appendages to bring these particles to the capture area (Paffenhöfer et al., 1982; Strickler, 1982; Moore et al., 1999; Jiang et al., 2002b). Similarly, chemoreception is tightly linked to mate location (Kiørboe et al., 2005).

In calanoid copepods, remote chemical stimuli are perceived by sensory structures called aesthetascs or chemoreceptive sensilla and located in part on the first antennae, one of the primary organ involved in the perception of the surrounding environment (Bundy and Paffenhöfer, 1993). Aesthetascs are thin-walled, cylindrical and innervated structures whose basal part contains the unsheathed ciliary segments of sensory cells. Receptors are also present on head and mouth appendages, allowing copepods to make feeding decision in the presence of chemical information (Paffenhöfer et al., 1982). Ultrastructural studies have shown variation in receptor morphology. For instance, two kinds of receptors were found in the mandibles and mandibular palps and in the first and second maxillae of the copepod *Diaptomus pallidus* (Friedman and Strickler, 1975). Receptors in the mandibles were enclosed in a cuticular sheath and contained one or two ciliary dendrites. In the first and second maxillae and in the mandibular palps, sensilla were formed by a setae containing several ciliary or non-ciliary dendrites. A pore system was observed in their distal region, permeating their cell wall and supporting chemoperceptive capabilities (Paffenhöfer and Loyd, 2000). Aesthetascs on copepod antennules are structurally similar to those described for other crustaceans, though different patterns of innervation have been observed between species (Bundy and Paffenhöfer, 1993). Aesthetascs are bulbous in shape with a basal part through which the unsheathed ciliary segments of the sensory cells extend to fill the lumen of the sensilla (Gill, 1986; Bundy and Paffenhöfer, 1993). It is worth noting that the chemosensory system of the antennules is enhanced at the final molt in many adult male planktonic copepods (Boxshall and Huys, 1998).

Mechanoreception

In copepods, steady propulsion is typically accomplished by the vibration of the feeding appendages (van Duren and Videler, 2003). However, upon detecting hydrodynamic disturbances created by approaching predators, copepods jump by sequentially striking their antennae and the four or five pairs of legs backwards followed by a recovery stroke where all legs are moved forward simultaneously (Buskey et al., 2002; van Duren and Videler, 2003; Borazjani et al., 2010). Escape jumps bring the copepod to a safe distance from the predator. They are facilitated by the sensory system extending from the body and located, for the main part, on the first antennae.

Detection of hydromechanical signals is permitted by setae which are the extension of modified ciliary structures and the site of signal transduction. They are located on the first antennae and function as mechanoreceptors, conveying information about an approaching zooplankter or a particle in the feeding current (Strickler and Bal, 1975). Ultrastructural studies in several species have revealed their cellular organization (Weatherby and Lenz, 2000). Setae are highly rigid structures which contain synaptic junctions, synaptic vesicles and a band of cross-linked microtubules. They are connected to the antennae by a complex of microtubules which might serve to connect mechanically the bipolar nerve cell with the moving tip of the seta and to transmit electrical signals derived from vibrations and movements of the seta (Strickler and Bal, 1975; Bundy and Paffenhöfer, 1993). Different patterns of innervation were observed between mechano- and chemoreceptors, the former being innervated by less dendrites (Gill, 1986). Different morphologies (either smooth or plumose) were observed in mechanoreceptive setae (Yen et al., 1992; Bundy and Paffenhöfer, 1993). Similarly, variations in the morphology and arrangement of setae were observed between species as well as within different sectors and locations on the antenna, suggesting different sensitivities to different cues (Yen et al., 1992). The integument can also be covered with structures resembling sensillae, suggesting that mechanoreception must also occur on other parts of the body (Gill, 1986). Finally, setae can be innervated by both chemo- and mechanosensory dendrites (Weatherby et al., 2000).

Neural activity in the antennae increases rapidly and with a high sensitivity in response to brief mechanical stimuli even at high frequency (Yen et al., 1992). Different species have distinct neural activity patterns, the species with large axones displaying rapid response behavior (Yen et al., 1992). Moreover, cross-sections through the first antenna of several species in more recently evolved copepods have revealed in some well-developed multilayered sheaths resembling vertebrate myelin surrounding nearly all axons of both sensory and motor nerves of the first antenna and the interneurons of the ventral nerve cord (Davis et al., 1999; Weatherby et al., 2000; Lenz et al., 2000). Significant differences in reaction times were observed, with myelinated species taking less time to initiate escape behavior (Davis et al., 1999). However contradicting results showed absence of difference in latency and similar kinetic performance between myelinated and nonmyelinated species (Wagget and Buskey, 2008).

2.3.2 Effects of anthropogenic contaminants

An increasingly popular approach to appreciate anthropogenic disturbances involves examining changes in behavior (Clotfelter et al., 2004; Zala and Penn, 2004). Prolonged exposures to low concentrations of pollutants are not likely to result in lethal toxicity, rendering conventional mortality tests less relevant. In this context, behavior is an appropriate metric, capturing differences occurring at sub-lethal concentration and conveying important information about impairment of subtle ecological processes (e.g. Krång, 2007; Ward et al., 2008; Henry et al., 2012).

Individual level

Numerous studies have addressed the behavioral response of various species to the presence of several classes of toxicant and at different concentrations, leading to a wide range of results which may be challenging to compare. Hyper- or hypo-activity have been commonly observed, depending on the species, dosage, exposure time and toxicant.

For instance, a several day exposure to cadmium at $10^{-8}\,\mathrm{mol\,L^{-1}}$ reduced the swimming velocity and food intake of *Daphnia magna* (Baillieul and Blust, 1999). Similarly, a $9\,\mathrm{h}$ exposure to copper at $30\,\mathrm{\mu g\,L^{-1}}$ caused a reduction in the swimming velocity of the same species, but no change was observed following a $24\,\mathrm{h}$ exposure to $5\,\mathrm{\mu g\,L^{-1}}$ (Untersteiner et al., 2003). The pyrethroid insecticide cypermethrin inhibited the feeding efficiency and swimming activity of *D. magna* at environmentally realistic concentrations ranging from $0.1\,\mathrm{\mu g\,L^{-1}}$ to $1\,\mathrm{\mu g\,L^{-1}}$ after a $6\,\mathrm{h}$ exposure time, with recovery of the swimming response exhibiting a concentration response pattern (Christensen et al., 2005). On the opposite, a $24\,\mathrm{h}$ exposure to methyl-paraoxon at $0.7\,\mathrm{\mu g\,L^{-1}}$ caused an increase in velocity in the same species (Duquesne and Küster, 2010). Nanoparticle (titanium dioxide and fullerenes) suspensions altered the behavior of *Daphnia* at sublethal concentrations, causing hopping frequency and appendage movement to increase (Lovern et al., 2007). The authors associated theses results with an increased risk of predation and reproductive decline. The study of Shimizu et al. (2002) indicated a strong increase in the trajectory complexity and a $60\,\mathrm{min}$ latency in the behavioral response of individual *Daphnia* to $CuSO_4$ and organophosphorus at $10\,\mathrm{\mu g\,L^{-1}}$ and to carbamate at $500\,\mathrm{\mu g\,L^{-1}}$, with the magnitude of the change in the fractal dimension related to the toxic chemical concentration and the exposure time.

Similar results have been obtained in other zooplankters. For instance, larvae of *Balanus amphitrite* exposed to sub-lethal levels of several classes of pollutants (antifouling biocides, neurotoxic pesticides and heavy metals) showed a clear alteration of their swimming activity (Faimali et al., 2006). The organophosphorus pesticide dimethoate had sublethal toxic effects on the freshwater rotifer *Brachorius calyciflorus*, strongly inhibiting its angular and linear swimming speed after a few hour exposure at $180\,\mathrm{\mu g\,L^{-1}}$ (Guo et al., 2012). In the study of Charoy et al. (1995), changes in the locomotory behavior of *B. calyciflorus* were used as sublethal indicators of toxic stress; copper, pentachloro-phenol, lindane and 3,4-dichloroaniline affected either the swimming speed, trajectory sinuosity and period of swimming after a $2\,\mathrm{h}$ exposure, with the $2\,\mathrm{h}\,\mathrm{EC}_{50}$ obtained with the behavioral test being of the same order of magnitude as the $24\,\mathrm{h}\,\mathrm{LC}_{50}$ resulting from conventional acute toxicity tests.

Indeed, results from behavioral tests clearly indicate that swimming activity is a valid endpoint in ecotoxicological studies and highlight the value of behavioral disruption as a sensitive indicator of environmental chemical contamination. For instance, tests with larvae of *Artemia* sp. and the rotifer *Brachionus plicatilis* demonstrated the higher sensitivity of behavioral tests compared to the classic mortality assays, with alterations in swimming speed detected at toxic compound concentrations as low as a few percents of their LC_{50} values (Garaventa et al., 2010). Similar results have been obtained elsewhere. Copper and the pesticide lindane strongly impaired *D. magna* grazing at $10\,\mu g\,L^{-1}$ and $50\,\mu g\,L^{-1}$ after a $48\,h$ exposure, whereas the EC_{50} for immobilization test were $47\,\mu g\,L^{-1}$ and $383\,\mu g\,L^{-1}$, respectively (Clément and Zaid, 2004). A study designed to assess the phototactic behavior of *D. magna* in presence of a wide range of chemicals commonly found in the aquatic environment showed that the lowest concentrations detected were between 2 and 43 times lower than the LC_{50} and EC_{50} (Martins et al., 2007). Behavior was also found to be more sensitive than acetylcholinesterase activity in shrimps exposed for $24\,h$ to sublethal concentrations of methamidophos (García de la Parra et al., 2006).

It is worth noting that behavioral alterations have also been observed in mobile epibenthic or partly zooplanktonic crustaceans. For instance, the freshwater amphipod *Gamarus pulex* showed decreased activity in presence of fluoxetine, ibuprofen and carbamazepine at low concentrations ($10\,ng\,L^{-1}$ to $100\,ng\,L^{-1}$) and in presence of a cationic surfactant (up to $100\,mg\,L^{-1}$) (De Lange et al., 2006). Behavioral responses, such as feeding rate, locomotor and ventilatory activities, were significantly reduced by cadmium exposure in the same species (Felten et al., 2008). Similarly, exposure to cadmium during 7 days and at sublethal and environmental relevant concentrations ($0.5\,\mu g\,L^{-1}$ and $1\,\mu g\,L^{-1}$) resulted in disrupted swimming activity and hyperbenthic behavior of *Neomysis integer*, with fewer individuals moving upstream against the current and more individuals found in the water column (Roast et al., 2011).

Community level

The effects of anthropogenic contaminants on zooplankton behavior can be observed at larger scales. Biological interactions play a major role in regulating the population dynamics of zooplankton. Several toxic substances, especially pesticides, have been shown to affect the structure of the zooplankton community by perturbing interactions between organisms and impacting their fitness (Jones et al., 1991; Hanazato, 2001).

For instance, the combined effects of predators and chemicals has been studied in mesocosms, showing changes in predator-prey interactions and in the population structure after application of lindane (Peither et al., 1996) or carbaryl (Chang et al., 2005; Hanazato and Yasuno, 1990). Zooplankters change their swimming behavior when exposed to pesticides and these changes may alter relationships between predators and preys. A study investigating changes in the vulnerability of six herbivorous freshwater rotifer species to predation by a predatory rotifer in the presence of sub-lethal concentrations of pentachlorophenol reported toxicant-induced changes in swimming behavior and a subsequent alteration in predation risk (Preston et al., 1999). Similarly, the behavioral response of *Daphnia* to carbaryl exposure was investigated by analyzing trajectories of free-swimming animals exposed to the chemical (Dodson et al., 1995). Animals showed extreme and continuous escape behavior in response to acutely toxic levels of carbaryl and an increase in escape-like behavior in response to sublethal levels, with an increased probability of *Daphnia* being eaten by fish. A study examining the influence of PCBs on the response of *Daphnia* to the presence of fish kairomones or cyanobacteria revealed that depth selection was affected by these two stressors through disruption of natural reaction, leading to an inadequate behavioral response (Bernatowicz and Pijanowska, 2011).

Studies on copepods

Behavioral toxicology is disproportionately biased towards studying freshwater organisms. Despite their crucial position in the dynamics of marine ecosystems, the number of studies that address the potential impact of anthropogenic contaminants on marine copepods is still limited. These studies have concentrated mainly in acute lethal responses or observation of feeding impairment (Saiz et al., 2009) and only a few have considered free swimming behavior as a metric.

Nauplii of *E. affinis* were found sensitive to sublethal copper concentrations ranging from $1\,\mu g\,L^{-1}$ to $50\,\mu g\,L^{-1}$, with an initial hyperactivity between 24 h and 72 h followed by a depressed activity after longer exposure (Sullivan et al., 1983). After a sub-lethal exposure to an aqueous solution of 4-nonylphenol and nonylphenol-ethoxy-acetic-acid, males and females *E. affinis* displayed an increase in swimming speed and activity, along with more frequent sinking events, which resulted in a helter-skelter swimming behavior (Cailleaud et al., 2011b). The water-soluble fraction of diesel oil at sub-lethal concentrations (0.01 %, 0.1 % and 1 %) affected the swimming path complexity of both adult males and females of the marine copepod *Temora longicornis* and impacted mating behavior and success by reducing the ability of males to detect and follow female pheromone trails (Seuront, 2011b). The two species *E. affinis* and *T. longicornis* showed avoidance of patches contaminated with water-soluble fraction of diesel oil at similar concentrations (Seuront, 2010). The presence of copper, chromium and endosulfan at sublethal concentrations resulted in higher escape ability of the copepods *Notodiaptomus conifer* and *Argyrodiaptomus falcifer* to suction flows (Gutierrez et al., 2012) . However the authors questioned the immediate apparent advantage, since the increase in one physiological or behavioral process may be at the expense of another.

2.3.3 Effects of natural parameters

Because of their poor swimming ability in regards to hydrodynamics, copepods are at the mercy of the intrinsic properties of the surrounding water. Swimming, feeding behavior and predator-prey interactions are fundamental activities in the ecology of copepods and integral components of their fitness. A greater understanding of how environmental constraints influence these behavioral processes is required.

Hydrodynamic disturbances

Copepods are preyed upon by a variety of invertebrate and vertebrate predators and have evolved behavioral adaptations to avoid predation. Several studies have presented different copepod species with local hydrodynamics disturbance created by artificial mechanisms or by moving organisms in an effort to elicit and quantify these escape reactions.

Results obtained with high-speed cameras have indicated that copepods exhibit rapid escape behavior with latencies as low as 2 ms in response to small hydrodynamic disturbances, displaying multiple and repeated power strokes of the swimming legs (Yen and Strickler, 1996; Lenz and Hartline, 1999; Buskey et al., 2002, 2012). The threshold needed to elicit an escape reaction varies between copepod species and developmental stages, with animals captured from more energetic regimes requiring a higher threshold than those captured from more pacific locations (Fields and Yen, 1997; Titelman, 2001). The signal which triggers escape reactions is the rapidly rising water deformation produced by the suction-induced disturbance created by the predator (Holzman and Wainwright, 2009). Bradley et al. (2013) created small hydromechanical stimuli to elicit behavioral responses in *Eurytemora affinis* and in *Parvocalanus crassirostris* and showed that (i) maximum response distances were greater and response latencies were shorter in copepodits than in nauplii (ii) the maximum escape speed increased with copepod size (iii) the duration of the escape response decreased with the developmental stage. Direct numerical simulation have shown that escape velocities and force and power output needed to accelerate and overcome drag in escaping copepods were very high compared with those of other aquatic animals, with weight-specific force more than an order of magnitude higher (Kiørboe et al., 2010).

Escape reactions are energetically costly and copepods react selectively to hydrodynamic disturbances of different strengths. They show ability to differentiate small signals emitted by a fellow zooplankter from large signals emitted by a predator, displaying measured reactions towards small deformations of the fluid (Yen and Strickler, 1996; Strickler and Balázsi, 2007). Copepods are also able to assess the direction of flow and to exhibit appropriate behavior, orienting their escape jumps away from the source of disturbance (Fields, 2002; Titelman and Kiørboe, 2003). The ability to interpret hydromechanical signals based on their intensity seems to come from different physical and subsequent physiological responses of the antennule setae to various intensities of water flow (Fields et al., 2002). In the copepod *Gaussia princeps* for instance, shortest hairs were physiologically less sensitive than the longer setae despite being more easily displaced by fluid flow and the authors suggested a role in the detection of rapid fluid motion. On the opposite, the combination of high resistance to displacement and acute physiological sensitivity allowed the long seta to respond to hydrodynamic disturbances of low intensity while filtering high-frequency background noise (Fields et al., 2002).

Turbulence and ambiant flow

Copepods in pelagic environments are subject to turbulent flow motions which appear to affect their distribution. As a result, copepods are not randomly distributed in the water column. Several field studies have shown that turbulence avoidance behavior can lead to enhanced subsurface concentrations. It has been observed that some copepods travel to deeper depths in the water column during turbulent events at the surface, while others prefer turbulent conditions and remain closer to the surface (Mackas et al., 1993; Lagadeuc et al., 1997; Visser et al., 2001; Visser and Stips, 2002). For instance, turbulence from a moderate wind event triggered active movements of copepods and influenced their *in situ* vertical distribution in the surface mixed layer (Incze et al., 2001). Similarly, copepods can resist to relatively strong hydrodynamic conditions and swim actively to maintain their position. Comparisons of three dimensional tracks obtained by acoustic tracking with the simultaneous recordings of local currents have revealed that under both downwelling and upwelling conditions, copepods passively drift with horizontal currents but swam against strong vertical currents with a complete depth retention, accumulating in regions of persistent fronts (Genin et al., 2005). Zooplanktonic crustaceans respond behaviorally to changes in hydrostatic pressure (Lincoln, 1971; Forward et al., 1989) and the authors indicated a pressure-sensing mechanism as a possibility for directional swimming and depth maintenance in zooplankton. In copepods indeed, mechanoreceptors on the first antennae appear to function as gravity receptors (Strickler and Bal, 1975).

At the individual level, turbulence also affects copepod feeding or escape behavior by changing the perception of their surrounding environment. For instance, moderate turbulence increased encounter rates between copepods and algae at low concentration, rising perceived food concentration to levels comparable to satiation and inducing switch to feeding bouts of shorter duration (Marassé et al., 1990; Saiz, 1994). Similarly, the jumping frequency was higher, jumps were faster and feeding bouts more frequent in *Acartia clausi* under turbulence compared to calm conditions (Saiz and Alcaraz, 1992). These results tend to confirm the hypothesis of enhanced encounter rate between predators and preys under water motion (Rothschild and Osborn, 1988). However feeding enhancement is not automatically positive and moderate to high turbulence intensities can cause detrimental effects on feeding efficiency, most likely driven by a lower capture success. For instance, small-scale turbulence affected the feeding of the ambush copepods *Oithona davisae* in different ways (Saiz et al., 2003). Positive effects on feeding were evident only at low turbulence intensity and at higher turbulence levels there was either no effect or impairment of feeding. It also appears that turbulence has a major effect on ambush copepods at realistic intensities and marginal effects on suspension feeders i.e. on copepods which set up feeding currents (Kiørboe and Saiz, 1995). Small scale turbulence has also been demonstrated to interfere with the ability of copepods to detect hydrodynamic signals associated with predators (Gilbert and Buskey, 2005), although contradicting results have shown similar escape reactions under non-turbulent and turbulent conditions (Waggett and Buskey, 2007). Finally, turbulence can trigger unnecessary escape reactions whose frequency, however, appears to lessen after repeated stimulation (Hwang et al., 1994).

Presence of food

The behavioral response of copepods to the presence and absence of phytoplankton cells or exudate is diverse. It appears that most adaptations to the presence of algae aim at increasing food intake while minimizing energy expenditure and consequently, the concentration of phytoplankton seems to determine the level of activity.

For instance, the swimming behavior of the copepod *Pseudocalanus minutus* in the presence of *Skeletonema costatum* at $10^4\,\mathrm{cell\,mL^{-1}}$ was characterized by a decrease in average swimming speed and activity compared to its swimming behavior in filtered seawater, yet the presence of filtered phytoplankton exudate alone resulted in an increased swimming speed (Buskey, 1984). Strong changes in swimming behavior were observed in *Daphnia* and *Acartia* in the presence of food (Dodson et al., 1997; Tiselius et al., 1997). Similarly, adults of *Temora longicornis* showed low swimming speeds at very low food concentrations, higher swimming speeds at intermediate concentrations and low swimming speeds at very high food concentrations (van Duren and Videler, 1995). The swimming speed and complexity of trajectories of *Calanus sinicus* were likewise reduced in the absence of food (Chen et al., 2012). Tiselius (1992) observed increased motility in *Acartia tonsa* transferred to a homogeneous suspension of the diatom *Thalassiosira weissflogii* but fast behavioral responses to ephemeral patches of diatoms and a combination of decreased motility and more convoluted paths after entering a patch, indicating that copepods have strong ability to remain within areas of high food concentration (Tiselius, 1992). The results of Lombard et al. (2013) demonstrated that copepods - in this case *T. longicornis* - were able to remotely detect and follow sinking food particles and eventually grab them and feed on them.

Light intensity and endogenous rhythms

Most copepod species possess simple naupliar eyes which are sensitive to slight variations in light intensity. Rapid changes in light intensity (both flashes and shadows) elicited vigorous escape reactions in copepods (Buskey et al., 1983; Buskey and Hartline, 2003). This behavior may have adaptive value, reflecting an avoidance mechanism to moving predators. The pattern of response seems to differ for calanoid copepods from freshwater, estuarine and oceanic environments (Buskey et al., 1987). Shafts of light caused copepods to swarm after an initial photophobic response, even at low intensity, with copepods reversing their swimming direction upon encountering a light intensity gradient near the edge of a light shaft (Buskey et al., 1995). The presence of endogenous rhythms in the swimming behavior of marine copepods was investigated by Seuront (2011a) who showed an increase in the degree of convolution of trajectories at night and who suggested a possible endogenous swimming rhythm aiming at increasing food foraging activity.

Temperature and seasons

Different species of *Daphnia* responded to small changes in temperature by changing the duration and frequency of upward swimming in direct proportion to the rate of change of temperature (Gerritsen, 1982). Hirche (1987) investigated in several Arctic copepod species the potential contribution of swimming activity to total metabolism at different temperatures and found that the increase in metabolic rate with rising temperature was not related to a higher swimming activity, as the average swimming speed remained unchanged at all temperatures and in all species. There were however species-specific differences in the time spent actively swimming, with copepods either showing no change or increasing or decreasing the duration of periods of active swimming. Moison et al. (2012) studied the effect of seasonal temperature variation on the swimming speed of the copepod *Temora longicornis* from the English Channel and observed a higher swimming speed, a temperature-dependent increase in swimming activity and a lower activity in the low temperature range in the summer population compared to the winter population, revealing in this species the ability to adjust to environmental changes in temperature. Differences in swimming parameters (turning angles, swimming speed and sinking rate) between seasons were similarly reported in *Daphnia pulicaria* by Ryan and Dodson (1998), yet without significant correlation with temperature. The results of Ziarek et al. (2011) on the opposite demonstrated that the individual swimming behavior of *D. pulicaria* was affected by ambient temperature - and by light - with a predominance of vertical movements in cold water, regardless of the light condition, and a predominance of lateral motions in warm water and in the dark.

Chemical cues

Planktonic organisms are able to detect the presence of a potential predator through kairomones and to change their swimming behavior in an attempt to reduce their visibility or to escape predators (Lass and Spaak, 2003). The daily vertical migration of *Daphnia* for instance appears to be an adaptive predator avoidance triggered or enhanced by chemical cues (Dodson, 1988; Ringelberg, 1991). Chemically-induced migrations have also been observed in other species, for instance in previously non-migrating freshwater copepods in response to *Chaoborus* kairomones (Neill, 1990) or in *Chaoborus* larvae in response to the presence of chemical cues from planktivorous fish (Tjossem, 1990). Changes in behavior can also be observed at smaller scales. For instance, the copepod *Diaptomus sicilis* reduced its swimming speed and jump frequency in presence of the predatory copepod *Limnocalanus macrurus* (Ramcharan and Sprules, 1991). Similarly, chemicals released into the water by the predatory zooplankter *Epischura nevadensis* strongly reduced the filtering rate of the copepod *Diaptomus turrelli* (Folt and Goldman, 1981). This behavior has been observed in other species (Cieri and Stearns, 1999) and may be linked to the evolution of a mechanism for avoiding predation. Kairomones from *Chaoborus* and *Perca* induced changes in behavioral parameters (swimming speed, trajectory length and vertical distribution) in clones of *Daphnia galeata*, with genotype-specific responses pointing out the importance of genetic variability in small-scale swimming behavior (Weber and van Noordwijk, 2002). Finally, it is worth noting that changes in aggregation behavior and escape behavior in *Daphnia* can be induced not only by predator kairomones but also by damaged conspecifics in what appears to be an effective predator avoidance strategy (Pijanowska and Kowalczewski, 1997).

Copepods live in a highly diluted environment which challenges them to locate mate. They have evolved behavioral adaptations to enhance mate location and mating probability. Females attract males by producing hydromechanical or chemicals signals which males detect and follow (Strickler, 1998; Tsuda and Miller, 1998; Weissburg et al., 1998; van Duren et al., 1998; Bagøien and Kiørboe, 2005a). Chemical signals are transmitted through plumes (Kiørboe et al., 2005) or trails (Doall et al., 1998; Bagøien and Kiørboe, 2005b) and affect conspecific males which suddenly increase their activity and path complexity (Tsuda and Miller, 1998) and follow the trail for relatively large distances and durations (Doall et al., 1998; Yen et al., 2011; Dur et al., 2011). Males attempt to determine the swimming direction of a female by repeatedly sampling its pheromone trail. They follow the trail, increasing their swimming speed as the concentration of pheromone increases. Once close enough, the male senses the mechanosensory disturbance caused by the female and attempts to capture her (Yen et al., 1998). Males sometimes start tracking the trail in the wrong direction (Bagøien and Kiørboe, 2005b; Doall et al., 1998; Yen et al., 1998) or lose a complicated or broken trail, in which case they increase their swimming speed and perform frequent shifts of direction in order to scan the surrounding water (Bagøien and Kiørboe, 2005b). Males and females generally have distinct motility patterns. In the species producing pheromones, males usually display faster and less convoluted trajectories than females, maximizing the chance of encountering a female signal (Dur et al., 2010, 2011). Finally, female copepods have been observed to adapt their swimming behavior in reaction to chemical signals from males, creating hydromechanical signals aimed at increasing encounter probability (van Duren and Videler, 1996).

These observations tend to demonstrate the fundamental role of chemoreception in copepod mating behavior. However, studies have been conducted in still water. Copepods in pelagic environments are subject to turbulent flow motion. The fate of chemical plumes in the presence of turbulence and the mechanisms allowing copepods to efficiently locate a mate using partial information have yet to be fully understood. Turbulence affects the structure of pheromone plumes which breaks into random and disconnected patches of concentrated chemical cues separated by gaps with no detectable signal. Male moths appear to fly faster and straighter upwind and locate source more frequently in the presence of turbulent or pulsed plumes than continuous and narrow plumes (Mafra-Neto and Cardé, 1995) but no studies of this kind have been conducted on copepods. Vertical migrations induced by salinity variations (Hough and Naylor, 1991), avoidance of surface water during the day (Lampert, 1989) or passive retention mechanisms in estuaries (Schmitt et al., 2011) are additional processes enhancing copepod patchiness. Random encounters in areas of high density or turbulent-induced increase in contact rate (Rothschild and Osborn, 1988) are possible explanations to the mating success of copepods in turbulent environments.

Chapter 3

Salinity affects copepod swimming behavior

Three dimensional observation of salinity-induced changes in the swimming behavior of the estuarine calanoid copepod *Pseudodiaptomus annandalei*.

3.1 Introduction

Maintenance and fitness optimization of copepod populations in macrotidal estuaries is a complex process because of the highly dynamic environment, leading to frequent variations in environmental conditions. Alternating tides reconfigure the estuarine hydrodynamics and cause fluctuations in freshwater and seawater inputs. They lead to important and rapid changes in salinity which put a real selection pressure on local organisms. Salinity represents a major parameter in the ecology of estuarine copepods, affecting their metabolism, life cycle and *in situ* distribution (Collins and Williams, 1981; Roddie et al., 1984; Soetaert and Rijswijk, 1993; Laprise and Dodson, 1994; David et al., 2005; Calliari et al., 2006; Holste and Peck, 2006; Devreker et al., 2009; Lin et al., 2011). Changes in salinity may also trigger vertical migrations involved in the maintenance of estuarine populations within preferential salinity areas despite strong hydrodynamic conditions. Vertical tidal migrations are directed toward the surface during low tide and flow and toward the seabed during high and ebb tide and allow copepods to avoid advection by seaward and landward flows toward areas with less optimal salinities (Morgan et al., 1997; Kimmerer et al., 1998; Schmitt et al., 2011). Similarly, an *in situ* study in the Seine Estuary has demonstrated the influence of salinity on the distribution of *Eurytemora affinis* during a tidal cycle, with a differential distribution of developmental stages as a function of salinity and depending on their swimming abilities (Devreker et al., 2008)

A previous work showed that salinity affected the swimming behavior of *E. affinis*, a copepod commonly found in the oligo- to mesohaline part of temperate estuaries (Michalec et al., 2010). Different salinities resulted in different swimming speeds and activities after a few hours of acclimation, with maximum values at low salinities and a steady decrease at higher salinities. These results supported laboratory and field studies indicating a preference of *E. affinis* for low to medium salinities and were attributed to an increase in energy allocated to osmoregulation rather than to swimming. However, copepods in the field experience gradual salinity increases whose effects were not tested and remain unclear. Moreover, a previous study found in the same species a steady increase in swimming speed and activity as salinity increased. The author suggested an endogenous behavioral adaptive strategy to salinity fluctuation (Seuront, 2006).

The calanoid copepod *Pseudodiaptomus annandalei* seems to be well adapted to salinity fluctuations, as suggested by its abundance in many tropical estuaries. However, laboratory experiments showed a higher offspring number, naupliar survival rate and fecundity at salinity 15 and a marked decrease in survival of adults above this salinity (Chen et al., 2006). Similarly, a recent work based on the population from the Danshuei estuary suggested that medium salinities were more appropriate for reproduction and survival at environmentally-realistic temperatures (Beyrend-Dur et al., 2011). Results from these two studies tend to indicate the existence in this species of an appropriate salinity range for physiological parameters.

Here we studied the movement of *P. annandalei* in the three-dimensional space and we tested for the existence and the extent of the effect of a realistic and progressive salinity increase on its swimming behavior. We aimed to determine whether the swimming activity (i) would vary together with salinity, suggesting an endogenous behavioral strategy to salinity fluctuation (Hough and Naylor, 1991; Seuront, 2006) (ii) would reflect once again the physiological preference of the species (Michalec et al., 2010) or (iii) would remain unaffected by salinity, a possible result considering the euryhaline nature of the species considered.

3.2 Methods

3.2.1 Studied organisms

Copepods were sampled in June 2010 from the oligo- to mesohaline part of the Danshuei estuary i.e. between GuanDu bridge and ZhongYan bridge, during the early flood tide and by using horizontal tows of a WP2 plankton net drifting in surface water. The surface water temperature was 27 °C and the salinity was 5. Copepods were kept in aerated containers filled with water from the sampling site and quickly brought to laboratory. They were sorted under a microscope and transferred into 5 L aquariums filled with filtered artificial sea water adjusted to a salinity of 5 with distilled water, under a 12L:12D light cycle and at 27 ± 2 °C. Prior to but not during experiments and at the same time every day, copepods were fed in excess on a unialgal diet of *Isochrysis galbana* from the laboratory cultures, harvested during the exponential growth phase and grown under a 14L:10D light cycle in f/2 medium and at a temperature of 27 ± 3 °C.

3.2.2 Experimental setup

Two synchronized analog CCD B/W video cameras (JVC TK S-250U) fitted with Nikkor 24 mm lenses at f/4 were mounted in front of a $10 \times 10 \times 11\,\text{cm}^3$ acrylic glass aquarium with 2 mm thick walls. The cameras were connected to a frame grabber and recorded at 30 frames per second. The aquarium was filled with 1 L of filtered water at salinity 5 and at 27 °C. Its inner faces were coated to prevent reflection and one face only was left transparent. The illumination consisted of two arrays of infrared (870 nm) light-emitting diodes mounted above the aquarium.

In the photogrammetric method, camera calibration is performed by observing an object whose geometry in the three-dimensional space is known with very good precision. The calibration object usually consists of a block with dots of known coordinates which provide correspondences between points in the images and points in the object space. The shape and dimension of the target object and the spacing between the reference dots and their size are determined based on the experimental conditions. In this study the calibration object was made from a gray polyvinyl chloride block in which 7 stages were machined at different z and y directions. On each stage, 15 white reference dots were evenly distributed along the x direction. The spacing between the dots was 5 mm in the x direction, 10 mm in the y direction and 10 mm in the z direction. The dots were 1 mm wide and 0.5 mm depth and were filled with white paint.

3.2.3 Filming conditions

Experiments were carried out at night to match the activity pattern of copepods, in the absence of food to prevent any synergetic effect and in a dark room under infrared illumination to avoid phototropism. Since *P. annandalei* shows clear substratum preference (Shang et al., 2008) and sticks to the walls or bottom of the aquarium, a relatively large number of copepods was used to ensure a sufficient amount of trajectories. Copepods were checked for integrity under microscope and damaged individuals were discarded. For each adult state (i.e. males, non-ovigerous females and ovigerous females), groups of 75 individuals (mean total body length: 1 mm for females and 0.9 mm for males) were gently transferred from the culture into the aquarium. Copepods were allowed to acclimate for 15 min before the recording started. Every 60 min of recording, 125 mL of water was gently and quickly removed from the aquarium and a similar volume of artificial hyper-saline water was added in order to increase the salinity by 5 while keeping the volume constant. After each addition, the fluid was gently stirred and copepods were allowed to recover for 5 min from the disturbance caused by light and turbulence before the recording resumed. The final salinity was 25 over the course of a 5 h recording, roughly corresponding to the range of salinity in the top and bottom layers of the mesohaline zone of the Danshuei estuary (Liu et al., 2007). Water temperature was monitored and remained constant at $27\,^{\circ}\mathrm{C}$ over the course of the experiments. The image sequences were digitized on a hard disk in a compressed format. Experiments were conducted twice for each adult state, on non-consecutive nights and within a few days after sampling.

3.2.4 Trajectory extraction

In the tracking procedure and to limit assignment mismatches, the algorithm requires as an input upper bounds for the magnitude of velocity and acceleration and these were set in this study at $45\,\mathrm{mm\,s^{-1}}$ and $1800\,\mathrm{mm\,s^{-2}}$, respectively. Copepods are not spherical in shape and constantly reorient themselves while swimming, adding noise to their recorded positions. Trajectories were extracted from the image sequences then smoothed using a spatially variant Gaussian filter to remove background noise. Each position was replaced by a weighted average of its neighbors estimated by a kernel operating symmetrically along each dimension. The coefficients of the filter decreased monotonically with the distance from the central coordinate. Trajectories were displayed and checked for tracking errors. Trajectories shorter than 30 frames were rejected. Trajectories lying within 5 mm of the bottom, surface and walls of the aquarium were similarly rejected in order to avoid border effects.

3.2.5 Trajectory analysis

A comparison of a number of variables is needed to quantify swimming behavior efficiently. In this study, scale-dependent and independent metrics were considered. Trajectory visualization and analysis were conducted using home-made MATLAB scripts.

Instantaneous and mean swimming speed

The magnitude of the instantaneous velocity (hereafter referred to as instantaneous speed) was estimated as the distance between two successive positions of a copepod in the 3D space divided by the time step. Instantaneous speeds were averaged for each trajectory. For each experimental condition we obtained a new sampling distribution on which we conducted analysis. Sampling distributions were not normally distributed (Kolmogorov-Smirnov test) and their contents could not be paired between treatments (same individuals but uncorrelated trajectories). Comparisons between experimental conditions were conducted using the Kruskal-Wallis test. The magnitude of the horizontal and vertical components of the velocity vector were also considered. The tangential acceleration was estimated as the difference between instantaneous speeds of two consecutive displacements. The behavior of copepods is intermittent, with a succession of fast and slow swimming behavior and rest periods. Probability density functions of speed and acceleration values were computed to show their relative frequencies and the importance of extreme values. For a given experimental condition, we estimated the mean swimming speed by averaging instantaneous speed values.

Swimming state frequency

The behavior of copepods can be considered as a succession of swimming states and codified as a finite sequence of successive symbols (Schmitt et al., 2006; Moison et al., 2009; Dur et al., 2010). Each symbol corresponds to a swimming state and is repeated within the sequence as many times as the swimming state occurs and according to its duration. To create the swimming state categories, instantaneous velocities were separated based on the shape of their probability density functions into 3 different groups corresponding to different behaviors (i) break for swimming speed values below $1\,\mathrm{mm\,s^{-1}}$, when the copepod does not swim but maintains its position in the water column, (ii) slow swimming for speed values between $1\,\mathrm{mm\,s^{-1}}$ and $5\,\mathrm{mm\,s^{-1}}$ when the copepod swims actively and cruises at a steady pace and (iii) fast swimming for speed values over $5\,\mathrm{mm\,s^{-1}}$ when the copepod swims at high speeds or displays escape reactions of large amplitude. Swimming state frequencies were estimated from the instantaneous speed series transformed into a symbol sequence.

Degree of space occupation

The fractal dimension is a scale-independent metric commonly used to assess the degree of space occupation of an organism trajectory (e.g. Uttieri et al., 2005, 2007). In this study, the fractal dimension was used to compare the degree of convolution between experimental conditions and was computed using the classical box counting method. The mesh size followed a geometric sequence and the minimal size was set to $1\,\mathrm{mm}$. For each trajectory, the origin of the grid lay at the intersection of the planes defined by the minimal x, y and z coordinates in the three-dimensional space (Buczkowski et al., 1998). The embedding volume was cubic and its size was equal to the maximal displacement of the copepod along the three dimensions. Trajectories shorter than $1\,\mathrm{cm}$ in the three-dimensional space were rejected. Fractal dimension was given by the slope of the power fit of the log-log plot of the number of boxes versus mesh size. For each trajectory the script computed a three-dimensional linear regression line through a principal component analysis. Principal component analysis minimizes the perpendicular distances from the data to the fitted model and is an appropriate method to fit a linear regression to scattered three-dimensional data. The discrepancy between the observed fractal dimension of the straight line and its expected value was used as a calibration factor in the estimation of the fractal dimension of the trajectory.

3.3 Results

Variable numbers of trajectories and instantaneous speed values were obtained for each experimental condition and are summarized in Table 3.1. For each adult state and at each salinity condition, a total number of trajectories ranging from 1479 to 8456 were considered for the data analysis. The number of instantaneous speed values ranged from $271\,305$ to $951\,346$ per condition. No concordance was found between number of speed values and salinity or adult state.

3.3.1 Instantaneous and mean swimming speed

Salinity had a significant effect on the mean swimming speed in the three adult states (Kruskal-Wallis test, p <0.01). In ovigerous females, mean speed steadily and significantly decreased from $3.5\,\mathrm{mm\,s^{-1}}$ to $2\,\mathrm{mm\,s^{-1}}$ when salinity increased from 5 to 25 (Fig. 3.1). In males, speed was low at salinities 5 and

Table 3.1: Number of trajectories and number of instantaneous speed values for the three adult states of *P. annandalei* and for the five salinity conditions tested.

		5	10	15	20	25
Males	Trajectories	7701	1479	2730	3489	6247
	Speed values	951 346	272 428	271 305	288 101	589 533
Females	Trajectories	1496	2332	3501	4723	4144
	Speed values	278 678	355 747	373 902	451 803	471 147
Ovigerous females	Trajectories	2996	2832	3977	5008	8456
	Speed values	403 605	356 518	531 823	555 373	929 280

25 with $1.7\,\mathrm{mm\,s^{-1}}$ and $1.9\,\mathrm{mm\,s^{-1}}$ and peaked at salinities 15 and 20 with $3.4\,\mathrm{mm\,s^{-1}}$ and $3.3\,\mathrm{mm\,s^{-1}}$, respectively. Non-ovigerous females showed a similar though less-marked trend. Speed increased from $1.8\,\mathrm{mm\,s^{-1}}$ at salinity 5 to $2.1\,\mathrm{mm\,s^{-1}}$ at salinity 15 and salinity 20, then decreased to $1.6\,\mathrm{mm\,s^{-1}}$ at salinity 25. Males swam faster than non-ovigerous females over the range of salinity tested. At low salinities, ovigerous females swam faster than the two other adult states.

The probability density functions of instantaneous speed values of males are shown in Figure 3.2 A to illustrate differences in their distributions between salinity conditions. Correlations between the probability density functions and their mean values can be seen, with a higher frequency of slow movements when the mean speed is low, and a more important proportion of fast movements when the mean speed is large. The horizontal component of the velocity was on average 1.5 times larger than the vertical component for all three adult states (Fig. 3.2 B displays the result for ovigerous females at salinity 15), showing a predominance of horizontal movements in *P. annandalei* behavior. Figure 3.2 C shows the probability density function of measured accelerations for the three adult states at salinity 20. Large accelerations are more common than one would expect relative to a Gaussian distribution. All adult states showed important variations in their swimming speed, as illustrated in Fig. 3.3 which shows a trajectory displayed by an ovigerous female at salinity 10. At the present temporal resolution, the maximal magnitude of the instantaneous velocity and acceleration was observed in females with $57\,\mathrm{mm\,s^{-1}}$ and in ovigerous females with $940\,\mathrm{mm\,s^{-2}}$, respectively. Copepods display complex behaviors with speed and acceleration of intermittent amplitude and accelerate on timescales shorter than the temporal resolution used in this study (Kiørboe et al., 2010). We remind here that the capture of large events such as spontaneous jumps with a higher temporal resolution would amplify intermittency.

3.3.2 Swimming state frequency

The swimming state frequencies were significantly different for each adult state between salinity conditions (Pearson χ^2 test, $p < 0.01$) (Fig. 3.4). Break and slow swimming behavior were mainly involved in the swimming activity. Break ranged from $24\,\%$ to $54\,\%$ in males, from $24\,\%$ to $38\,\%$ in females and from $5\,\%$ to $34\,\%$ in ovigerous females. Slow swimming ranged from $36\,\%$ to $50\,\%$ in males, from $62\,\%$ to $72\,\%$ in non-ovigerous females and from $60\,\%$ to $79\,\%$ in ovigerous females. Fast swimming accounted for less than $4\,\%$ of the swimming activity in females, from $5\,\%$ to $15\,\%$ in ovigerous females and from $10\,\%$ to $28\,\%$ of the total swimming activity in males. Ovigerous females were generally more often swimming slowly than non-ovigerous females and males. Frequencies correlated well with swimming

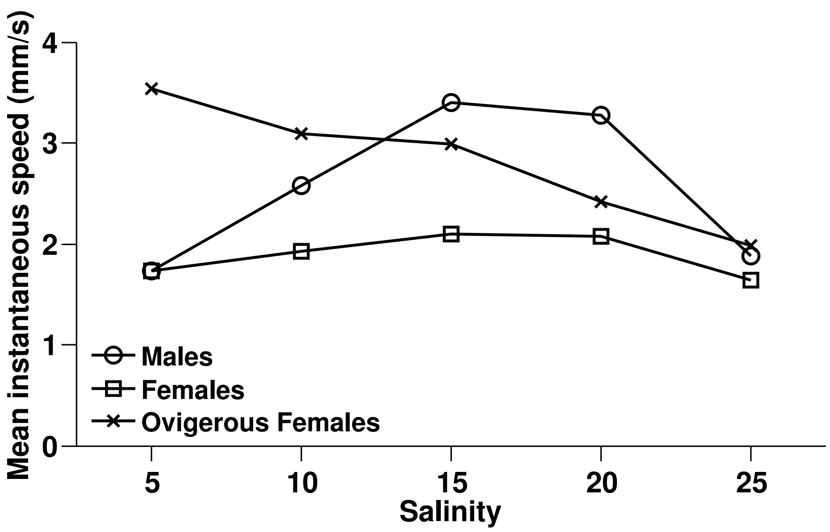

Figure 3.1: Mean instantaneous swimming speeds of *P. annandalei* males (circle), females (square) and ovigerous females (cross) for the five salinity conditions tested.

speeds in the three adult states. Higher swimming speeds came with higher frequencies of slow swimming and lower frequencies of break events and, in males and ovigerous females, higher frequencies of fast swimming behavior.

3.3.3 Degree of space occupation

The mean fractal dimension was similar between adult states and between salinity conditions (Fig. 3.5). Observation of trajectories revealed similarities and differences between the three adult states at all salinity conditions. Males traveled predominantly in a rectilinear way, both in the vertical and horizontal directions. Trajectories of females were on average more convoluted (Fig. 3.6). Trajectories of ovigerous females appeared on average more tortuous, with smooth changes in direction. For the three adult states but especially in females and ovigerous females, periods of slow swimming were interrupted by periods of break and sinking activity. Straight and convoluted trajectories were observed in the tree adult states.

3.4 Discussion

Due to alternating tides, estuarine copepods face strong and rapid salinity variations. It is still unclear whether and how salinity affects their swimming behavior at the scale of the individual, where key processes such as mating, feeding and predator avoidance occur. In this study we found a marked effect of salinity increase on the swimming behavior of the three reproductive stages of *P. annandalei*. For males and females, speeds were maximal at salinities 15 and 20 and lower at the two extreme salinities. For ovigerous females, speed was maximal at salinity 5 then steadily decreased as salinity increased. Differences were explained by variations in the frequency of break, slow and fast swimming behavior. The degree of space occupation was unaffected. There are limitations to measuring fractals in ecology (Turchin, 1996; Halley et al., 2004) and we remind here that the main objective was to compare the geometry of trajectories rather than to determine the true value of their fractal dimension.

Salinity variations place a strong osmoregulatory demand on estuarine organisms and have been linked in several studies to changes in behavior and metabolic activity. For instance, in marine copepods, wa-

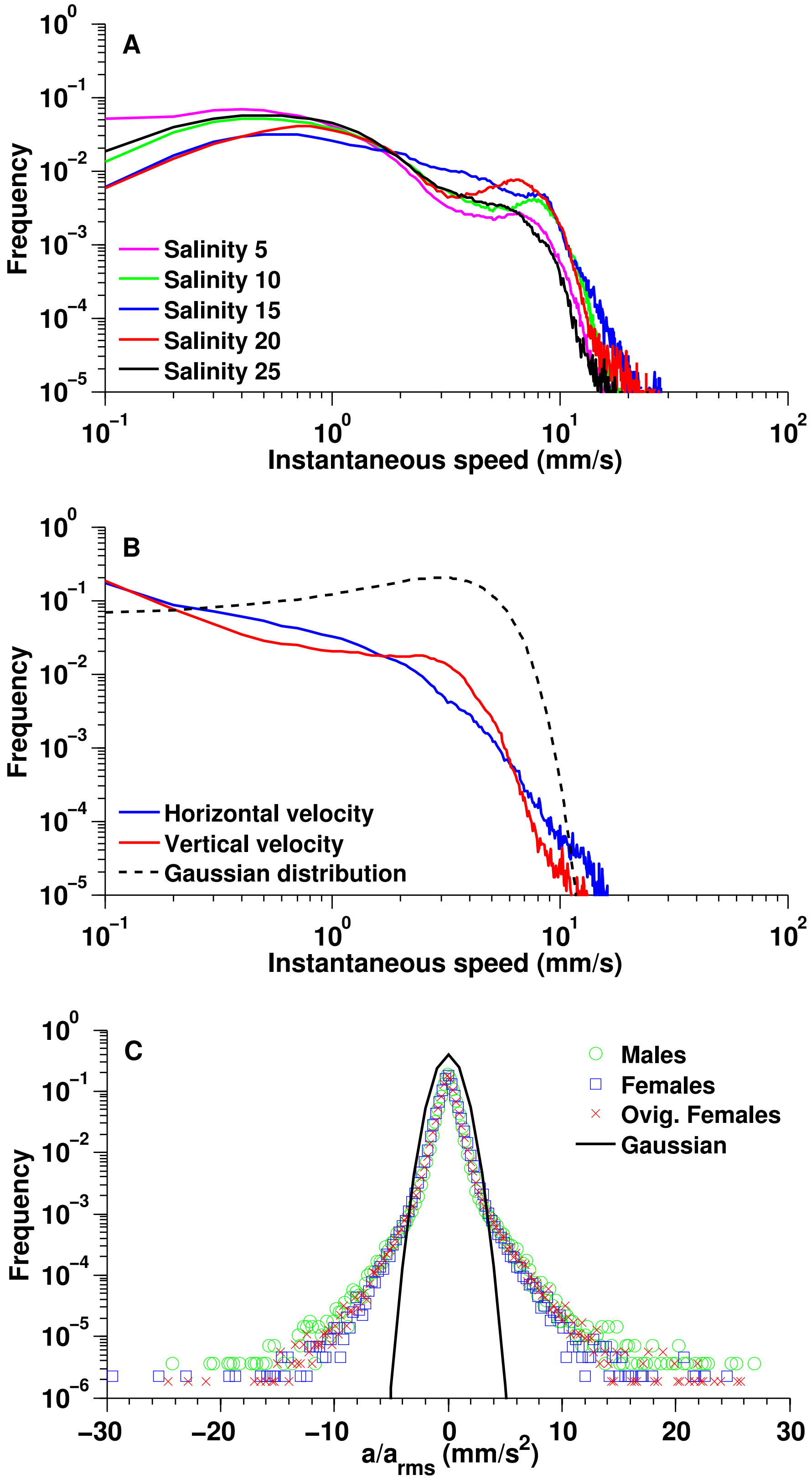

Figure 3.2: Instantaneous speed probability density functions of *P. annandalei* males for the five salinity conditions (A). Probability density functions of the horizontal (blue) and vertical (red) components of the velocity for ovigerous females at salinity 15 and probability density function of a Gaussian distribution of the same parameters (black dashed line) (B). Normalized acceleration probability density functions of males (green, circle), females (blue, square) and ovigerous females (red, cross) at salinity 20 ($rms = 25$, 22.9 and 24.3, respectively) and probability density function of a Gaussian distribution of the same parameters (solid line, black) (C).

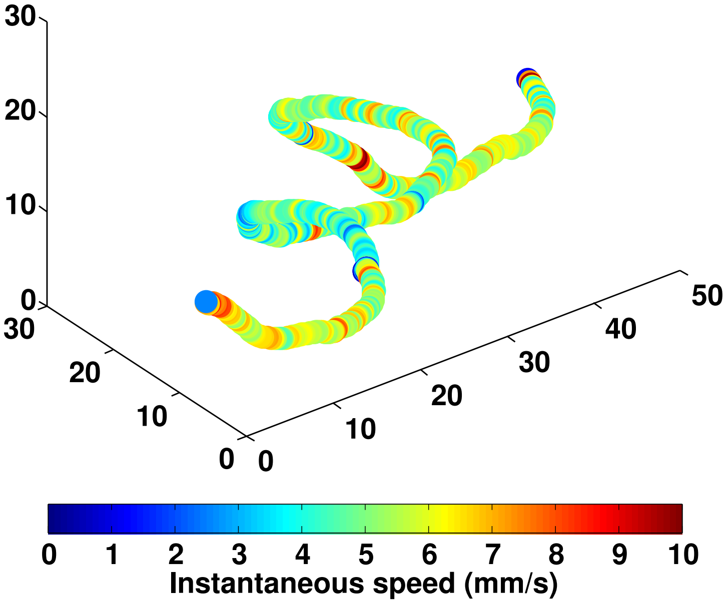

Figure 3.3: Illustration of a trajectory displayed by an ovigerous female *P. annandalei* at salinity 10, totalizing 863 frames and showing variations in the magnitude of instantaneous velocities. Coordinates are in mm.

ter of reduced salinity caused depressed swimming activity and variations in behavior according to the salinity tolerance of the species (Lance, 1962). Similarly, reduced salinity water caused major changes in the feeding rate of *Calanus helgolandicus* (Lance, 1964). Similar results have been observed in the harpacticoid copepod *Tigriopus brevicornis*, an inhabitant of high-shore rock pools, whose metabolic rate increased at the lowest salinity tolerated by the species with no increase in swimming activity, indicating the possible energetic requirement to maintain a constant osmotic body composition at very low salinities (MacAllen and Taylor, 2001). The effect of salinity on two biomarker enzymatic activities i.e. acetylcholinesterase and glutathione S-transferase was investigated in *E. affinis* (Cailleaud et al., 2007c). The authors found maximal expression related to an optimal salinity range and assumed an increasing energy devoted to osmotic regulation and a decrease in energy allocated to other biological processes. Similarly, hypo- and hyper-osmotic shocks caused in the same species a 2-fold induction of the transcript level of a cytosolic heat shock protein (Xuereb et al., 2012). Modifications in metabolism under stressful salinity conditions have been studied in the copepod *Acartia clausi* with detrimental effects on total metabolism at salinities beyond the optimal range (Calliari et al., 2006). The copepods *Acartia tonsa* and *A. clausi* increased their respiration rate when salinity conditions diverged from the optimal salinity zone of these species, indicating a need of supplementary energy for osmoregulation (Gaudy et al., 2000). Similar results were observed earlier in *Eurytemora hirundoides* (Gyllenberg and Lundqvist, 1979) and in copepods of the genus *Gladioferens* (King, 1976).

Field sampling in the Danshuei Estuary revealed that *P. annandalei* was the dominant species in the mesohaline zone and was unlikely to be found at higher or lower salinities, suggesting the existence of a salinity preference for this species (Hwang et al., 2010). Laboratory studies have shown a physiological preference of this species for medium salinities (Chen et al., 2006; Beyrend-Dur et al., 2011). In our study, the maximal swimming activity of males and females was observed at medium salinities. The daily energy required to adjust to osmotic stress accounts for more than 11 % of the total energy (Goolish and Burton, 1989). The depressed swimming activity at salinities 5 and 25 in females and males can be interpreted as the result of osmotic stress in nearly freshwater or under marine conditions. A decrease

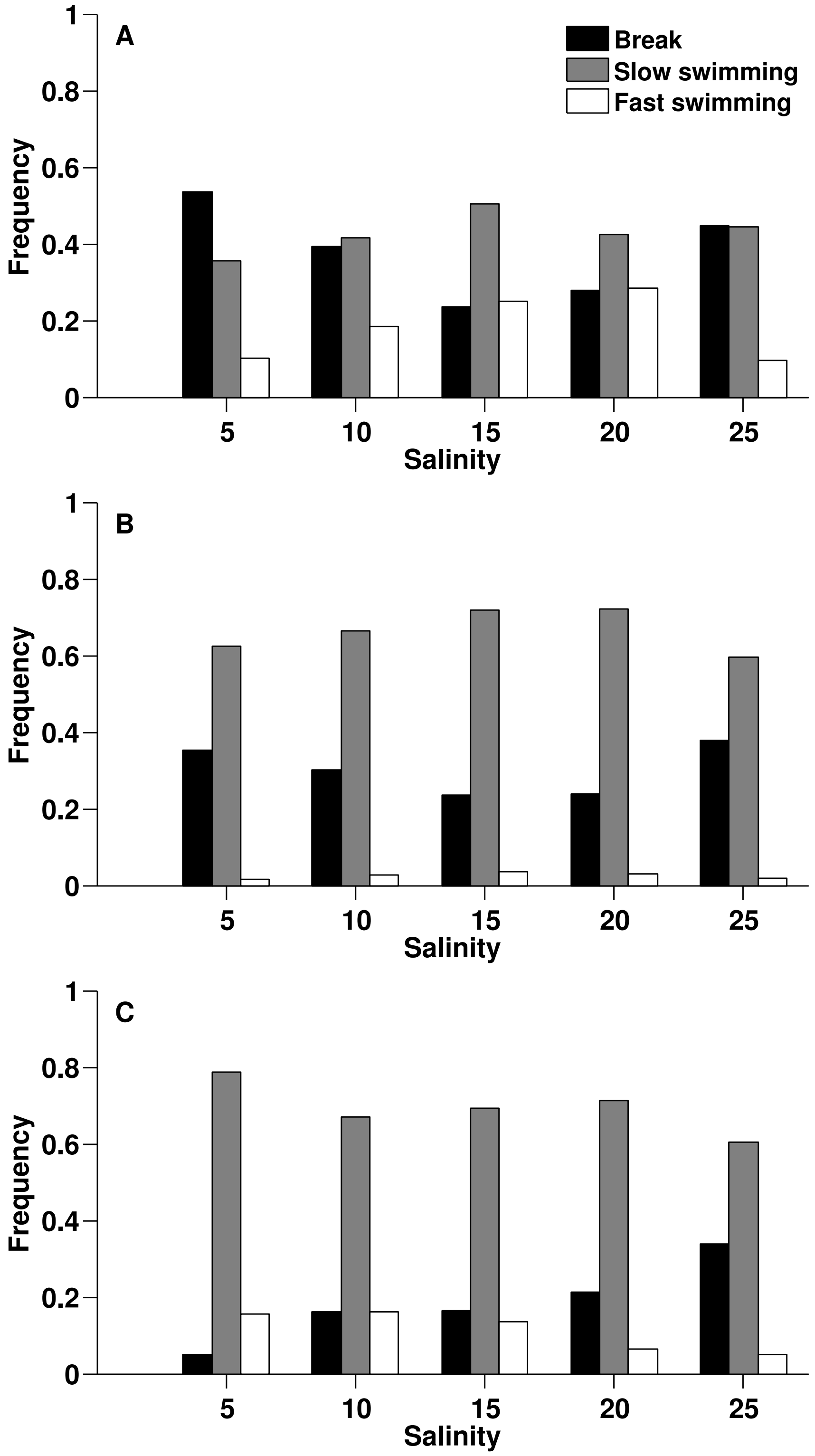

Figure 3.4: Frequency of break (black), slow (gray) and fast swimming state (white) of *P. annandalei* males (A), females (B) and ovigerous females (C) for the five salinity conditions tested.

in energy allocated to swimming and an increase in energy devoted to osmoregulation when salinity diverges from the optimal range of the species is a plausible explanation of our results. Further investigations are needed to link the behavioral response observed in this study to changes in total metabolism.

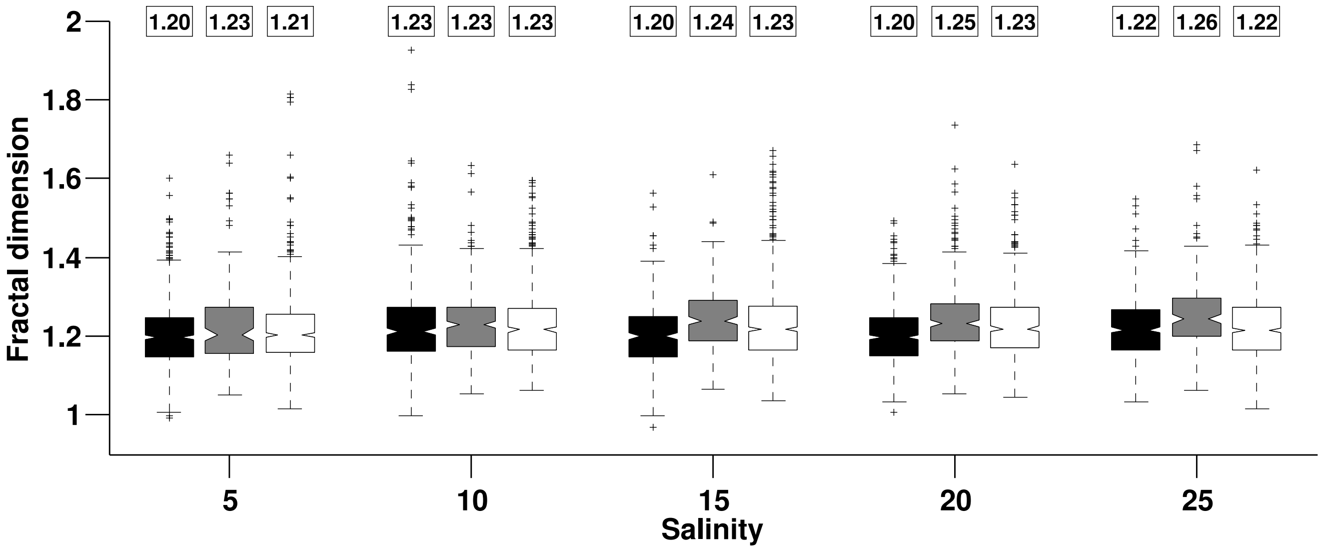

Figure 3.5: Boxplot of the fractal dimension of the trajectories of *P. annandalei* males (black), females (gray) and ovigerous females (white) for the five salinity conditions tested. On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles and the whiskers extend to 1.5 times the inter-quartile range. Outliers are plotted individually. The mean fractal dimension is indicated above each box.

It is worth noting that the exacerbated response observed in males is consistent with previous studies reporting on the higher sensitivity of male copepods to different stressors, for instance toxicants (Medina et al., 2002; Stringer et al., 2012) or a combination of pesticide exposure and hypo-osmotic stress (Bollmohr et al., 2009). Our results are also in agreement with a previous study showing a lower tolerance to salinity variation in *P. annandalei* males than in females (Chen et al., 2006). Ovigerous females displayed a different response than males and females and spent more time actively swimming than resting. Unfortunately studies on the swimming behavior of ovigerous females are rare, making comparison difficult. The study of Svetlichny et al. (2012) reported on higher respiration rates in females carrying ovisacs than in females without ovisacs for the copepods *Calanipeda aquaedulcis* and *Arctodiaptomus salinus*, indicating extra energy expenditure due to carrying ovisacs and egg respiration.

We found that males globally traveled faster than females and ovigerous females within the preferential salinity range. Chemical cues seem to be involved in mating in *P. annandalei* (Dur et al., 2011). In species using chemical signals for mating, male swimming speed usually exceeds female speed (Doall et al., 1998; Kiørboe and Bagøien, 2005; Kiørboe, 2008), as males perform active searching to maximize the chance of encountering a female signal (Tsuda and Miller, 1998; van Duren et al., 1998; Weissburg et al., 1998). However in a previous study conducted with the same species, females swam faster than males at salinity 15 (Dur et al., 2010). The two studies were conducted with two populations having different histories of salinity exposure. We used copepods from the Danshuei Estuary, sampled and kept at salinity 5. We conducted experiments within a few days after sampling to preserve the biological response. Conversely, Dur et al. (2010) used copepods sorted from a laboratory culture and maintained for several years at salinity 15. It is difficult to compare studies using different populations with different life histories and exposure to salinity. Acclimation is known to be a critical element in osmoregulation capabilities and tolerance to changes in salinity (Lance, 1963; Roddie et al., 1984) and differences in the acclimation process may have led to different responses to salinity variation.

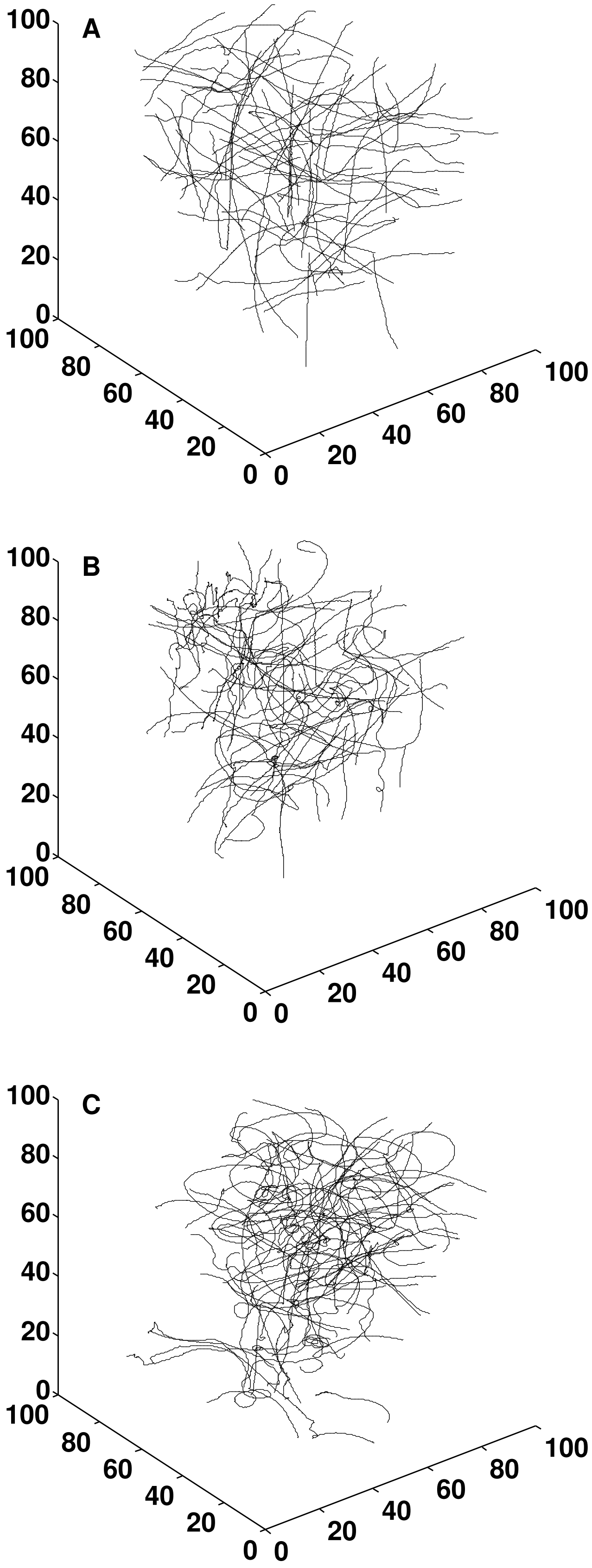

Figure 3.6: Subsets of trajectories displayed by *P. annandalei* males (A), females (B) and ovigerous females (C) showing differences in their degree of space occupation. Females and ovigerous females swam in a more convoluted way. Conversely, males travelled in a more rectilinear way. Sinking behavior, when copepods are not swimming but sink slowly due to gravity, is visible in some trajectories.

Chapter 4

Responses to waterborne pollutants

Behavioral responses of the estuarine calanoid copepod *Eurytemora affinis* to sub-lethal concentrations of waterborne pollutants.

4.1 Introduction

Alkylphenol-polyethoxylates (APEs) form a class of nonionic surfactants widely used as intermediates and components of industrial detergents, pesticides and petroleum products. Nonylphenol (NP) polyethoxylates represent the main part of APE production. These compounds and their degradation products represent a potential ecotoxicological problem in aquatic ecosystems due to their large occurrence in surface waters and persistence in sediments (Vazquez-Duhalt et al., 2005; Soares et al., 2008). Moreover, their complex fate leads to the formation of biodegraded products even more toxic than their parents counterparts (Ying et al., 2002; Zoller, 2006). Several studies have reported on the toxicity (Bechmann, 1999; Forget-Leray et al., 2005) and bioaccumulation (Cailleaud et al., 2007b, 2011a) of nonlylphenol and metabolites in marine and estuarine copepods.

Despite a recent trend toward decreased contamination due to environmental regulation, cadmium (Cd) is still one of the major pollutants in the aquatic environment. In estuaries, cadmium exhibits non-conservative behavior in the dissolved phase, characterized by higher concentrations in the low salinity zone due to its desorption from particles (Chiffoleau et al., 1999). Its abundance in sediments and in the water column as well as its accumulation, toxicity and enzyme-disruptive activity have been widely studied in a wide range of aquatic organisms including copepods (e.g. Toudal and Riisgard, 1987; Wright and Welbourn, 1994; Chiffoleau et al., 2001; Dabrin et al., 2009; Mebane et al., 2012).

Polycyclic aromatic hydrocarbons (PAHs) enter the aquatic environment from multiple sources such as industrial effluents, atmospheric transport, spillage or disposal of petrol products and incomplete combustion of crude oil and its refined products (Boehm, 2006). They are present in the environment as mixture of alkylated and parent species. High molecular weight PAHs derive mainly from fossil fuel combustion, have low solubility and are preferentially found in sediment (Wang et al., 2002; Luo et al., 2006). Petrogenic PAHs are characterized by the relative abundance of low and intermediate molecular weight compounds of two to four rings. Low molecular weight PAHs are the most toxic. They are preferentially bound to suspended particulate matter or found in the dissolved phase where they often represent a large part of the total PAH contamination (e.g. Fernandes et al., 1997; Cailleaud et al., 2007a, 2009). Polycyclic aromatic hydrocarbons can accumulate in copepods (Carls et al., 2006; Cailleaud et al., 2007a; Berrojalbiz et al., 2009) and affect their feeding, survival, reproduction and motility (Cowles and Remillard, 1983; Lotufo, 1997; Barata et al., 2005; Bellas and Thor, 2007; Calbet et al., 2007; Saiz et al., 2009).

Automated tracking of the movement of small organisms in three dimensions allows the acquisition of a large number of trajectories, forming a reliable basis for the analysis of their dynamics. Several studies have developed methods to keep tracks of moving organisms, for instance fruit flies (Straw et al., 2011; Ardekani et al., 2013), sessile barnacles (Maleschlijski et al., 2012) or schooling fish (Butail and Paley, 2012). In ecotoxicology however, and despite the growing interest in using zooplankton behavior as an integrated measure of sub-lethal exposure to pollutants, quantitative characterization of three dimensional trajectories is uncommon, primarily because there is no standard technique to recover the position information of a large number of animals moving simultaneously.

In this study we tested for the existence and the extent of contaminant-induced changes in the three-dimensional swimming behavior of the three adult states of the estuarine copepod *Eurytemora affinis* from laboratory cultures. We recorded the free swimming behavior of copepods before, during and after a brief and sublethal exposure to 4-NP, Cd and a mixture of three environmental-relevant PAHs. We hypothesized that *E. affinis* would behave differently in the presence of different classes of chemical toxicants and that, considering the low concentrations, behavior would return to normal immediately after transfer to clean water.

4.2 Methods

4.2.1 Studied organisms

Copepods were sampled from the oligohaline part of the Scheldt Estuary (near Lillo-Fort) in the northern part of Belgium, in March 2012 and using a WP2 plankton net drifting in sub-surface water. Individuals were brought to the laboratory and maintained for several generations in large and aerated aquariums under optimal conditions for reproduction and development, i.e. at salinity of 15 (filtered and sterilized sea water from the English channel adjusted to salinity with deionized water) and a temperature ranging from $15\,^{\circ}\mathrm{C}$ to $18\,^{\circ}\mathrm{C}$ (Devreker et al., 2009). Copepods were fed in excess on a unialgal diet of *Rhodomonas baltica* from the laboratory culture, harvested during the exponential growth phase and centrifuged. Algae were grown in $6\,\mathrm{L}$ flasks under a fluorescent light:dark cycle of 14L:10D in Conway medium.

4.2.2 Test solutions

The first test solution consisted of 4-nonylphenol (4-NP, CAS: 68152-92-1, analytical standard, VWR France) prepared in acetone and diluted in distilled water at a concentration of $20\,\mathrm{mg\,L^{-1}}$. The final nominal concentration of 4-NP in the experimental aquarium was $2\,\mathrm{\mu g\,L^{-1}}$. The second test solution consisted of cadmium chloride (CAS: 10108-64-2, analytical standard, VWR France) prepared in distilled water at a concentration of $0.45\,\mathrm{mg\,L^{-1}}$. The final nominal concentration of cadmium in the experimental aquarium was $45\,\mathrm{ng\,L^{-1}}$, either in free ionic form or chloride complexes. The third test solution consisted of a mixture of pyrene (CAS: 129-00-0), phenanthrene (CAS: 85-01-8) and fluoranthene (CAS: 206-44-0) (analytical standard, Fisher Scientific France) prepared in equal proportions in acetone and diluted in distilled water at a concentration of $0.4\,\mathrm{\mu g\,L^{-1}}$. The final nominal concentration of PAHs in the experimental aquarium was $40\,\mathrm{ng\,L^{-1}}$. The concentrations used in this study lay within the range observed in several major European estuaries for cadmium (Chiffoleau et al., 1999, 2001; Garnier and Guieu, 2003; Waeles et al., 2004; Baeyens et al., 2005), PAHs (Fernandes et al., 1997; Cailleaud et al., 2007a, 2009) and NP (Cailleaud et al., 2007b; Soares et al., 2008). The final nominal concentration of acetone in the aquarium after injection of 4-NP and PAHs was below $0.8\,\mathrm{ppm}$ and below $0.8\,\mathrm{ppb}$, respectively. The effect of acetone was tested for each adult state in separate control observations. Test solutions were prepared immediately before experiments. The chemical structures of the contaminants are given in Fig. 4.1.

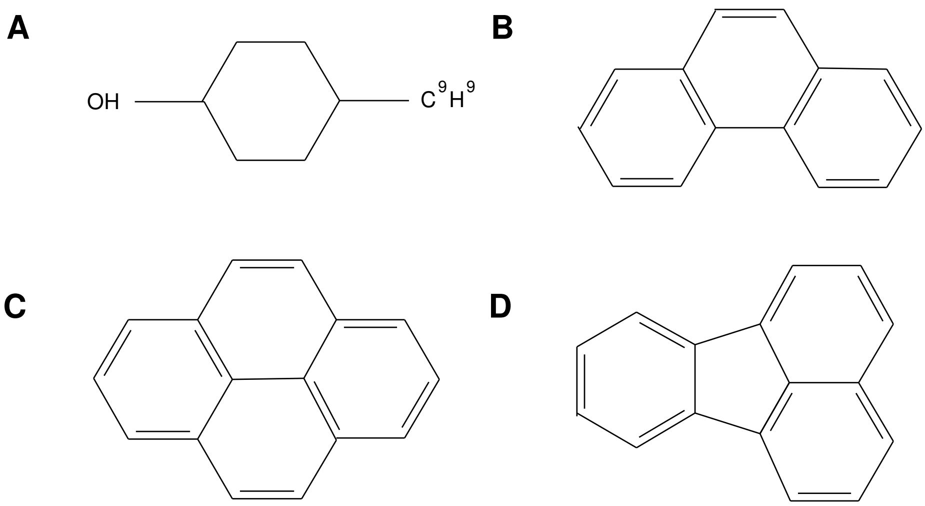

Figure 4.1: Planar representations of the chemical structures of 4-nonylphenol (A), phenanthrene (B), pyrene (C) and fluoranthene (D).

The hydrophilic part of APEs is produced by ethoxylation i.e. by ethylene oxide addition to a phenol group. Ethoxy chains of medium lengths are responsible for the surfactant properties of APEs (Schick, 1962). It should be noted that although 4-NP features both hydrophilic (the phenol group) and hydrophobic (the hydrocarbon tail) subunits, it exhibits much lower surfactant properties than its ethoxylated counterparts (Brix et al., 2001). Its low solubility and little surface activity suggest that, at the present concentration, 4-NP had no effect on the cohesive force of water (Ahel and Giger, 1993; Brix et al., 2001).

4.2.3 Experimental setup

To record the behavior of the copepods, two synchronized CCD B/W video cameras (AVT GE1050) were mounted in front of the experimental vessel which was filled with 1 L of filtered water at salinity 15 and at 17 °C (Fig. 4.2). The observation vessel consisted of a $10 \times 10 \times 11\,\mathrm{cm}^3$ acrylic glass aquarium with 3 mm thick walls. The inner faces were coated and one face only was left transparent. The cameras were fitted with 25 mm lenses (Pentax CCTV at f/5.6). They were triggered by a TTL signal and controlled through a GigE network adapter via an instance of the StreamPix5 (Norpix) acquisition software. The recording frequency was 60 frames per second at one megapixel resolution and the exposure time was set at 12 ms. The illumination consisted of two arrays of infrared (850 nm) light-emitting diodes mounted above the observation vessel. Supports for the aquarium, cameras and illumination were manufactured and the system was set up on an optic table. For the recovery of the camera parameters and scene geometry, a different calibration block was manufactured. It consisted of a machined PVC block on which 135 reference dots of known coordinates were evenly distributed along the three directions (Fig. 4.2 and 4.3).

4.2.4 Filming conditions

Experiments were carried out at night, in the absence of food and in a dark room. For each adult state, groups of 25 healthy individuals (mean total body length: 0.9 mm for females and 0.8 mm for males) were gently transferred from the culture into the aquarium. Copepods were allowed to acclimate for 5 min in the dark before the recording started. After 25 min of recording, 100 µL of the test solution was

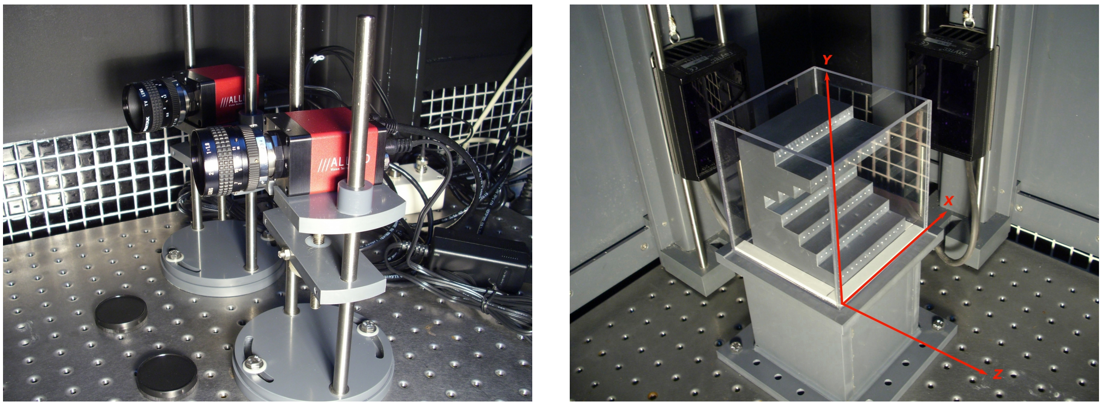

Figure 4.2: Photographs of the particle tracking system implemented at Lille 1 University, Laboratory of Oceanography and Geosciences. Close view of the cameras and lenses (left). Calibration block and IR diode arrays (right). The origin of the world coordinate system lay on the inner face of the aquarium.

added. The fluid was gently stirred and copepods were allowed to recover for 5 min from the disturbance caused by light and turbulence. The behavior was recorded for an additional 25 min. Finally, copepods were gently transferred to a clean and identical aquarium filled with uncontaminated water and allowed to recover for 5 min. The behavior was recorded for the next 25 min. Water temperature increased from 17 °C to 19 °C before transfer to clean water. Each copepod was used only once and experiments were conduced on consecutive nights. The data rate for a 60 Hz camera with a resolution of 1024 × 1024 pixels in a 8-bit grayscale format is about 62 MB s^{-1} and for a sequence of one hour, two cameras delivers a total amount of about 453 GB image data. The image sequences were recorded in real time on solid-state disks in an uncompressed 8-bit grayscale format.

4.2.5 Trajectory extraction and analysis

Trajectories were smoothed using a polynomial filter. Trajectories shorter than 30 frames or lying within 5 mm of the bottom, surface and walls of the aquarium were rejected. Trajectories shorter than 1 cm in the three-dimensional space were similarly rejected. Instantaneous speeds were averaged for each trajectory and we conducted analysis on the sampling distribution formed by trajectory-averaged swimming speeds, as described in section 3.2.5. For each experimental condition, we estimated the mean swimming speed by averaging the corresponding sampling distribution. Comparisons between experimental conditions were conducted using the Wilcoxon Mann-Whitney test. We estimated the swimming state frequencies and the fractal dimension of the trajectories following the procedure described in section 3.2.5.

4.3 Results

Copepods and especially *E. affinis* display complex behavior of intermittent amplitudes and accelerate on a temporal scale shorter than the resolution used in this study. Sudden changes in the magnitude of acceleration resulted in broken trajectories whose parts were linked together using a temporal and spatial matching assignment (Saha et al., 2012). The number of trajectories and instantaneous speed values was variable between adult states and experimental conditions and is summarized in Table 4.1. The number of trajectories ranged from 118 to 634 and the number of instantaneous speed values ranged from 123 933 to 550 788 per condition. We observed no concordance between the number of trajectories and the adult state or experimental condition.

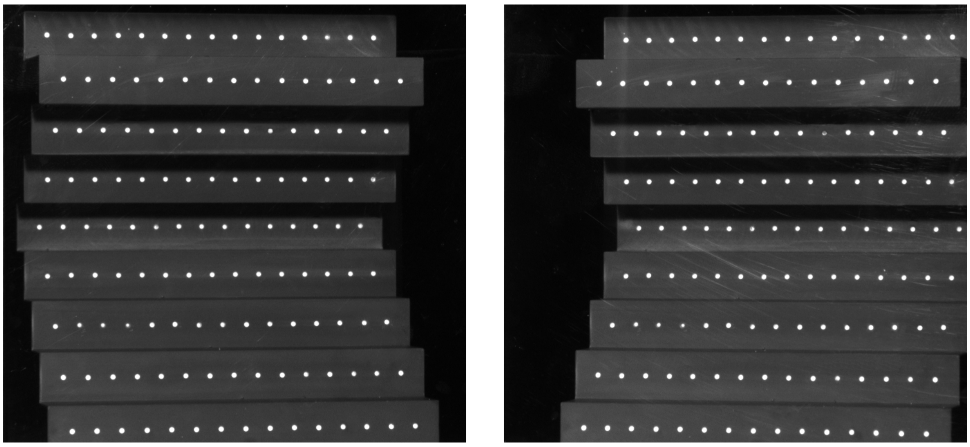

Figure 4.3: View of the calibration block in the images of the left and right cameras. The inner faces of the aquarium were coated to prevent reflection. Calibration targets and moving copepods appeared as white dots on a dark background. The calibration returned a final rms fitting error of 1.22 µm.

4.3.1 Instantaneous and mean swimming speed

The combined effects of increased water temperature caused by illumination and exposure to acetone at 0.8 ppm were tested in control observations. No significant differences in mean swimming speed were observed with copepods swimming in control water. Males swam at $4.4 \pm 1.3\,\mathrm{mm\,s^{-1}}$ in clean water and at $4.5 \pm 1.9\,\mathrm{mm\,s^{-1}}$ during exposure to acetone and the difference was not statistically significant ($p = 0.7$). Sampling distributions of females were similar before and during exposure ($p = 0.2$) with a mean value of $2.4 \pm 1.3\,\mathrm{mm\,s^{-1}}$ and $2.4 \pm 2.5\,\mathrm{mm\,s^{-1}}$. Ovigerous females swam at $2.5 \pm 1.5\,\mathrm{mm\,s^{-1}}$ in clean water and at $2.6 \pm 1.8\,\mathrm{mm\,s^{-1}}$ in the presence of acetone.

Males were very sensitive to the presence of 4-NP, increasing their swimming speed from $4.5\,\mathrm{mm\,s^{-1}}$ to $5.8\,\mathrm{mm\,s^{-1}}$ ($p < 0.01$) then slowing down to $4.7\,\mathrm{mm\,s^{-1}}$ during the recovery period ($p < 0.01$) (Fig. 4.4). The swimming speed of females increased from $3.1\,\mathrm{mm\,s^{-1}}$ to $3.4\,\mathrm{mm\,s^{-1}}$ ($p < 0.01$) and remained similar at $3.5\,\mathrm{mm\,s^{-1}}$ after transfer to uncontaminated water ($p = 0.8$). Similarly, ovigerous females significantly increased their swimming speed from $4.2\,\mathrm{mm\,s^{-1}}$ prior exposure to $4.7\,\mathrm{mm\,s^{-1}}$ in the presence of 4-NP ($p < 0.01$) then swam at a similar speed during the recovery period ($p = 0.4$).

In the Cd treatment, male swimming speed increased from $4.1\,\mathrm{mm\,s^{-1}}$ to $4.7\,\mathrm{mm\,s^{-1}}$ ($p < 0.01$) then remained similar at $4.6\,\mathrm{mm\,s^{-1}}$ during the recovery period ($p = 0.09$). Females increased their swimming speed from $3.2\,\mathrm{mm\,s^{-1}}$ to $3.7\,\mathrm{mm\,s^{-1}}$ ($p < 0.05$) then slowed down to $3.4\,\mathrm{mm\,s^{-1}}$, though sampling distributions during and after exposure appeared to be similar ($p = 0.2$). The swimming speed of ovigerous females significantly increased from $3.1\,\mathrm{mm\,s^{-1}}$ before exposure to $3.9\,\mathrm{mm\,s^{-1}}$ ($p < 0.01$) and $4.3\,\mathrm{mm\,s^{-1}}$ ($p < 0.05$) in the presence of Cd and after exposure, respectively.

In the PAH treatment, male swimming speed increased from $3.3\,\mathrm{mm\,s^{-1}}$ to $3.9\,\mathrm{mm\,s^{-1}}$ ($p < 0.01$) then decreased to $3.6\,\mathrm{mm\,s^{-1}}$. The decrease, however, was not significant ($p = 0.5$). The mean swimming speed of females was similar ($p = 0.7$) before and during exposure at $3.1\,\mathrm{mm\,s^{-1}}$ then increased significantly to $3.5\,\mathrm{mm\,s^{-1}}$ during the recovery period ($p < 0.01$). Ovigerous female swimming speed increased from $3.0\,\mathrm{mm\,s^{-1}}$ before exposure to $3.4\,\mathrm{mm\,s^{-1}}$ ($p < 0.05$) and $3.7\,\mathrm{mm\,s^{-1}}$ in the presence of PAHs and during the recovery period, respectively. Swimming speed distributions, however, appeared to be similar during and after exposure ($p = 0.1$).

Table 4.1: Number of trajectories and number of instantaneous speed values for the three adult states of *E. affinis* before and during exposure to acetone and before, during and after exposure to 4-NP, Cd and PAHs.

		Control		Test		Recovery	
		Trajectories	Values	Trajectories	Values	Trajectories	Values
Solvent	Males	346	153 469	423	184 986	-	-
	Females	147	130 515	314	316 517	-	-
	Ovigerous females	297	294 048	319	321 138	-	-
4-NP	Males	465	290 357	491	240 644	583	346 405
	Females	346	333 450	329	317 651	312	280 127
	Ovigerous females	275	210 594	240	187 678	189	135 318
Cd	Males	634	433 390	537	277 227	364	160 012
	Females	219	216 902	149	137 211	168	130 543
	Ovigerous females	205	227 198	179	109 769	225	166 196
PAHs	Males	484	371 229	398	295 483	518	356 704
	Females	608	550 788	469	460 250	331	244 947
	Ovigerous females	182	190 282	118	123 933	249	223 841

For the three adult states and for the three contaminants, the mean swimming speed was higher during the recovery period than before exposure. In all but one case, the mean swimming speed was higher during exposure than before the introduction of contaminants. Probability density functions of instantaneous swimming speed values are shown in Fig 4.5 for the three adult states before, during and after exposure to pollutants. Distributions of speed values exhibit heavy-tails showing the relative abundance of extreme events and indicating intermittency, a characteristic feature of the behavior of copepods. In males, the shape of the probability density functions shows differences in the distribution of speed values between experimental conditions, with a clearly visible decrease in the frequency of low values (below $2\,\mathrm{mm\,s^{-1}}$) in the presence of pollutants. Differences are much less marked in ovigerous and non-ovigerous females. They were more comprehensively quantified hereafter.

4.3.2 Swimming state frequency

Swimming states frequencies were significantly different before, during and after exposure to pollutants (Pearson χ^2 test, $p < 0.01$). For the three adult states and at all experimental conditions, the frequency of fast swimming state was higher during or after exposure than prior exposure (Fig. 4.6). Similarly, the frequency of break state was the lowest during exposure, except in females exposed to Cd. The three adult states spent most of their time in the slow swimming state, whose frequency showed not constant trend. A notable increase in the frequency of fast swimming behavior, as in males during and after exposure to 4-NP and Cd, came with a marked reduction in the frequency of slow swimming behavior. A noticeable reduction in the frequency of break events, as in ovigerous females in the Cd and PAHs treatments, came with an increase in the abundance of slow swimming behavior. The frequency of slow swimming state remained globally constant in some cases, for instance in males in the PAH treatment, differences occurring simultaneously in the abundance of break and fast swimming behavior.

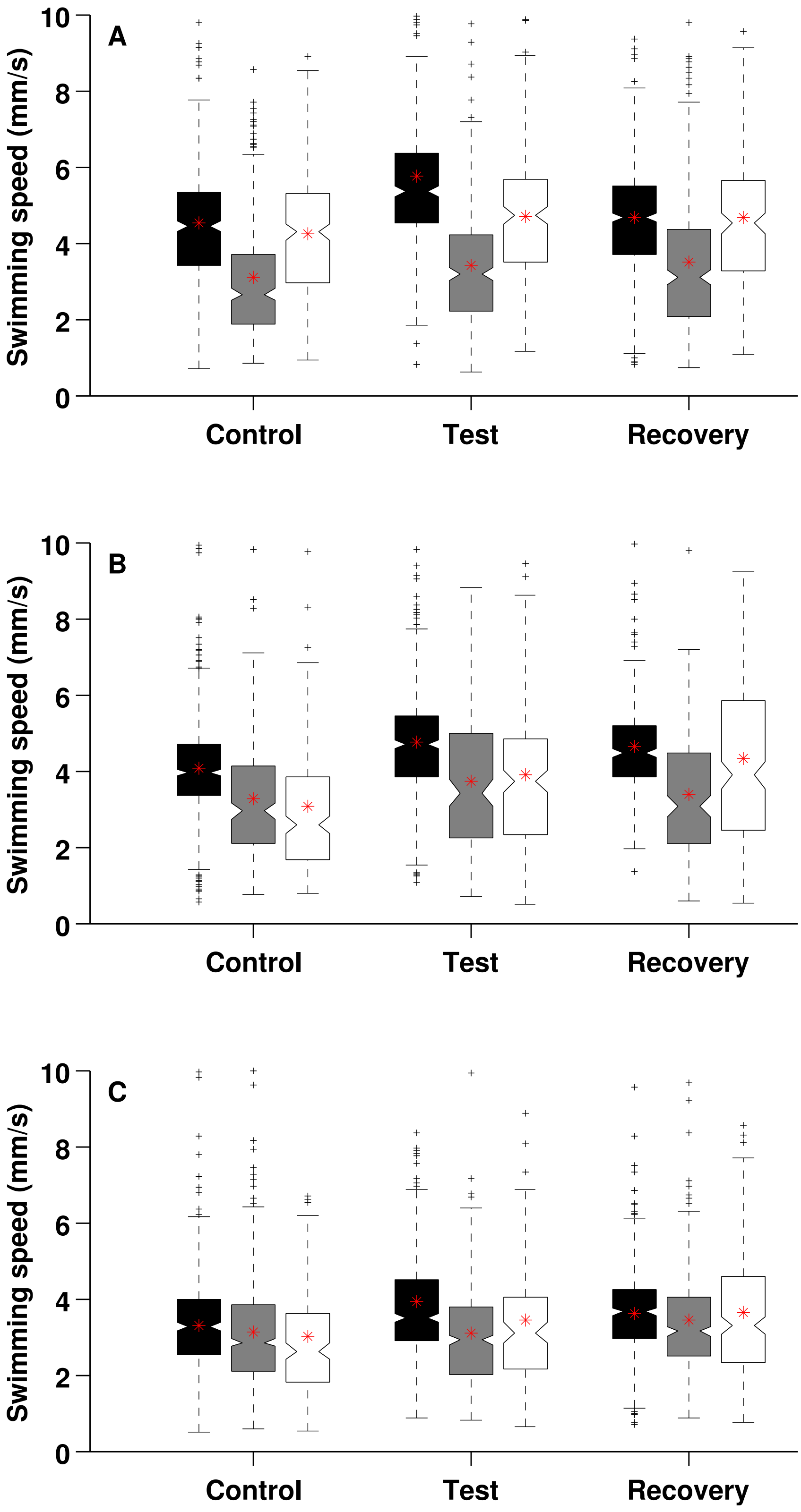

Figure 4.4: Box-plot of trajectory-averaged swimming speed values of *E. affinis* males (black), females (gray) and ovigerous females (white) before, during and after exposure to 4-NP (A), Cd (B) and PAHs (C). On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles and the whiskers extend to 1.5 times the inter-quartile range. Outliers are plotted individually. Red markers indicate mean swimming speeds.

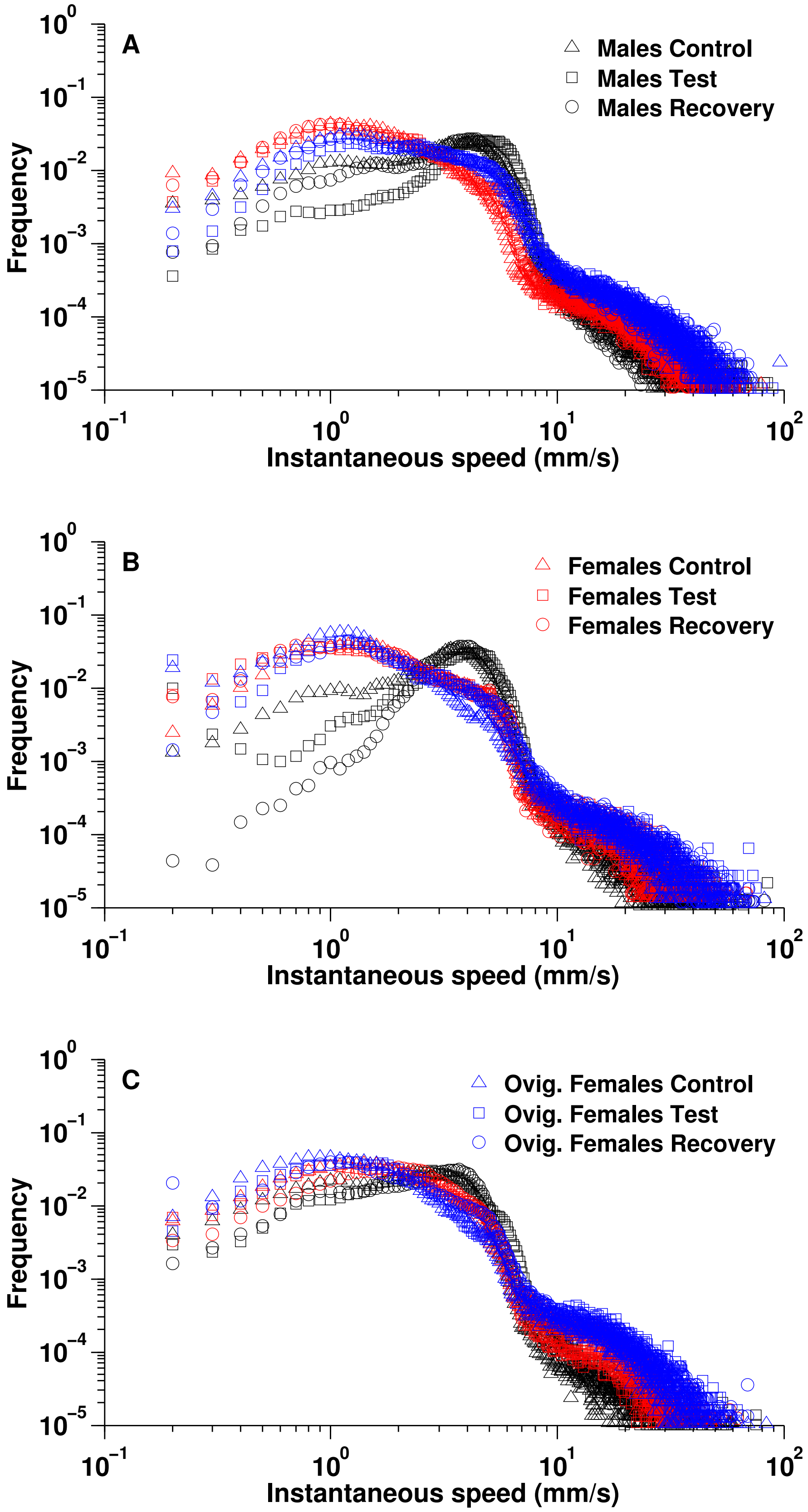

Figure 4.5: Probability density functions of the instantaneous swimming speed values of *E. affinis* males (black), females (red) and ovigerous females (blue) before (triangle), during (square) and after (circle) exposure to 4-NP (A), Cd (B) and PAHs (C).

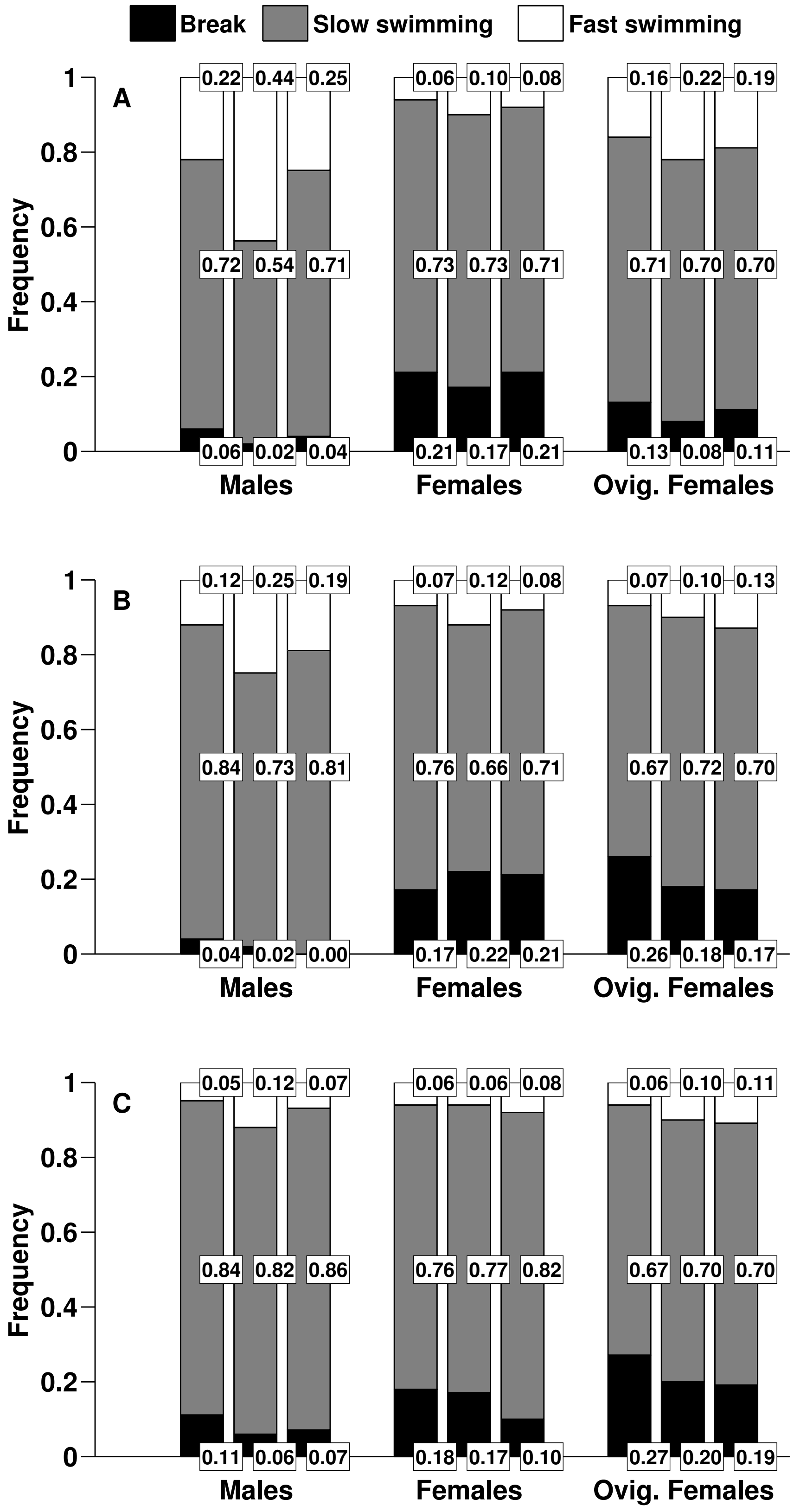

Figure 4.6: Swimming state frequencies of *E. affinis* males, females and ovigerous females before (left bar), during (middle bar) and after (right bar) exposure to 4-NP (A), Cd (B) and PAHs (C). Copepods spent most of their time in the slow swimming state.

Table 4.2: Mean (± standard deviation) fractal dimension of the trajectories of the three adult states of *E. affinis* before, during and after exposure to 4-NP, Cd and PAHs.

		Control	Test	Recovery
4-NP	Males	1.26 ± 0.11	1.27 ± 0.12	1.27 ± 0.11
	Females	1.33 ± 0.15	1.32 ± 0.15	1.32 ± 0.14
	Ovigerous females	1.35 ± 0.13	1.35 ± 0.14	1.37 ± 0.14
Cd	Males	1.25 ± 0.09	1.24 ± 0.08	1.25 ± 0.09
	Females	1.29 ± 0.13	1.31 ± 0.15	1.28 ± 0.12
	Ovigerous females	1.32 ± 0.12	1.32 ± 0.15	1.32 ± 0.13
PAHs	Males	1.26 ± 0.11	1.27 ± 0.13	1.26 ± 0.11
	Females	1.31 ± 0.13	1.29 ± 0.12	1.33 ± 0.12
	Ovigerous females	1.32 ± 0.14	1.33 ± 0.13	1.35 ± 0.14

4.3.3 Degree of space occupation

There was no difference in the mean fractal dimension before, during and after exposure to pollutants (Table 4.2) (Wilcoxon Mann-Whitney test, $p > 0.1$), suggesting that the complexity of trajectories did not change. However, we observed marked differences in the degree of space occupation between adult states ($p < 0.01$). The fractal dimension was constantly lower in males and higher in non-ovigerous and ovigerous females. Qualitative observations confirmed that males swam in a smoother and more rectilinear way, and that trajectories of females and especially ovigerous females were more convoluted (Fig. 4.7). Ovigerous females swan in a typical motion characterized by periods of slow swimming and sinking interrupted by sudden bursts in velocity. Examples are shown in Fig. 4.8.

4.4 Discussion

A large number of studies have described the effects of contaminants on the behavior of aquatic organisms. However test species, experimental conditions, contaminants and incubation times varied greatly, making results difficult to compare. Behavioral assays using copepods remain uncommon, as most studies focused on conventional mortality or reproductive tests. In this work we assessed the immediate effects of a brief exposure to environmental-realistic concentrations of pollutants on the free swimming behavior of a key estuarine copepod. Effects consisted of an increase in swimming activity and speed and did not immediately subside after transfer to clean water. The degree of trajectory convolution showed no variation.

Data on the molecular and cellular toxicity of APEs are scarce and attention has been focused mainly on their endocrine disrupting activity (Vazquez-Duhalt et al., 2005). Nonylphenol disrupts the functioning of the (Ca^{2+}-Mg^{2+})-ATPase (Michelangeli et al., 1990) and seems to be a potent acetylcholinesterase (AChE) inhibitor (e.g. Talorete et al., 2001; Li, 2008a,b). Toxicants can act through different modes of action, causing a tremendous variety of species-specific effects (Russom et al., 1997). Linking behavioral responses to the precise mechanism of action and properties of chemicals remains delicate. However, altered behavior following AChE inhibition has been observed in several species such as amphipods

(Xuereb et al., 2009), *Daphnia* (Duquesne and Küster, 2010), bettles (Jensen et al., 1997) or salmons (Sandahl et al., 2005) and included both increased or diminished locomotor activity.

The mechanisms responsible for the toxicity of Cd are not well understood. They seem to involve reactive oxygen species causing lipid peroxidation (Stohs and Bagchi, 1995). Behavioral effects following exposure to Cd have been demonstrated in several species of small crustaceans. To the best of our knowledge, all studies have reported on depressed activity. For instance, Baillieul and Blust (1999) observed a decrease in swimming speed and activity in *D. magna* after exposure to Cd^{2+} at $10^{-2}\,\mu\mathrm{g\,L^{-1}}$ for 10 days. Similarly, the exposure to water contaminated with Cd at $15\,\mu\mathrm{g\,L^{-1}}$ for $120\,\mathrm{h}$ led to a reduction in the percentage of *Gammarus pulex* in movement (Felten et al., 2008). Finally, Sullivan et al. (1983) observed depressed swimming activity in *E. affinis* nauplii exposed to Cd at $130\,\mu\mathrm{g\,L^{-1}}$ for $24\,\mathrm{h}$.

Our results are also at variance with the more established expectation that PAHs have a narcotic effect on aquatic organisms. Narcotic effect is defined as an apparently reversible alteration of the membrane fluidity and protein function caused by the accumulation of hydrophobic pollutants in the lipid bilayer region (van Wezel and Opperhuizen, 1995). This nonspecific partitioning of the chemical in nerve tissue results in anesthetic effects. The narcotic properties of naphthalene, fluoranthene and pyrene have for instance been reported in Barata et al. (2005) who observed a lack of motility of the copepod *Oithona davisae* after a $48\,\mathrm{h}$ exposure to crude oil at concentrations 3 to 5 orders of magnitude higher than the ones used in our work. Similarly, Cowles (1983) found a decrease in swimming speed and activity in *Centropages hamatus* females exposed to crude oil for $64\,\mathrm{h}$. Conversely, we observed hyperactivity following immediate exposure.

Biological systems often display an increased response at low levels of toxicity and a negative response as concentrations or exposure time increase (Calabrese and Blain, 1995). Initial hyperactivity in response to low doses of toxicants or during the first stage of exposure have been reported in a variety of organisms, for instance *Daphnia* exposed to organophosphorus pesticides (Ren et al., 2007) or amphipods in presence of Cd (Sornom et al., 2012) and pesticide (Kirkpatrick et al., 2006). Apparent low-dose stimulations have also been observed in *Artemia* sp. and in the rotifer *Brachionus plicatilis* exposed to zinc pyrithione and to a carbamate pesticide, with an increase in swimming speed at low concentrations and an inhibited behavior at high concentrations (Garaventa et al., 2010). Similarly, Kienle and Gerhardt (2008) observed hyperactivity in the amphipod *Corophium volutator* exposed to 25 and $50\,\%$ water accommodated fraction of weathered crude oil, whereas exposure to $100\,\%$ led to narcosis. The hyperactivity observed in this study for the three classes of pollutants supports the assumption that aquatic organisms react to the first stages of a stressful situation by increasing their swimming activity in a typical escape reaction, as observed in *Daphnia* in presence of copper (Lopes et al., 2004) or in *E. affinis* exposed to hydrocarbons (Gyllenberg, 1986). On the opposite, higher levels of pollution or longer exposure are expected to result in lower swimming activity, indicating loss of coordination or reflecting the metabolic cost of detoxification (Cailleaud et al., 2009) and the higher energy required for maintenance (De Coen and Janssen, 1997).

In our study, three classes of contaminants with different toxic characteristics induced a similar response. This suggests that avoidance behavior is independent of the toxic properties of the chemical, as was also the case in *Daphnia magna* exposed to deltamethrin, chlorothalonil and nitrofen (Ren et al., 2009). We also observed that ovigerous females exposed to Cd and to PAHs displayed more pronounced effects during the depuration period. These results are unexpected, considering the reversible toxicity of nonpolar narcotics (van Wezel and Opperhuizen, 1995). The elimination of contaminants by an organism is influenced by metabolism, storage and excretion (Escher and Hermens, 2002). Detoxification is energetically costly (Pook et al., 2009) and in female copepods, carrying ovisacs requires extra energy

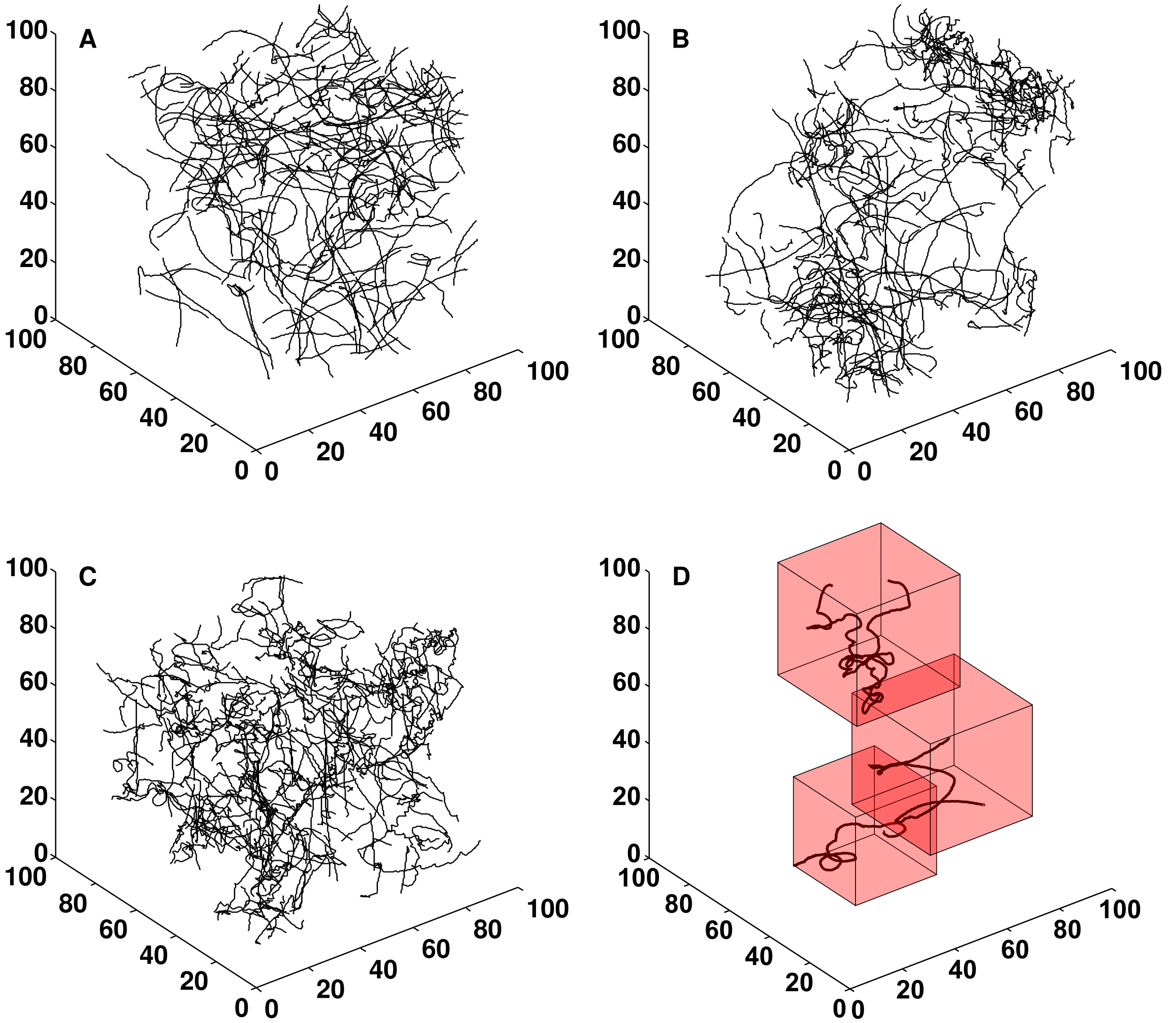

Figure 4.7: Subsets of trajectories displayed by *E. affinis* males (A), females (B) and ovigerous females (C) in uncontaminated water, showing differences in their degree of convolution. Females and ovigerous females swam in a typical hope-and-sink pattern and display complex trajectories. Conversely, males traveled faster and displayed smooth changes in direction. To estimate the fractal dimension, trajectories were embedded in a cubic volume and a regression line was computed with a principal component analysis and used for calibration (D).

expenditure (Svetlichny et al., 2012). It can be postulated that reproduction caused energetic resources to be allocated away from detoxification mechanisms, resulting in the persistence of intrinsic activity at the target source (Escher and Hermens, 2002) and in a exacerbated escape response.

Although not considered in the present study, it is likely that copepod hyperactivity leads to impairments in behavioral processes and to a reduced fitness. Studies have for instance linked pollutant-induced increase in swimming speed or altered activity with changes in specific predator-avoidance behavior in aquatic organisms (Sullivan et al., 1978; Dodson et al., 1995). Because the motion of a prey can affect the distance at which a planktivorous predator can detect it (Wright and O'Brien, 1982) and since speed and activity determine both prey conspicuousness to predators and encounter rates (Buskey et al., 1993;

Kiørboe and Visser, 1999), a higher swimming speed can result in increased predation. Stripped bass, for instance, fed at higher rates on nauplii *E. affinis* that were hyperactive due to exposure to copper (Sullivan et al., 1983). Using the encounter model developed by Gerritsen and Strickler (1977), Buskey et al. (1993) showed that as prey swimming speed increased, encounter rates with predators increased. These theoretical results were further confirmed by Mahjoub et al. (2011) who observed that ovigerous females swimming at $3.8\,\mathrm{mm\,s^{-1}}$ suffered $63\,\%$ higher mortality than non-ovigerous females swimming at $3.3\,\mathrm{mm\,s^{-1}}$, a difference attributed in part to the stronger hydromechanical disturbance of ovigerous females, but also to their higher encounter rate with fish larvae. Hyperactivity can also impair the ability of copepods to detect food in their feeding current. Jiang et al. (2002b) demonstrated that a copepod swimming slowly can use chemoreception to remotely detect individual algae entrained by the flow field around itself, whereas a fast-swimming copepod is not able to rely on chemoreception to remotely detect individual algae. The same authors (Jiang et al., 2002a) also showed that swimming slowly (around $1\,\mathrm{mm\,s^{-1}}$) was more energetically efficient in terms of relative capture volume per energy expended than swimming faster (around $4\,\mathrm{mm\,s^{-1}}$). Hyperactivity may finally have a considerable effect on the energy budget of copepods. Buskey (1998) for instance measured changes in the respiration rate of the copepod *Dioithona oculata* with activity and found a significant metabolic cost of swimming at higher speed to maintain position in currents, with a mean respiration rate in copepods swimming at $8.6\,\mathrm{mm\,s^{-1}}$ twice as much as in copepods swimming at $3.5\,\mathrm{mm\,s^{-1}}$. In our study however, we observed modest changes in swimming speed and it is unknown whether significant impairment in feeding efficiency or increased metabolic expense would occur.

Under real environmental conditions, toxicity might increase even further. First, waterborne pollutants may transfer to copepods though different pathways. They can be directly absorbed from water or ingested after consumption of contaminated phytoplankton (Sobek et al., 2006; Magnusson et al., 2007), trophic exposure usually resulting in higher accumulation and stronger effects (Connell and Sanders, 1999; Magnusson et al., 2007). For instance, Hook and Fisher (2001) found different metal distributions in the copepods *Acartia hudsonica* and *A. tonsa* and different toxic effects following different routes of uptake, with exposure to dissolved metal resulting in deposition in the exoskeleton and modest reproductive effects whereas exposure to contaminated algae resulted in high amount of metal accumulation in internal tissues and marked reproductive impairments. Second, the toxicity of PAHs increases with their degree of alkylation and after photo-activation (Ott et al., 1978; Boese et al., 1998; Duesterloh et al., 2002). Their accumulation in copepods strongly depends on their octanol-water partition coefficient and when supplied in mixtures, their toxic effect appears additive (Boese et al., 1999; Barata et al., 2005; Berrojalbiz et al., 2009; Jensen et al., 2012). Third, the toxicity of a chemical varies with both temperature and salinity (Staton et al., 2002; Kwok and Leung, 2005). We cultured *E. affinis* at salinity 15 which is an optimal value for reproduction and development (Devreker et al., 2009). We conducted experiments at the same salinity to avoid any synergistic effect of pollution and salinity variation (Michalec et al., 2010). However, individuals *E. affinis* were found less resistant to Cd contamination at low salinities, probably due to the increase in osmotic stress in hypotonic conditions (Hall et al., 1995). Salinity also influences the concentration of free cadmium ion, responsible for the toxicity of cadmium, by governing its complexion with inorganic ligands. The interactive effects of varying salinity conditions, contaminated food particles and chronic exposure to complex mixtures of pollutants will finally depend on the ability of copepods to accumulate, metabolize and excrete these pollutants (Bryan et al., 1979).

One point worth noting here is that we observed differences between adult states, regardless of the presence of pollutant. Females and ovigerous females displayed convoluted and nestled trajectories. They swam in a typical pattern characterized by periods of slow swimming interrupted by sudden jumps. Males swam faster than the two other adult states and in a smooth motion. *Eurytemora affinis* relies

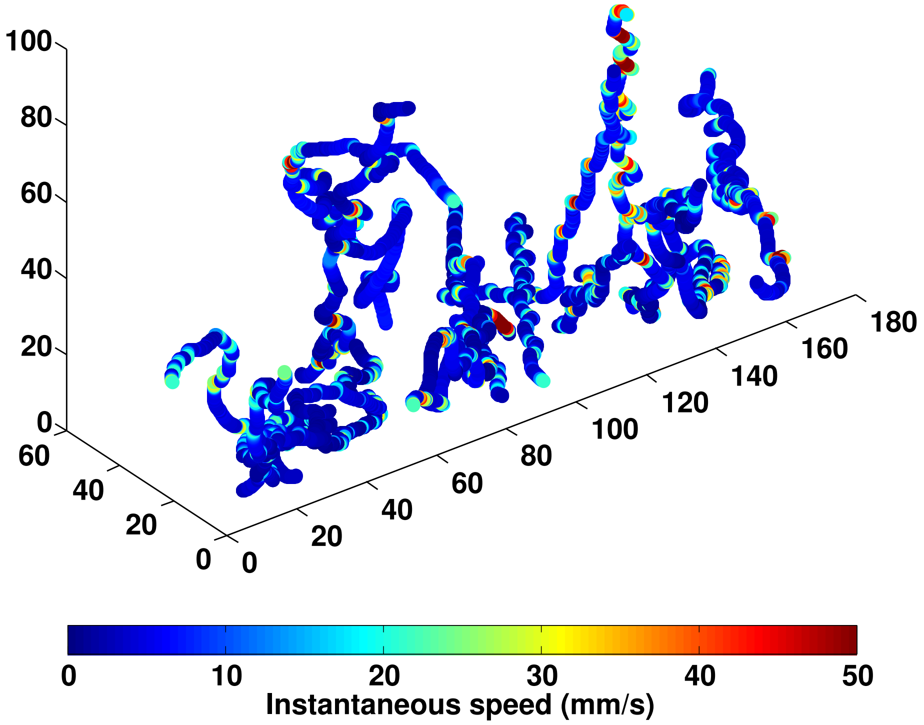

Figure 4.8: Examples of complex trajectories displayed by *E. affinis* ovigerous females, regardless of the presence of pollutants. Convoluted and helical trajectories are two recurrent features of their swimming behavior. Helical trajectories are formed by tight loops travelled at low speed separated by sudden bursts in velocity. Most instantaneous speed values are found below $5\,\mathrm{mm\,s^{-1}}$. The intermittent nature of the displacement is illustrated by the importance of extreme events, with instantaneous swimming speeds up to $48\,\mathrm{mm\,s^{-1}}$ in these trajectories.

on chemical communication for mating (Katona, 1973) and these behavioral features reflect strategies aiming at increasing encounter rate by permitting males to maintain a more linear behavior for longer displacement and to investigate a larger volume (Doall et al., 1998; Kiørboe, 2008).

In conclusion, our results indicate that copepod swimming behavior is a sensitive indicator of sub-lethal concentrations of chemical toxicants. Because behavior governs critical processes in the ecology of copepods, any disruption in their swimming activity can have negative ecological consequences. The mechanisms involved in pollutant-induced disturbance and the effect of impaired swimming activity on interactions within the zooplankton community should be the subject of further studies.

Chapter 5

Effects of a diatom toxin

Changes in the dynamics of *Pseudodiaptomus annandalei* (Copepoda, Calanoida) adults exposed to the diatom toxin 2-*trans*, 4-*trans* decadienal.

5.1 Introduction

Diatoms are unicellular algae and key primary producers in aquatic ecosystems. Certain species produce secondary metabolites, collectively termed oxylipins, upon mechanical wounding caused by mesozooplankton grazing (Wichard et al., 2005a). Oxylipins are secreted either as a single compound or as a mixture of several toxins. Polyunsaturated aldehydes (PUAs) form a well-studied group of diatom metabolites. They derive from the lipoxygenase-mediated degradation of free polyunsaturated fatty acids released by phospho- and galactolipids hydrolysis immediately after cell breakage (Pohnert, 2002; d'Ippolito et al., 2004; Wichard et al., 2007). PUAs are assumed to serve as a defense mechanism which aims at interfering with the reproductive success and recruitment of their predators (Pohnert, 2005), thereby allowing the diatom bloom to persist despite sustained grazing pressure. Their toxicity is for the most part related to a reactive Michael acceptors structural element, the α,β,γ,δ-unsaturated aldehyde group (Pohnert, 2000), but the polarity of the aldehyde and the length of the carbon chain also seem to be important features influencing their biological activity (Adolph et al., 2003).

PUAs have teratogenic (Uye, 1996; Ianora et al., 2004), anti-mitotic and apoptotic properties (Poulet et al., 1994; Buttino et al., 1999). Their negative effects on copepod egg production, embryogenesis, egg hatching success and offspring survival and development have received considerable attention and are well documented in both laboratory and field studies (Ianora et al., 1996; Ban et al., 1997; Miralto et al., 1999; Turner et al., 2001; Miralto et al., 2003; Ianora et al., 2004; Halsband-Lenk et al., 2005; Pierson et al., 2005). Some authors, however, did not observe any adverse effect of diatom diet or any negative relationship between occurrence of diatoms and hatching success or naupliar survival (Irigoien et al., 2002; Taylor et al., 2007; Dutz et al., 2008; Wichard et al., 2008; Koski et al., 2008). Discrepancies between studies might be explained by species- and strain-specific differences in PUA production and biological activity (Pohnert et al., 2002; Adolph et al., 2003; Wichard et al., 2005a; Amin et al., 2011) or by variations in copepod sensitivity and detoxification mechanisms between species or populations (Amin et al., 2011; Lauritano et al., 2012).

Seasonal microalgal blooms are a recurrent phenomenon in polar and temperate latitudes. They occur when abundant nutrients are brought to the euphotic zone by deep convection currents, supporting the rapid growth of phytoplankton (Legendre, 1990; Dore et al., 2008). Seasonal blooms are often dominated by PUA-producing diatoms flourishing at abnormally high concentrations (Tiselius and Kuylenstierna, 1996; Miralto et al., 1999; Scholin et al., 2000) and on which copepods actively graze (Teegarden et al., 2001; Schultes et al., 2006). Damaged diatoms rapidly secrete PUAs at species-specific concentrations ranging for instance from $4\,\mathrm{fmol\,cell^{-1}}$ in *Thalassiosira rotula* to $49\,\mathrm{fmol\,cell^{-1}}$ in *Asterionella formosa* and depending on their physiological condition (Pohnert, 2000; Wichard et al., 2005b; Ribalet et al., 2007; Taylor et al., 2009). Moreover, the enzymes involved in the biosynthesis of toxins retain activity in seawater over several minutes after cell breakage (Pohnert, 2000, 2002). Consequently, copepods feeding on diatom blooms are expected not only to ingest large quantities of aldehydes (Ianora et al., 2004), but also to encounter patchy and locally high levels of toxins (Vidoudez et al., 2011). However, despite the prevalence and ecological importance of diatom blooms in oceans (Legendre, 1990), the behavioral response of copepods to the presence of diatom-derived toxins remains unclear.

The compound 2-*trans*, 4-*trans* decadienal (hereafter referred to as decadienal) is relatively often identified among the aldehydes produced by diatoms and is the best investigated member of this structure class (Miralto et al., 1999; Pohnert, 2000; Caldwell et al., 2004; Taylor et al., 2005; Caldwell et al., 2011) (Fig. 5.1). Decadienal is known to have antiproliferative activity in a broad range of organisms (Adolph et al., 2004). In copepods, decadienal arrested embryonic development and impaired egg hatching success (Miralto et al., 1999; Ianora et al., 2004). It has also been found to reduce survival in polychaete and echinoderm larvae (Caldwell et al., 2005) and to induce apoptosis in copepod and echinoderm embryos (Romano et al., 2003). Decadienal inhibited CDK1-cyclin B activity and DNA replication in early embryos of the sea urchin *Sphaerechinus granularis*, hindering tubulin polymerization and inducing aberrations in mitotic spindles (Hansen et al., 2004).

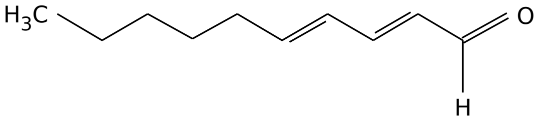

Figure 5.1: Planar representation of the chemical structure of the diatom toxin 2-*trans*, 4-*trans* decadienal.

Motivation of this study was to test for the existence and the extent of toxin-induced changes in the swimming behavior of copepods. We carried out experiments in which individuals *Pseudodiaptomus annandalei* were briefly exposed to different concentrations of decadienal and we investigated changes in their dynamics.

5.2 Methods

5.2.1 Studied organisms

Copepods were sampled from the mesohaline part of the Danshuei Estuary in Northern Taiwan by using horizontal tows of a WP2 plankton net drifting in mid-water. Copepods were transferred into large and aerated containers filled with estuarine water and quickly brought to the laboratory. They were cultured for several generations in mildly aerated 20 L cylindrical tanks filled with autoclaved sea water adjusted to a salinity of 20 with distilled water, under a fluorescent light:dark cycle of 12L:12D and at a temperature of 27 ± 2 °C. Several hours before experiments and at the same time everyday, copepods were fed in excess on a unialgal diet of *Isochrysis galbana* from the laboratory culture, harvested during the exponential growth phase and centrifuged. Algae were grown in 6 L flasks under a 12L:12D light cycle in f/2 medium and at a temperature of 26 ± 3 °C.

5.2.2 Test solutions

Most studies assessing the effect of decadienal as a model aldehyde have used concentrations ranging from 3 µmol L^{-1} to 20 µmol L^{-1} (Miralto et al., 1999; Romano et al., 2003; Ianora et al., 2004). Effective concentrations varied greatly depending on the species and endpoint considered, ranging for instance from 3×10^{-2} µmol L^{-1} for the survival of *Arenicola marina* and *Nereis virens* larval stages (Caldwell et al., 2005) to 7.3 µmol L^{-1} for the cleavage of sea urchin embryos (Pohnert et al., 2002) or 20 µmol L^{-1} for the survival of *Artemia salina* nauplii (Taylor et al., 2005). In this study we tested for the effects of decadienal at 3 µmol L^{-1}, 6 µmol L^{-1} and 12 µmol L^{-1}. Three stock solutions were prepared by diluting 2-*trans*, 4-*trans* decadienal (technical grade 95 %, Acros Organics) in technical grade ethanol. To produce the desired final concentrations during exposure, 200 µL of the stock solution was added to the observation vessel. The final nominal concentration of ethanol during exposure was 200 ppm. The effect of carrier solvent was tested for each adult state in separate control observations.

5.2.3 Experimental setup

We conducted experiments in a $10 \times 10 \times 11\,\mathrm{cm}^3$, 3 mm thick plexiglass aquarium filled with water at salinity 20 and at 27 °C. The inner faces of the aquarium were coated to prevent reflexion. Two synchronized and externally triggered B/W CCD video cameras (AVT Guppy F-033B) fitted with Nikkor 24 mm lenses were set up in front of the only transparent wall (Fig. 5.2). The cameras recorded at 30 frames per second in a VGA format and were controlled via an instance of the StreamPix5 software (Norpix) through an IE1394 interface. The shutter speed was set at 30 ms. The observation volume was illuminated by two arrays of infrared light-emitting diodes mounted above the aquarium. A calibration block (described in section 3.2.2) was placed inside the aquarium and images were taken.

5.2.4 Filming conditions

Experiments were carried out at night, in the absence of food and in a dark room to avoid phototropism. Copepods were sorted under a microscope and damaged individuals were discarded. For the three adult states, groups of 25 healthy copepods (mean total length: 1 mm for females and 0.9 mm for males) were gently placed in the observation vessel. They were allowed to acclimate for 5 min in the dark before the recording started. The test solution was added after 25 min of recording. The fluid was gently stirred and copepods were allowed to recover for 5 min from the disturbance caused by light and turbulence. The behavior was recorded for an additional 25 min. Water temperature was constant at 27 °C over the course of the experiments. Each copepod was used only once and experiments were conduced twice for each adult state, on non-consecutive nights. The image sequences were recorded in real time in an uncompressed 8-bit grayscale format.

5.2.5 Trajectory analysis

Trajectories were extracted from the image sequences and smoothed via a spatially variant polynomial filter to remove background noise. Trajectories were displayed and checked for tracking errors. Trajectories shorter than 30 frames were rejected. Trajectories lying within 5 mm of the bottom, surface and walls of the aquarium were similarly rejected in order to avoid border effects. Finally, trajectories shorter than 1 cm in the 3D space were rejected to ensure a minimal degree of space occupation.

Instantaneous and mean swimming speed

We estimated the magnitude of the instantaneous velocity as the distance between two successive positions of a copepod divided by the time step. As described in section 3.2.5, comparisons between experi-

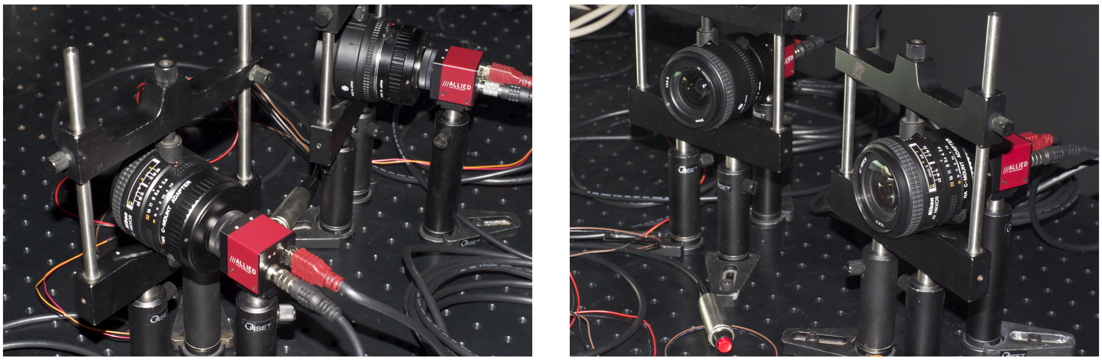

Figure 5.2: Photographs of the particle tracking system implemented at National Taiwan Ocean University, Institute of Marine Biology, showing the two IE1394 cameras. The cameras were triggered by a simple volt-free contact and were computer-synchronized.

mental conditions were conducted using the Wilcoxon Mann-Whitney test on the sampling distributions formed by trajectory-averaged swimming speeds. For each experimental condition, the mean swimming speed was estimated by averaging the corresponding sampling distribution.

Moment function of the displacement

Multifractal analyses have been widely used to describe the statistical properties of intermittent variables in multi-scale systems, for instance the rate of dissipation of turbulent kinetic energy (Meneveau and Sreenivasan, 1991), fluctuations in daily river flows (Pandey et al., 1998) or human heartbeat dynamics (Ivanov et al., 1999). Multifractal analysis aims at characterizing the fluctuation in time or space of the local regularity of a process through analysis of the power law behavior of its structure functions and the determination of their scaling exponents.

By definition, the norm of a displacement is scale-dependent. It is a non-stationary process with stationary increment, meaning that its statistical properties vary only with the time increment. Schmitt and Seuront (2001) performed multifractal analysis on copepod behavior and demonstrated that the walk of *Temora longicornis* belonged to multifractal anomalous diffusion characterized by a nonlinear moment scaling function for the distance versus time. Following Schmitt and Seuront (2001), let us note $X(t)$ the position of a copepod at time t and let us consider the moments of order $q > 0$ of the norm of the displacement δX_τ for all time increments τ. By hypothesis, a scaling behavior is present over at least some ranges of order of τ and for every q. In this case, the moments of order $q > 0$ of the norm of the displacements depend only on the time increment τ as follow:

$$E[||X(t+\tau) - X(t)||^q] \approx \tau^{\zeta(q)} \quad \forall l \leq \tau \leq T$$

where E refers to the mathematical expectation and $\zeta(q)$ is the exponent which governs the local power-law scaling of the absolute moments of the fluctuations $\delta_\tau X(t)$ at any scale l up to a scale T. The exponents $\zeta(q)$ are estimated from the slope of the power fit of the log-log plot of $E[||\delta X_\tau||^q]$ versus τ for each value of q. The scaling of $\zeta(q)$ for different q values quantifies the statistical properties of the different subsets and reveals the properties of the swimming behavior. It is a scale-invariant indicator of the strategy used by a copepod to explore its environment.

When the exponents $\zeta(q)$ are linear in q, a single scaling exponent is involved in all fractal subsets and the function is said to be monofractal. In particular, when $\zeta(q) = q/2$ the path has the property of a

Brownian motion characterized by displacements of random amplitudes. In a normal diffusion most displacements are small and extreme amplitudes are rare. When $\zeta(q) \neq q/2$ the path has the property of an anomalous diffusion (Castiglione et al., 1999). Monofractal anomalous diffusions are called fractional Brownian processes. If $\zeta(q) > q/2$ the process is referred to as super-diffusive, whereas sub-diffusion corresponds to $\zeta(q) < q/2$. Usually only the second moment is estimated and it is assumed that a process corresponds to a normal diffusion if $\zeta(2) = 1$ with the assumption that all $\zeta(q) = q/2$ and to an anomalous diffusion if $\zeta(2) \neq 1$. However, $\zeta(q)$ can be non linear in q and it is better to characterize the process with the whole moment structure function instead of a single exponent (e.g. Schmitt and Seuront, 2001, and references therein). If $\zeta(q)$ is non linear in q, then the walk involves non-unique exponents for different values of q and is said to be multifractal.

Lévy flights, for instance, are Markovian stochastic processes whose stationary increments are distributed according to a probability density $\lambda(x)$ of the asymptotic power-law form $\lambda(x) \simeq |x|^{-1-\alpha}$ with $0 < \alpha < 2$. Lévy flights represent a case of anomalous diffusion characterized by a bilinear moment structure function, with $\zeta(q) = q/\alpha$ when $q < \alpha$ and $\zeta(q) = 1$ when $q > \alpha$. Due to the divergence of their variance, extremely long jump may occur. In a Lévy flight, trajectories are self-similar and all scales show clusters of small displacements separated by long jumps. Recent analyses have questioned the strength of the empirical evidence for Lévy flights in ecology (Edwards et al., 2007) but other statistically robust empirical studies have confirmed its occurrence among moving organisms such as honeybees (Reynolds et al., 2009) or albatrosses (Humphries et al., 2012), demonstrating that Lévy flights in dynamic natural environments represent a beneficial search strategy when prey are sparsely and heterogeneously distributed.

The first moment is best known as the mean and estimates the value around which central clustering occurs. The second moment is the variance and characterizes the variability in the distribution. The third moment is the skewness of the distribution and characterizes its degree of asymmetry. High skewness for instance indicates a tail extending out toward large values. The fourth moment is the kurtosis and measures the sharpness of the distribution relative to a standard distribution, that is, the relative contribution of shoulders and tails in explaining variability. High kurtosis corresponds to a narrow distribution with heavy tails, while low kurtosis corresponds to a distribution with a relatively flat center and heavy shoulders. The calculation of higher moments exploits the far tails of the distribution and requires a very large number of trajectories.

5.3 Results

5.3.1 Swimming activity

A general assessment of copepod swimming activity was provided by the number of trajectories and instantaneous speed values, which reflected directly the number of copepods actively swimming in the observation vessel. In control water, *P. annandalei* showed strong substratum preference and low level of spontaneous swimming activity. To ensure a statistically relevant analysis of their dynamics, trajectories were pooled from all control sequences. Exposure to decadienal diluted in ethanol caused hyperactivity in the three adult states, as evidenced by a marked and dose-dependent increase in the number of trajectories and swimming speed values (Table 5.1). Exposure to ethanol alone resulted in an increase in activity in males and non-ovigerous females, though much less marked than in the presence of decadienal at $6\,\mu\text{mol}\,\text{L}^{-1}$ and $12\,\mu\text{mol}\,\text{L}^{-1}$. Conversely, ovigerous females did not increase their activity in the presence of ethanol.

Table 5.1: Number of trajectories and number of instantaneous speed values for the three adult states of *P. annandalei* before and during exposure to ethanol and to different concentrations of decadienal.

		Control	Solvent	$3\,\mu\mathrm{mol\,L^{-1}}$	$6\,\mu\mathrm{mol\,L^{-1}}$	$12\,\mu\mathrm{mol\,L^{-1}}$
Males	Trajectories	131	266	107	572	994
	Speed values	40 782	50 014	37 554	263 659	309 505
Females	Trajectories	46	92	129	395	570
	Speed values	25 494	53 619	62 441	169 820	187 082
Ovigerous females	Trajectories	415	103	645	673	1001
	Speed values	128 079	12 510	168 172	295 381	391 189

5.3.2 Instantaneous and mean swimming speed

In males swimming in control water, the distribution of speed values appears to be bimodal, with two maxima for values around $1\,\mathrm{mm\,s^{-1}}$ and $5\,\mathrm{mm\,s^{-1}}$ (Fig. 5.3). Exposure to ethanol amplified bimodality and caused the peaks to switch toward lower (below $1\,\mathrm{mm\,s^{-1}}$) and higher (around $8\,\mathrm{mm\,s^{-1}}$) speed values. Exposure to decadienal at $3\,\mu\mathrm{mol\,L^{-1}}$ reduced the strong bimodality induced by ethanol and caused a reversal of the distribution toward medium speed values. Exposure to the toxin at $6\,\mu\mathrm{mol\,L^{-1}}$ and $12\,\mu\mathrm{mol\,L^{-1}}$ completely removed bimodality and resulted in a clustering of speed values between $1\,\mathrm{mm\,s^{-1}}$ and $3\,\mathrm{mm\,s^{-1}}$.

In non-ovigerous females, probability density functions showed no difference between experimental conditions for speed values above $1\,\mathrm{mm\,s^{-1}}$, with most of the data comprised between $0.7\,\mathrm{mm\,s^{-1}}$ and $4\,\mathrm{mm\,s^{-1}}$. A modest increase in the frequency of speed values below $1\,\mathrm{mm\,s^{-1}}$ was found in females exposed to either decadienal diluted in ethanol or to the solvent alone, compared to females swimming in clean water.

In ovigerous females, exposure to ethanol caused a switch in the distribution toward both higher (around $6\,\mathrm{mm\,s^{-1}}$) or extremely low (below $0.2\,\mathrm{mm\,s^{-1}}$) speed values compared to the control condition. Fewer values were distributed between $0.3\,\mathrm{mm\,s^{-1}}$ and $3\,\mathrm{mm\,s^{-1}}$, indicating that copepods were either immobile of swimming relatively fast. Exposure to decadienal caused a decrease in the abundance of large speed values and a dose-dependent increase in the frequency of medium and lower speed values, with most of the data clustering between $1\,\mathrm{mm\,s^{-1}}$ and $3\,\mathrm{mm\,s^{-1}}$ and heavy tails extending toward larger speed values.

The mean swimming speed is indicated in Table 5.2 for each experimental condition. In males and ovigerous females, exposure to ethanol at $200\,\mathrm{ppm}$ resulted in a marked increase in swimming speed (Wilcoxon Mann-Whitney test, $p < 0.01$). Conversely, exposure to decadienal at $3\,\mu\mathrm{mol\,L^{-1}}$, $6\,\mu\mathrm{mol\,L^{-1}}$ and $12\,\mu\mathrm{mol\,L^{-1}}$ cancelled the effects of ethanol ($p < 0.01$) and reduced swimming speed bellow control values ($p < 0.01$). The decrease appeared to be dose-dependent between $3\,\mu\mathrm{mol\,L^{-1}}$ and $6\,\mu\mathrm{mol\,L^{-1}}$ ($p < 0.01$). Surprisingly, males and ovigerous females swam significantly faster when exposed to decadienal at $12\,\mu\mathrm{mol\,L^{-1}}$ than at $6\,\mu\mathrm{mol\,L^{-1}}$ ($p < 0.01$). However, the sampling distribution of trajectory-averaged values obtained during exposure to decadienal at $12\,\mu\mathrm{mol\,L^{-1}}$ was significantly different from the ones obtained at $3\,\mu\mathrm{mol\,L^{-1}}$ ($p < 0.01$) and in clean water ($p < 0.01$).

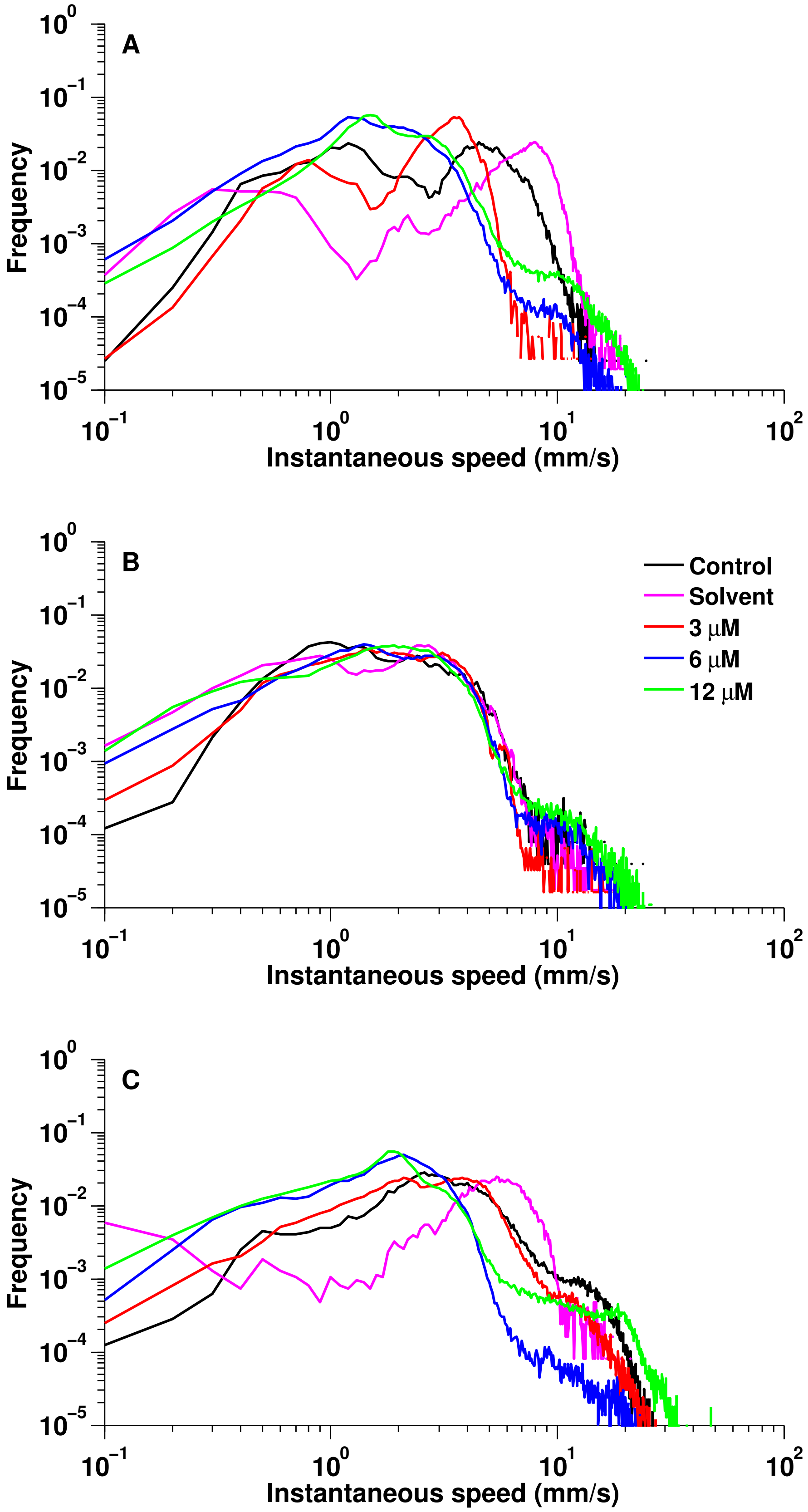

Figure 5.3: Probability density functions of the instantaneous swimming speed values of *P. annandalei* males (A), females (B) and ovigerous females (C) swimming in clean water (black) and during exposure to ethanol (magenta) and to decadienal at 3 µmol L^{-1} (red), 6 µmol L^{-1} (blue) and 12 µmol L^{-1} (green).

Table 5.2: Mean instantaneous speed (± standard deviation) of *P. annandalei* males, females and ovigerous females before and during exposure to ethanol and to different concentrations of decadienal.

	Control	Solvent	$3\,\mu\text{mol}\,\text{L}^{-1}$	$6\,\mu\text{mol}\,\text{L}^{-1}$	$12\,\mu\text{mol}\,\text{L}^{-1}$
Males	5.3 ± 1.9	7.6 ± 1.4	3.5 ± 1.2	2.3 ± 0.8	2.7 ± 0.9
Females	2.8 ± 1.2	3.2 ± 1.2	3.1 ± 1.0	2.7 ± 1.0	2.8 ± 1.0
Ovigerous females	4.9 ± 2.2	5.7 ± 1.4	4.2 ± 1.3	2.5 ± 0.8	3.2 ± 1.0

Sampling distributions from non-ovigerous females swimming in control water and in the presence of ethanol appeared to be similar ($p = 0.1$) even though their mean values were different. Similarly, no difference was found between their swimming speed in clean water and during exposure to decadienal at any concentration. The trend toward increasing p-values, however, suggests that exposure to decadienal tends to cancel the effects of ethanol ($p = 0.1$ between control and solvent and $p = 0.2$, $p = 0.8$ and $p = 0.9$ between control and exposure to decadienal at $3\,\mu\text{mol}\,\text{L}^{-1}$, $6\,\mu\text{mol}\,\text{L}^{-1}$ and $12\,\mu\text{mol}\,\text{L}^{-1}$). Indeed, females swam at a similar speed in the presence of ethanol alone and during exposure to decadienal at $3\,\mu\text{mol}\,\text{L}^{-1}$ ($p = 0.7$) but significantly slower at $6\,\mu\text{mol}\,\text{L}^{-1}$ and $12\,\mu\text{mol}\,\text{L}^{-1}$ ($p < 0.01$).

5.3.3 Moment function of the displacement

We conducted multifractal analysis to study how the moments of the norm of the displacement grew with time. For the three adult states and at each experimental condition, displacements exhibited long power-law correlation behavior up to approximately 8 s (Fig. 5.4), suggesting that their amplitude is structured in a scale-independent way. Scale invariance in foraging patterns usually reflects the exploitation of complex environments which might themselves have fractal properties. For the three adult states swimming in control water, the shape of the spectra of dimension clearly differs from a Lévy motion and indicates that the amplitude of the displacement is moderately multifractal (Fig. 5.5).

In males and ovigerous females, exposure to ethanol resulted in a more ballistic motion and in a rather monofractal behavior, as evidenced by a shift of the moment structure functions toward $\zeta(q) = q$. By contrast, exposure to the toxin reduced or cancelled the effect of ethanol and caused a displacement of the curve toward Brownian motion, particularly visible for $q > 3$. The moment structure functions of males and ovigerous females exposed to decadienal at $3\,\mu\text{mol}\,\text{L}^{-1}$ and $6\,\mu\text{mol}\,\text{L}^{-1}$ appeared to be similar and remained, in ovigerous females, above the control curve. Exposure to the toxin at $12\,\mu\text{mol}\,\text{L}^{-1}$ substantially increased multifractality in ovigerous females and caused a more pronounced shift of the moment structure function toward $\zeta(q) = q/2$, below control values for $q \geq 4$. The behavior of non-ovigerous females in control water, in the presence of decadienal at $3\,\mu\text{mol}\,\text{L}^{-1}$ and $6\,\mu\text{mol}\,\text{L}^{-1}$ or during exposure to the solvent alone remained similar. Exposure to the aldehyde at $12\,\mu\text{mol}\,\text{L}^{-1}$ caused a relatively modest shift toward Brownian motion and increased multifractality, as evidenced by a slightly more pronounced curvature of the moment structure function for high q values.

5.4 Discussion

Diatoms are broadly present in freshwater and marine ecosystems. A considerable literature has developed on the deleterious effects of diatom PUAs on the physiology and viability of copepod eggs. In contrast, considerably less is known on their influence on swimming behavior. In this study we showed

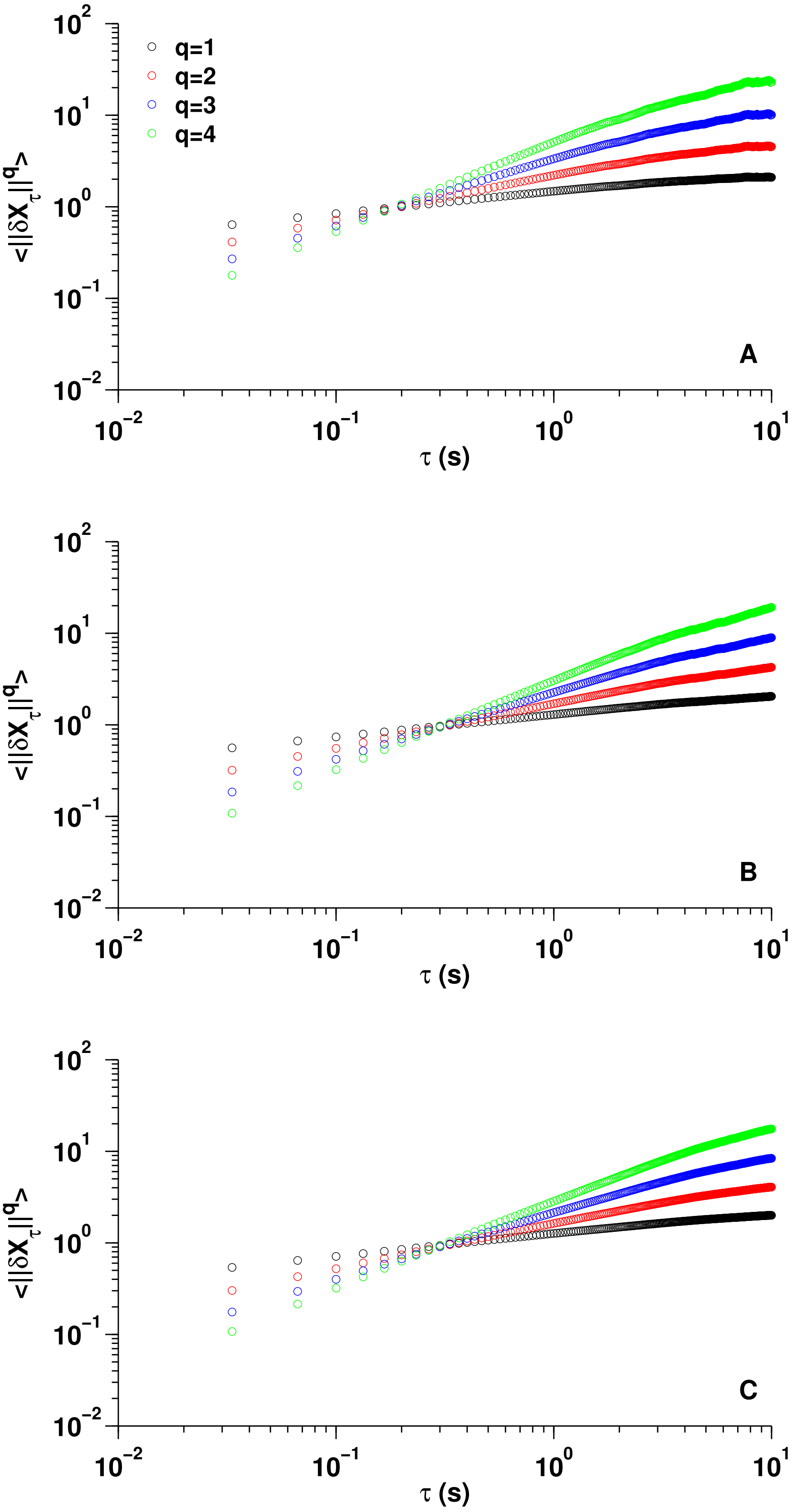

Figure 5.4: Scaling of $E[||\delta X_{\tau}||^{q}]$ versus τ for different q values and up to 10 s for *P. annandalei* males swimming in control water (A), non-ovigerous females exposed to ethanol (B) and ovigerous females exposed to decadienal at 12 µmol L^{-1} (C) for the first (black), second (red), third (blue) and fourth (green) moments.

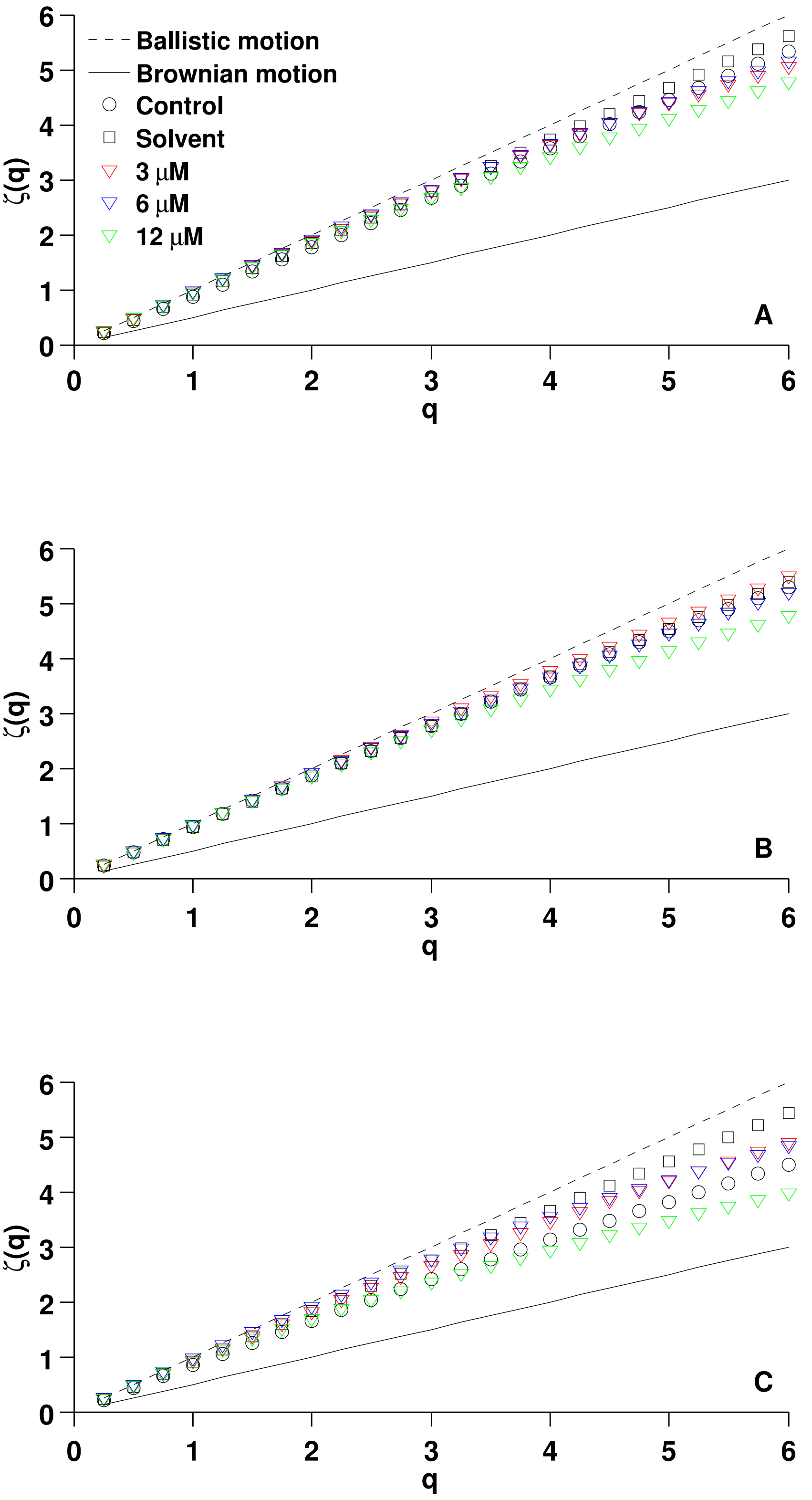

Figure 5.5: Structure function scaling exponent $\zeta(q)$ for *P. annandalei* males (A), females (B) and ovigerous females (C) swimming in clean water (circle, black) and during exposure to ethanol (square, black) and to decadienal at $3\,\mu\mathrm{mol\,L^{-1}}$ (triangle, red), $6\,\mu\mathrm{mol\,L^{-1}}$ (triangle, blue) and $12\,\mu\mathrm{mol\,L^{-1}}$ (triangle, green) compared with results expected in the case of ballistic motion (black, dashed line) and Brownian motion (black, solid line).

that decadienal used as a model aldehyde affected the dynamics of adults *P. annandalei*. Changes were immediate and included a dose-dependent hyperactivity, a decrease in swimming speed and a trend toward Brownian motion.

In the water column, phytoplankton is often patchily distributed over a distance of a few centimeters to meters. Field and laboratory studies investigating the response of copepods to the presence or the small scale variability of phytoplankton cells have revealed that copepods readily migrate and aggregate at localized diatom patches (Bainbridge, 1953; Tiselius, 1992; Atkinson and Shreeve, 1995; Bochdansky and Bollens, 2004), supporting the more established expectation that diatoms - even the toxic ones - do not exert any repellent effect on the adult stages of their grazers. These results were further confirmed by Lincoln et al. (2001) who observed similar ingestion rates in *Acartia tonsa* and *Temora longicornis* feeding on the non-toxic diatom *Pseudo-nitzschia pungens* and on *Pseudo-nitzschia multiseries*, which produces the toxin domoic acid. Similarly, the lack of toxic effect on behavior was demonstrated by Amin et al. (2011) who observed no avoidance of patches containing the PUA-producing diatom *Skeletonema marinoi* by *A. tonsa*, *T. longicornis* and *Pseudocalanus elongatus* and no or very little alterations in their swimming behavior.

There is, however, scarce evidence that certain secondary metabolites secreted by diatoms or other phytoplankton cells affect the feeding or swimming behavior of crustacean grazers. For instance, laboratory experiments have shown that the marine diatom *Phaeodactylum tricornutum* secrete apo-fucoxanthinoids acting as feeding deterrents when preyed upon by the copepod *Tigriopus californicus* (Shaw et al., 1995, 1997). Feeding deterrents are compounds which, upon detection by chemoreceptors, reduce or inhibit zooplankton grazing or cause selective grazing. Aldehydes from wounded benthic diatoms at concentrations ranging from $3.5\,\mu\mathrm{mol\,L^{-1}}$ for 2(E),4(E),7(Z)-decatrienal to $42\,\mu\mathrm{mol\,L^{-1}}$ for 2(E),4(E)-decadienal caused pelagic grazers of the genera *Daphnia*, *Cyclops* and *Eudiaptomus* to reduce their swimming activity (Jüttner, 2005). Similarly, Cohen et al. (2007) observed significant behavioral effects in *Centropages typicus* exposed for 24 h to brevetoxins of the marine dinoflagellate *Karenia brevis*, with a suppressed swimming activity at $0.15\,\mu\mathrm{g\,L^{-1}}$ and an increased swimming speed and loss of phototactic behavior at $15\,\mu\mathrm{g\,L^{-1}}$. Finally, Watson et al. (2007) reported on a market short-term increase in swimming velocity in *Daphnia* exposed to decatrienal at concentrations ranging from 2.5 to $25\,\mu\mathrm{mol\,L^{-1}}$.

In our study, the increase in swimming speed and the more ballistic motion following short exposure to ethanol at 200 ppm tend to indicate a typical escape reaction allowing copepods to escape unfavorable conditions. Escape reactions have been commonly observed in small crustaceans during the first stages of exposure to anthropogenic pollutants (e.g. Lopes et al., 2004; Kirkpatrick et al., 2006; Ren et al., 2007; Sornom et al., 2012) and regardless of the toxic characteristics of the chemicals (Ren et al., 2009). They are believed to result from damage or irritation of chemoreceptor cells (Blinova and Cherkashin, 2012). The decrease in swimming speed and the more erratic behavior induced by exposure to relatively high concentrations of decadienal, on the opposite, suggest that physiological incapacitation following loss of motor control has already occurred (Weis et al., 2001). Loss of motor control was also observed in *Calanus pacificus* presented with a diet of the toxic dinoflagellate *Ptychodiscus brevis* (Sykes and Huntley, 1987).

Direct exposure to PUAs is lethal to copepods (Taylor et al., 2007). Unfortunately there are few detailed reports on their toxic effects on physiological processes in adult individuals. *Calanus helgolandicus* fed for two days on the PUA-producing diatom *Skeletonema marinoi* displayed marked down-regulation of tubulin gene expression levels (Lauritano et al., 2011a) and significant reduction of the expression levels of genes involved in stress response, detoxification mechanisms and apoptosis regulation (Lauritano et al., 2011b). Diatom aldehydes are well-known Michael acceptors displaying potent cell toxicity

(Adolph et al., 2004). Michael acceptor elements create a variety of hazardous effects in vivo through alkylation of crucial cellular proteins (Refsgaard et al., 2000). However, the precise mechanisms that could account for the short-term behavioral toxicity observed here remain unknown.

Our results also show that non-ovigerous females *P. annandalei* were less affected by decadienal than males and ovigerous females. Females *P. annandalei* were also found less sensitive to salinity variation than males and ovigerous females (Chen et al., 2006; Michalec et al., 2012), indicating in this species a possible gender-specific difference in the response to environmental stressors. More generally, several studies have pointed out the apparent greater sensitivity of male copepods. In acute toxicity tests, males *Tisbe holothuriae* were significantly more vulnerable to decadienal than females (Taylor et al., 2007). Similarly, males of the estuarine copepod *Amphiascus tenuiremis* were almost four times more sensitive to the phenylpyrazole insecticide fipronil than non-ovigerous females (Chandler et al., 2004). It is generally agreed that the increased surface area to volume ratio contributes to the greater sensitivity of small organisms to external stressors. The marked response of ovigerous females observed here, however, suggests a distinct reason for the difference in sensitivity to decadienal.

These preliminary results offer some new insights into how diatoms affect copepods. They prompt us to further explore the relationship between PUA-induced changes in behavior and grazing efficiency. Our results also point out the need to conduct behavioral tests at environmental concentrations. Future studies on the accumulation of waterborne PUAs in copepods and on their immediate effects on physiological processes in adults may clarify the molecular and cellular basis of the behavioral response.

Chapter 6

Conclusion and outlook

6.1 Main findings

In this work, the swimming behavior of two widespread species of estuarine calanoid copepods was investigated by means of 3D-PTV. The method allowed the observation of a large number of copepods moving simultaneously in a significant volume of water and proved to be efficient at increasing the yield of long trajectories, a prerequisite for the Langrangian description of the movement of copepods.

We have presented experimental measurements of the dynamics of copepods under the influence of three ecologically relevant environmental parameters. We have assessed the effects of a realistic salinity increase on the behavior of *P. annandalei* reproductive stages and assumed an increasing energy devoted to osmotic regulation and a decrease in energy allocated to swimming. We have investigated the response of adults *E. affinis* to a brief exposure to three commonly found pollutants at sub-lethal concentrations and observed a typical escape reaction permitting copepods to evade stressful conditions. Finally, we have measured the dynamics of individuals *P. annandalei* exposed to a common diatom toxin and observed a dose-dependent increase in their swimming activity, a decrease in their swimming speed and changes in their multifractal behavior. We have used both conventional scale-dependent and independent metrics to describe the kinematics of copepods and the degree of complexity of their trajectories.

6.2 Limitations of the study

The present study has certain methodological limitations that must be considered. First, the behavior of copepods is highly intermittent. Copepods accelerate on a temporal scale that is much lower than the temporal resolution of our cameras. The tracking algorithm used in this study predicted the position of a particle in the next time step from its previous positions and assuming constant velocity or constant acceleration. Sudden changes in the magnitude of acceleration of *E. affinis* resulted in broken trajectories whose parts were linked together using a spatial and temporal matching assignment and under the assumption of a constant velocity, most likely under-estimating the importance of extreme events. Second, the use of two cameras led to ambiguities in the stereo matching process, as often two or more particles were found along the epipolar lines. These ambiguities could not be resolved by a system based on two cameras only. The use of a third and fourth cameras in further studies will restrict the search area to the intersection of the epipolar line segments. It will also improve the accuracy of the coordinates of the moving particles (Maas et al., 1993). Third, the light source did not yield an optimal and homogeneous illumination. The amount of light reflected by moving copepods and the intensity of their image particle was variable depending on their location within the aquarium and on their orientation.

Studying behavior also has difficulties and limitations. Behavior is difficult to measure and highly variable. It depends on the physiological state of the organisms and is strongly affected by experimental conditions. It can also differ between populations of the same species. As a result behavioral effects tend to be less reproducible than conventional mortality test or measurements of biochemical or physiological processes. Finally, effects need to be assessed under ecologically relevant conditions, on different species and at different stages of their life cycle.

6.3 Openings for further research

Further investigations are needed to link the changes in behavior observed in response to salinity variation to changes in total metabolism and enzymatic activity, contributing to a better understanding of the ecology of copepods within estuaries. The response of copepods to complex mixtures of contaminants also needs to be studied more comprehensively. In particular, the influence of pollution on interactions between individuals deserves more attention. The ability to detect, identify and properly respond to chemical signals is a crucial component of the ecology of marine organisms. A large number of studies spanning several phyla have shown that sub-lethal concentrations of various classes of contaminants can disrupt the transfer of chemical information between organisms, impairing for instance conspecific chemical communication, predator-prey interactions or mating behavior (Lürling and Scheffer, 2007). However, despite the importance of copepods at the basis of the trophic network, the effects of pollutants on their mating behavior have never been investigated. Chemical signals play an important role in copepod mating behavior where they elicit an appropriate response in the opposite sex. Copepods in the environment and especially in estuaries are inevitably exposed to chemical pollution. Although most toxicants are present at sub-lethal concentrations, any interference between waterborne pollutants and the processing of chemical information may have deleterious consequences on the ability of copepods to communicate and may cause disturbance in behavioral interactions between mates.

The limitations and questions raised here will soon be under investigation at the ETH Zürich, Institute of Environmental Engineering and at Lille 1 University of Sciences and Technologies, Laboratory of Oceanography and Geosciences. The particle tracking software developed at the ETH will be used in a three-camera system to study fine-scale interactions between zooplankters and in particular the mating behavior of several species of calanoid copepods in the presence of waterborne anthropogenic contaminants. The possible effects of impaired behavioral interactions on the dynamics of small copepod populations will be evaluated in laboratory experiments. There are also plans to conduct investigations at the cellular and molecular levels - for instance quantifying the transcript level of marker genes and the activity of detoxification proteins - in order to better understand the mechanisms causing changes in behavior. We aim to develop a more integrated approach to ecotoxicology, studying how different levels of biological organization relate to one another.

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