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TITLE : Study of recruitment of fucoid embryos in the natural environment and influence of stress of desiccation and excess of light energy on their development

ABSTRACT

The intertidal zone is an extreme environment due to the alternation of emergence and immersion. The benthic organisms, specially the young stages have to be able to colonize quickly this zone and to survive to these extreme conditions. Among benthic species of estuary of St Laurent (Québec, Canada), brown algae are dominant and live their optimum ecological niche. The rigorous winter cause the recovery of the intertidal zone by ice. All organisms live slowly or dye under this ice cover. In adopting an ecophysiological approach, this work aims at the study on the one hand the colonisation of the zone and on the other hand the effect of climatic parameters on the development of juveniles. (1) To estimate the colonisation, counting of embryos on artificial substrata allow to observe a wide spatially and temporally range of recruitment that don't depend to the number of adults; but would be strongly influenced by climatic factors. The mortality rate is variable and proves more important on rocky shores exposed than in basin and under canopy. (2) By measuring fluorescence in laboratory, we followed the set up of photosynthetic apparatus into embryos and these embryos have a efficient photosynthetic apparatus after 6 days of growth. (3) Measurements of fluorescence directly in the field have to be realised on embryos of three species located in their niche under four meteorological conditions ; Their photosynthetic answer are similar traducing by a decline during low tide. However the ability of photosynthesis in these juveniles call back those of adults. The embryos located under canopy have a electron transport working longer but weaker than on exposed surfaces. (4) On a wall, five levels with juveniles have been determined; the measurements of fluorescence relative to measurements of desiccation and light showed that the yield of juveniles of higher levels dropped more rapidly than those of lower levels. The correlations proved the action of light get worse with the action of wind. The percentage of mortality increased in the higher levels and the growth rate decreased. The desiccation seems to be greater responsible of the zonation of algae.

KEY WORDS : Fucales, Embryos, Fucus, Photosynthesis, Fluorescence, PAM, Recruitment

RESUME

La zone intertidale est un environnement extrême en raison de l'alternance de périodes d'émersion et d'immersion. Les organismes benthiques comme les algues brunes doivent être capables de coloniser rapidement cette zone et de survivre dans ces conditions difficiles. Ce travail consiste à étudier, d'une part, la colonisation du milieu par ces algues et, d'autre part, l'effet des paramètres climatiques sur le développement de leurs jeunes organismes dans l'estuaire du St Laurent (Canada). (1) Des dénombrements d'embryons sur des substrats artificiels permettent d'observer une grande variabilité spatiale et temporelle du recrutement, indépendant du nombre d'adultes matures mais conditionné par des facteurs climatiques. Le taux de mortalité s'avère plus important sur les surfaces rocheuses exposées et dans les cuvettes que sous la canopée. (2) Par des mesures de fluorescence chlorophyllienne en laboratoire, nous avons suivi la mise en place de l'appareil photosynthétique de ces embryons qui s'avère fonctionnel après six jours de développement. (3) Des mesures de fluorescence chlorophyllienne réalisées sur le terrain montrent que l'activité photosynthétique de jeunes embryons de trois espèces différentes, confrontés à quatre conditions météorologiques définies, déclinent inégalement durant les marées basses. Ces différences de sensibilité au stress déterminent l'emplacement de ces trois espèces sur l'estran, celle qui occupe le niveau bathymétrique le plus élevé étant capable de maintenir une activité photosynthétique plus longtemps au cours d'une émersion que celles qui vivent à des niveaux inférieurs. De plus, pour chaque espèce, les embryons qui se développent sous la canopée sont moins affectés durant la marée basse mais, en raison de la faible irradiance, leur photosynthèse est plus faible. (4) Durant la marée basse, un gradient dans la rapidité de dégradation de l'activité photosynthétique de jeunes thalles situés à cinq niveaux différents sur une paroi verticale a été mis en évidence par des mesures de fluorescence chlorophyllienne, les thalles des niveaux supérieurs étant les plus affectés. Cette altération est étroitement corrélée à un gradient de dessiccation qui apparaît comme un des facteurs déterminant l'emplacement des algues sur l'estran.

DISCIPLINE : Océanographie

MOTS-CLES : Fucales, embryons, Fucus, Photosynthèse, Fluorescence, PAM, Recrutement

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CHAPITRE 1

Introduction Générale

1.1 Des végétaux aquatiques : les algues

La faculté des végétaux chlorophylliens à réaliser la synthèse de matière organique à partir des substances minérales est à l'origine de toute vie. C'est dans les océans que l'on rencontre les formes végétales les plus simples capables d'autotrophie par photosynthèse : les algues. De plus la majorité de ces algues est susceptible de se développer en milieu inorganique. Ces deux capacités nous donnent à penser que les algues sont les premiers organismes vivants apparus à la surface du globe. Parmi ces premiers organismes, on trouve des algues de dimension microscopique communément appelées phytoplancton qui sont libres dans la colonne d'eau et des algues de dimension macroscopique, sessiles nommées macro-algues. Dans les océans, les algues constituent presque à elles seules le peuplement végétal, elles forment avec les bactéries le premier maillon de la chaîne alimentaire et représentent la majorité des producteurs primaires.

1.2 Les Fucales

Les algues brunes sont les exemples les plus marquants de la preuve de cette zonation du rivage bien qu'elle soit visible pour d'autres organismes. Cet arrangement des fucales est particulièrement frappant en termes d'adaptation des végétaux dans le rivage supérieur qui est un habitat presque terrestre bien qu'encore à l'intérieur d'un environnement marin. Des zones distinctes d'algues brunes sont observées sur la plupart des rives rocheuses.

Les algues brunes, spécialement les fucales sont les sujets les plus étudiés de l'écologie des zones intertidales (Chapman 1995) parce que des profils similaires existent à travers les zones tempérées du monde, incluant les îles britanniques (Schonbeck & Norton 1980), La Nouvelle-Angleterre (Lubchenco 1980), la Nouvelle Zélande (Chapman 1966), la France (Cabioch et al. 1992) et l'estuaire du Saint-Laurent où elles sont dominantes (South & Cardinal 1973). Elles forment des ceintures successives qui sont constituées par une seule espèce et cette disposition en escaliers est définie avec le terme zonation.

La zonation de fucales se profile souvent de la sorte ; la zone supérieure est colonisée par *Fucus spiralis*. Le peuplement de la zone moyenne est tributaire du degré d'intensité du mouvement des vagues (*F. vesiculosus* L. en forte turbulence, *Ascophyllum nodosum* (L.) Le Jolis si l'agitation diminue et surtout en milieu calme). La zone *F. distichus* est étendue assez bas dans le rivage au sommet de la zone des *Laminaria*. Les fucales alors constituent un groupe écologique bien distinct, remarquablement adapté au mode subaérien de vie avec une grande capacité de résister à des conditions extrêmes.

Les fucales sont très bien adaptées à leur habitat, elles sont aussi résistantes aux températures extrêmes de l'hiver qu'aux températures de l'été pouvant atteindre les 30 degrés Celsius sans dommages apparents. Cette zonation prononcée est sans nul doute le résultat des capacités à résister à la dessiccation durant la germination et la croissance. L'exposition à l'air s'accompagne aussi d'une augmentation de l'irradiance et l'effet de cette insolation devrait contribuer à la zonation. *F. vesiculosus* L. et *F. serratus* L. sont adaptés aux fortes intensités et se comportent comme des plantes de soleil. Dans le même temps, ils peuvent avoir une photosynthèse efficace sous des faibles intensités car leur croissance est active durant les premiers mois de l'année même lorsque les températures ambiantes sont basses (Björkman 1981).

Le thème sous-jacent de toutes ces études est que la zonation intertidale est en relation avec les aptitudes physiologiques de l'algue à supporter les périodes d'émersion (Burrows & Lodge 1950, Dayton 1975, Lubchenco 1978, Lubchenco & Menge 1978, Lein 1980, Buschmann 1990, McCook & Chapman 1992). Des études postérieures tenteront de vérifier cette hypothèse.

1.3 La zonation et les facteurs responsables

Les zones côtières sont classées en trois zone : zone supratidale, intertidale et subtidale. Les facteurs dominants avec lesquels réagissent les algues sont : pour la zone supratidale, l'exposition à l'air ; pour la zone intertidale, l'alternance de l'émersion et l'immersion provoquée par le balancement des marées ; et pour la zone subtidale, l'immersion et la faible luminosité. Ces trois zones sont visibles sur tous les rivages du monde.

La zone intertidale est à première vue l'environnement le plus inhospitalier, à cause du mouvement des marées. Ce mouvement de vagues crée une nouvelle zonation qui est en relation avec la longueur du temps d'émersion/immersion ; cette durée est le facteur contrôlant l'implantation des organismes visualisée par des ceintures bien délimitées, occupées par des formes de vie similaire. L'immersion périodique est une caractéristique de la vie entre les marques des marées. Afin de survivre, l'organisme doit être capable de lutter avec un éventail complexe de facteurs environnementaux à tous les stades de sa vie. Alors que les mouvements de la mer montrent une périodicité régulière et sans faille, les niveaux hauts et bas ne sont pas identiques d'un jour à l'autre à cause des cycles des marées. Ce mouvement régulier de la mer apporte la zonation des organismes entre ces marques de marées ; le taux de marée (l'amplitude) détermine largement la zonation verticale de la région intertidale et la profondeur des zones occupées par les végétaux et les animaux. Divers autres facteurs influencent la composition de la flore intertidale et le profil de zonation qui est le résultat de ces effets de la marée et de l'action des vagues. Ceci inclut la nature géologique du substrat et sa texture physique, la salinité de l'eau, la température, l'intensité de la lumière et l'interaction avec la faune.

Quand une algue est laissée découverte par la marée descendante, l'équilibre osmotique que lui fournissait le milieu aquatique ainsi que le support, le maintien d'une température et d'une composition ionique constante, la fourniture de gaz dissous et de nutriments nécessaires à son métabolisme sont rompus. Les algues souffrent alors d'interruption dans leur activité métabolique et sont les victimes de rapides changements de température ; elles souffrent de dessèchement du thalle, de l'irradiation par le soleil et de l'eau douce amenée par la pluie. La période d'exposition à l'air sera déterminante sur l'influence des différents facteurs affectant l'algue. L'algue, croissant près de la limite supérieure, sera le sujet de ces changements du milieu pour une période variable de quelques heures (qui dépend du coefficient et du niveau bathymétrique) pour chaque marée dans la zone littorale subissant une marée alors qu'un organisme proche de la limite inférieure sera seulement exposé pour quelques minutes ou plus. Quand on considère les différentes conditions qui prévalent dans la zone de balancement des eaux, il semble peu probable qu'une seule espèce puisse être capable de croître à tous les niveaux de la zone intertidale et supporter le large spectre de changements physiques et biologiques d'une marée. Cette exposition à l'air est considérée pour un organisme marin comme une période de stress.

Le stress se définit selon la littérature par toutes situations où les contraintes externes limitent le taux de production de matière sèche de toutes les parties de la végétation en dessous de son potentiel génétique (Grime 1979) ou plus simplement par la réduction de la production en dessous de l'optimum des caractéristiques du végétal. Le stress peut se produire dans des conditions environnementales variées.

En effet, l'exposition à l'air provoque des augmentations de chaleur, de température et de lumière que l'algue doit supporter. En particulier pendant les marées basses, les macro-algues peuvent subir des dessèchements intenses et des stress lumineux et thermiques sous l'effet d'exondations prolongées. La sévérité des stress dépendra des conditions atmosphériques comme la pluie, les nuages et la force du vent. Il est important de noter que les périodes de marées basses retardent dans le temps chaque jour. Les stress peuvent varier durant la journée suivant le moment de la marée basse. Il y a une base biologique intrinsèque (différents taux de croissance.....) pour la zonation le long d'un gradient de stress physique. Ceci renforce la zonation spécialement depuis que les différents stress ont lieu à différents points le long du gradient ; pour simplifier, il y a de longues dessiccations dans les limites supérieures, des alternances rapides de dessiccation et d'immersion dans la zone intermédiaire et une faible irradiance dans les limites inférieures. Lorsqu'il existe un gradient dans la nature, il y a une zonation des organismes ; ces conditions stressantes à l'air sont responsables d'un phénomène visible sous forme de différentes bandes de répartition des organismes. Les profils de zonation sont mis en relation directe avec les facteurs environnementaux.

Les études antérieures sur la zonation sont largement descriptives (Isaac 1933, Zaneveld 1937, Kanwisher 1957, Biebl 1962, Zaneveld 1969) et mettent l'accent sur l'observation des bandes horizontales des espèces qui existent sur les roches des zones côtières. Ce sont les deux auteurs Stephenson et Stephenson (1949) qui ont été crédités de la popularisation de l'idée de ceintures universelles et suggèrent que les ceintures sont le résultat d'interactions entre l'établissement et la croissance des espèces d'une part et l'opération de complexes de facteurs environnementaux, de broutage et de compétition d'autre part. D'autres études ont essayé de mettre en évidence la relation entre les niveaux de marées et la distribution des organismes. Malheureusement les relations n'ont jamais été prouvées expérimentalement. Selon Colman (1933), certaines régions subissent des augmentations soudaines de la marée montante dans le temps et ceci conduit à des formes de zonation en escaliers. Ces changements soudains dans le temps d'immersion sont corrélés avec les cassures entre les zones majeures des algues. Colman (1933) a introduit le concept de niveaux critiques de marée (critical tide level=CTL) qui met en relation les limites de distribution et les durées d'exposition. Ce concept fut énoncé par Colman mais fut développé par Doty (1946) qui redéfinit le concept en décrivant les « facteurs niveaux » qui augmentent lors de la durée d'exposition.

Les études plus récentes sont en accord avec le schéma de Colman, spécialement celle de Underwood (1978) qui a travaillé en Grande-Bretagne concluant qu'il n'est pas évident que la distribution caractéristique des espèces soit basée sur les CTLs mais que chaque espèce possède son unique série de facteurs contrôlant ses limites hautes et basses. Travaillant en Nouvelle-Écosse, Swisbanks (1982) a été capable de démontrer l'application des CTLs dans son étude de zonation de même que d'autres auteurs tels que Foster (1971) et Schonbeck & Norton (1978). Toutes ces études montrent que l'on peut appliquer le concept de CTLs pour tous les types de marées qu'elles soient mixtes, diurnes ou semi-diurnes. Toutefois aucune ne met en évidence que la zonation soit causée par les CTLs.

Quelques études pertinentes de l'époque ont essayé d'expliquer la relation entre la position d'une algue et son aptitude à supporter les alternances d'émersion et d'immersion. Des études comme celles de Baker (1909) ou celle de Stocker & Holdheide (1937) ont essayé de mettre en relation la position de l'algue avec sa capacité à supporter l'exposition à l'air et la récupération de la plante après la période de dessèchement. Baker (1909) a conclu dans son article que les algues, placées les plus hautes dans les zones côtières, ont une plus grande aptitude à supporter de plus longues périodes de dessèchement que celles qui croissent plus bas. Celles qui sont les plus hautes auraient une croissance plus lente. Les études plus récentes montrent que l'alternance émersion/immersion jouerait un rôle dans la zonation et concluent que les facteurs comme l'accumulation du temps immergé et la durée de la plus longue immersion paraissent en relation pour certaines algues sur les côtes britanniques (Druehl & Green 1982). Ils en conclurent que la distribution verticale des algues était significativement corrélée avec les profils d'immersion et d'émersion.

Dès 1960, les études sur la zonation ont rapporté des expériences qui prouvent que la capacité physiologique d'un organisme à supporter les stress physiques associés à l'émersion comme facteur primaire contribue à la distribution verticale. En 1961, l'étude de Connell sur deux espèces de balanes a introduit le concept général que les limites supérieures de la plupart des organismes de la zone intertidale sont probablement déterminées par les facteurs physiques et que les limites inférieures seraient déterminées par les facteurs biologiques.

Cependant, ce concept introduit par Connell a été remis en question sur son universalité par Chapman (1986) et Underwood (1980). Underwood montre qu'il y aurait une combinaison du facteur biologique broutage avec des facteurs physiques qui limitent les algues foliacées. Bien que le broutage puisse limiter la colonisation supérieure de l'algue, la dessiccation peut ultimement limiter sa distribution. Dayton (1975) met en relation la structure de la communauté à une variété de causes qui incluent les stress physiologiques et physiques ainsi que la compétition entre les organismes et le broutage. Cette étude de Dayton démontre que la distribution ne dépend pas que d'un seul facteur. Lubchenco (1980) suggère que la combinaison de facteurs abiotiques et biotiques puisse être responsable de la zonation des algues.

Cette étude démontre l'importance du broutage et de la compétition pour les limites inférieures de certaines algues. Ce travail a été fait sur l'algue brune *Fucus vesiculosus* L. et l'enlèvement des brouteurs et des algues qui sont en compétition avec *Fucus* favorisait l'établissement et la croissance de cette algue. Le résultat indique que les facteurs biotiques limitent la distribution mieux que les stress physiques. Schonbeck & Norton (1980) montrent que *Pelvetia canaliculata* est incapable de croître dans la zone inférieure à *F. spiralis* parce qu'il est incapable de rentrer en compétition avec *F. spiralis*. En enlevant les *Fucus*, *Pelvetia* est capable de croître et de coloniser cette zone. Schonbeck & Norton (1979) montrent que les juvéniles de *Pelvetia* sont capables de survivre sous la canopée de *Fucus* et les auteurs suggèrent que le broutage des juvéniles de *Pelvetia* doit être important aussi pour empêcher l'établissement de l'espèce. Les deux auteurs montrent aussi que *Pelvetia* ne peut survivre à une immersion prolongée. *Ascophyllum nodosum* (L.) Le Jolis dans l'étude de Schonbeck & Norton (1980) domine la zone intertidale moyenne en dépit d'une croissance plutôt lente car il peut vivre sous la canopée de *Fucus* ssp. Chapman (1986) conclue que « la distribution et l'abondance d'espèces doivent dépendre des attributs physiologiques, des attributs démographiques et des interactions biotiques, et qu'une approche qui intègre ces trois aspects doit mener à une meilleure compréhension de la distribution et de l'abondance des algues ».

Il y a beaucoup d'études qui examinent les facteurs physiques et les réponses physiologiques (Johnson et al. 1974). La position d'une algue doit être en relation avec sa capacité à maintenir sa photosynthèse nette quand l'algue est émergée. Johnson et al.

(1974), Quadir et al. (1979) et Johnston & Raven (1986), Dring & Brown (1982), Hawkins & Hartnoll (1985), Madsen & Maberly (1990) trouvent que les algues ont un taux de photosynthèse plus élevé quand elles sont émergées. Pour Johnson, la photosynthèse nette de *F. distichus* est six fois plus forte quand l'algue est hors de l'eau comparée à la photosynthèse de l'algue immergée et 1,5 fois plus dans l'étude de Quadir (1979). La production primaire de *Ascophyllum* (Johnston & Raven 1986) est plus grande. Dans d'autres cas, celle-ci est plus élevée lorsque les algues sont submergées comme l'étude de Oates & Murray (1983) sur *Pelvetia fastigiata* et *Hesperophycus harveyanus*, dans *Colpomenia peregrina* (Oates 1985) et *Halosaccion americanum* (Oates 1986). Ramus et al. (1977) trouvèrent que *F. vesiculosus* et *A. nodosum* ont une photosynthèse nette plus élevée quand ils sont cultivés à 4m de profondeur plutôt qu'en surface. A cause des larges variations dans les études, les techniques de mesure de la photosynthèse doivent être prises en compte sérieusement. Dans certains cas, la photosynthèse lorsque les algues sont émergées est plus grande à des niveaux légers de dessiccation (Chapman 1966, Brinkhuis et al. 1976, ...). Certaines algues peuvent maintenir une forte photosynthèse même avec des forts taux de perte d'eau. Chez *Fucus distichus*, Quadir (1979) mesure encore une photosynthèse nette après 70% de perte d'eau. Alors que Brinkhuis (1976) montre que cette photosynthèse nette existe encore chez *Fucus vesiculosus* après une perte d'eau de 45%. Parmi d'autres, Dromgoole (1980) a étudié la perte d'eau chez les principales fucoides trouvées sur les côtes européennes. Chez *Pelvetia canaliculata*, le maximum de perte d'eau a lieu dans les six heures suivant l'exposition alors que chez *F. serratus* et *Ascophyllum* le maximum de perte d'eau peut être étendu sur 18 heures (en conditions de laboratoire). Chez *F. vesiculosus*, plus de 90 % du contenu initial de l'eau peut être perdu en 1 ½ heure. Une augmentation dans le taux de perte d'eau peut être observée avec les différentes espèces qui occupent successivement les zones. De nos jours, on sait que les limites supérieures de distribution des algues sont dues à la capacité de l'algue à supporter la dessiccation.

Il est classiquement admis que les réponses des algues marines à l'exposition à l'air sont négatives (Dring 1994). Les algues doivent avoir un haut degré de résistance aux conditions extrêmes telles que la dessiccation (Schonbeck & Norton 1979), dont les effets indirects sont l'échauffement (Smith et al. 1986), la perte d'eau entraînant une augmentation de salinité (Karsten & Kirst 1989) et l'insolation (Hanelt 1992). La dessiccation joue un rôle majeur. Pourtant Schonbeck & Norton (1979) et Dromgoole (1980) trouvèrent qu'il n'y avait qu'une petite corrélation avec la position de l'algue et sa perte d'eau durant l'exposition. Les algues dans la limite supérieure devraient perdre de l'eau plus rapidement que les algues placées en bas, et le taux de perte d'eau serait lié au rapport surface/volume plutôt qu'aux mucilages (Haas & Hill 1933) ou à l'épaisseur de la paroi cellulaire (Dorgelo 1976). Schonbeck & Norton (1979) suggèrent que ce soit la résistance à la dessiccation plutôt que l'évitement qui assure la place de l'algue. Jones & Norton (1979) conclurent que le dessèchement du tissu réduit la surface pour l'évaporation et conserverait l'eau. McMurtry (1988) note qu'après quelques heures d'exposition, les parties exposées de *Pelvetia fastigiata* et *Hesperophycus harveyanus* étaient sévèrement déshydratées alors que celles qui sont sous la canopée sont encore bien hydratées. Les algues ne peuvent éviter la dessiccation, il est certain que des mécanismes destinés à éviter une perte d'eau mortelle se mettent en place. Même si les algues photosynthétisent mieux lorsqu'elles sont légèrement déshydratées, lorsque la perte d'eau se poursuit, la photosynthèse va décroître.

Smith & Berry (1986) suggèrent que le succès des algues intertidales puisse être en relation aussi avec la restauration de la photosynthèse chez l'algue après le dessèchement. Dring & Brown (1982) montrèrent que les espèces *Pelvetia canaliculata* et *Fucus spiralis* dans les limites supérieures photosynthétisent encore après avoir perdu 90% d'eau alors que *Laminaria digitata* qui croit dans les limites inférieures stoppe sa photosynthèse après une perte d'eau de 55%. Pour estimer les causes physiologiques de la zonation, la mesure du taux de photosynthèse après dessiccation et/ou après la restauration de la photosynthèse sont les paramètres les plus couramment employés.

La perte d'eau durant la période d'exposition à l'air est un facteur important qui affecte la zonation. Bien que la dessiccation joue un rôle important, l'exposition à l'air libre entraîne aussi pour l'autotrophe une augmentation de l'intensité lumineuse qui peut avoir des effets directs ou indirects sur la photosynthèse nette (Davison & Pearson 1996).

Les réponses photosynthètiques durant l'émersion ont suggéré de considérables variations dans le flux de photons reçu causé par les variations journalières dues aux conditions météorologiques et les conditions de marée. Mais il est improbable que cette restriction de position des fucales dans la zone intertidale soit dépendante de l'illumination. Stocker & Holdheide (1937) ont trouvé qu'il y a un gain considérable de la photosynthèse durant les périodes d'exposition bien que la capacité de photosynthèse soit graduellement perdue en séchant. Dans cette étude de Stocker & Holdheide qui utilisèrent *F. spiralis* et *F. serratus*, la différence entre les jours ensoleillés et nuageux était extrêmement prononcée et il était probable que l'exposition sous le soleil provoquait une chute dans le taux d'assimilation qui était corrélée avec le contenu en eau parce que la plante exposée séchait rapidement et cessait d'assimiler. Pour pouvoir jouer avec les fluctuations de l'intensité lumineuse, les algues doivent trouver le moyen d'ajuster et d'adapter le taux de la lumière disponible (Ramus & Rosenberg 1980). Pour ces organismes marins, lors de la marée basse, il y a habituellement un excès d'énergie lumineuse sur l'appareil photosynthétique et différents mécanismes biochimiques et physiologiques sont impliqués pour protéger la plante (Demmig-Adams et al. 1989, Genty et al. 1989, Demmig-Adams & Adams 1996, Harker et al. 1999). Ces différents mécanismes sont définis sous le terme de photoinhibition qui entraîne une décroissance de l'efficacité photosynthétique et dans le pire des cas d'une cessation de toute photosynthèse (Krause 1988).

La compréhension de ce processus, qu'est la photoinhibition n'est pas ici le but de ce travail. La photoinhibition est un phénomène biochimique qui est depuis une dizaine d'années presque élucidé et qui montre comment l'excès d'énergie lumineuse qui ne sert pas à la fixation du carbone est dissipé sans affecter la plante. La photoinhibition est un phénomène reconnu chez les algues (Hanelt 1992, 1994, 1996, Franklin 1992, Larkum & Wood 1993) car l'appareil photosynthétique des algues qui sont émergées, subit l'excès d'énergie lumineuse et sa performance photosynthétique décline durant la marée basse (Henley et al. 1991, 1992), les conditions lumineuses, la perte d'eau, le manque de carbone inorganique nécessaires pour fabriquer les carbohydrates produisent un stress de photoinhibition substantiel. Herbert & Waaland (1988) suggèrent une corrélation entre la sensibilité à la photoinhibition et la zonation en profondeur de l'algue. Hanelt et al. (1993)

étudiant les réponses variées des algues de différentes zones, celles des zones inférieures montrant une moins forte photoinhibition que celles des limites supérieures. Les algues montrent un maximum de photoinhibition durant le midi et tôt dans l'après-midi.

Cette photoinhibition est un processus qui permet à la plante de s'acclimater aux contraintes environnementales ; on ne parle pas d'adaptation car il est noté que le procédé d'adaptation implique une modification héréditaire dans la structure ou la fonction. Ce procédé contraste avec l'acclimatation qui arrive durant la vie d'un organisme et qui n'est pas héréditaire. S'endurcir est souvent employé pour signifier une acclimatation qui apparaît très tôt dans les jeunes organismes.

1.4 Les jeunes stades : avenir de la population

La plupart des études sur la zonation citées ci-dessus portent exclusivement sur les réponses des organismes adultes. Alors qu'il est logique de penser que les limites de distribution des algues adultes proviennent du succès de survie des jeunes stades. Lorsque l'adulte devient mature, il développe à ses extrémités des réceptacles qui relâchent dans le milieu extérieur ses gamètes. Les gamètes vont s'associer afin de former une cellule diploïde, le zygote qui se fixe rapidement et va se développer en embryon (Figure 1.1). Bien qu'il ne manque pas d'informations biochimiques sur le développement de ces embryons, il existe une réelle absence d'informations sur les jeunes stades des algues. Ceci est bien compréhensible, les stades juvéniles étant difficiles à suivre du fait de leur petite taille dans un milieu marin.

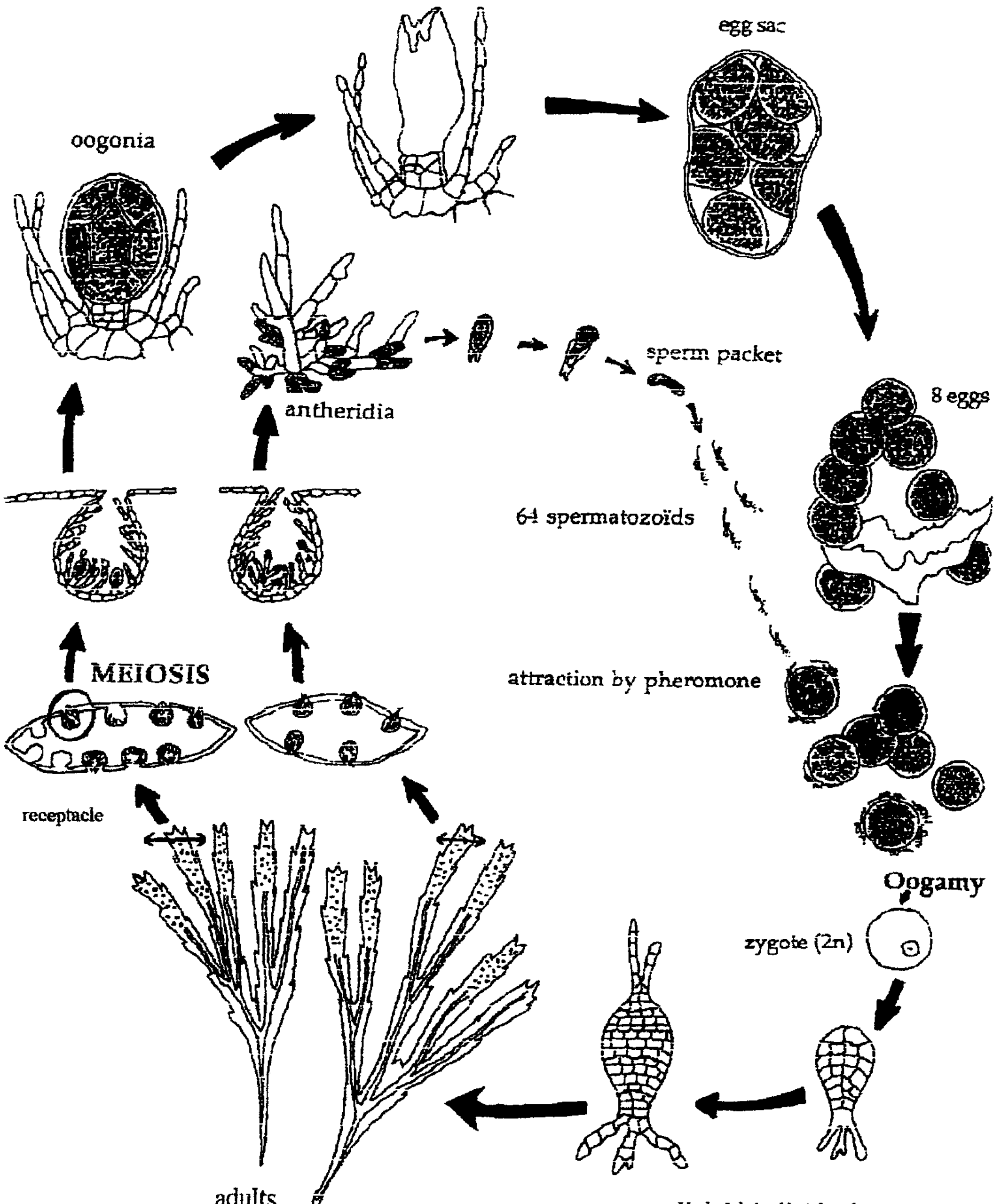


Figure 1.1 : Cycle reproducteur du *Fucus serratus* (modifié de Fristch 1965)

Cependant les jeunes stades sont les plus intéressants à étudier car ils sont plus sensibles et sont vraisemblablement les plus affectés par le large éventail des facteurs de stress qui ont lieu lors de la marée basse.

Le maintien des espèces passe par le recrutement de nouveaux individus qui s'avère nécessaire à la survie de ces populations. Une connaissance de leur dynamique de population est cruciale pour comprendre la biologie d'une espèce étudiée qui leur permet de vivre dans un écosystème marin. Un des éléments biologiques fondamentaux de cette population est le recrutement de nouveaux individus après la reproduction. Après s'être fixés, le succès de ces juvéniles dans des conditions environnementales qui changent constamment dépend de leur capacité à répondre aux conditions ambiantes. Le stade de germination ou de croissance est fondamental pour la survie parce que c'est le premier stade le plus sensible qui va être exposé aux stress de l'environnement intertidal, et la survie et la reproduction d'une algue dépendent de la survie des stades juvéniles. En effet, les stades zygotes et embryonnaires sont connus pour être les stades les plus sensibles. Terry & Moss (1981) montrèrent que les zygotes de quatre espèces de fucales ont germé sous un large spectre de lumière et de température et en déduisirent qu'une faible tolérance à l'un des autres nombreux stress environnementaux à chaque étape de la vie des algues doit déterminer la zonation des algues dans la zone intertidale. Brawley & Johnson (1991) ont suggéré que ce soit la dessiccation des jeunes plantes qui empêche l'apparition des algues en dehors de leur zones habituelles et c'est un fait que les embryons des fucales sont habituellement strictement confinés à leur stade jeune au niveau occupé par les thalles adultes. La survie des stades reproductifs (Brawley & Johnson 1991, Davison et al. 1993) et leur sensibilité au broutage et à l'exposition des vagues ont été identifiées comme des facteurs critiques additionnels déterminant la distribution des espèces dans la zone intertidale (Chapman 1995).

Cependant depuis une dizaine d'années, les études s'axent de plus en plus vers les mesures des effets des stress sur les jeunes stades. On sait que les juvéniles sont plus sensibles à des sources de mortalité comme le broutage et la dessiccation que leurs stades adultes (Santelices 1990, Vadas et al. 1992, Chapman 1995). Cependant il parait invraisemblable que la croissance des jeunes plants soit affectée par un degré similaire de dessiccation que les individus adultes. McLachlan (1974) a trouvé que la croissance et le développement des embryons de *Fucus* ssp. sont limités par la lumière et la température et a suggéré que la distribution de ces espèces soit contrôlée par des facteurs qui affectent les embryons et non pas les adultes. La croissance des embryons de *Phaeostrophion irregulare* est très sensible à la dessiccation (Mathieson 1982) qui pourrait limiter sa distribution. Il est généralement admis que les jeunes stades sont sensibles au broutage et à la dessiccation et que la distribution des adultes est largement le résultat d'un profil de mortalité parmi les jeunes (Santelices 1990, Brawley & Johnson 1991, Chapman 1995). Il existe bien sur, beaucoup de facteurs qui affectent les jeunes individus tels que les brouteurs (Lubchenco 1983, Hartnoll & Hawkins 1985, Barker & Chapman 1990, Benedetti-Cecchi & Cinelli 1992) mais Chapman (1989) trouva que les brouteurs ont un effet très faible sur les jeunes individus de *Fucus spiralis*. Les crevasses dans les roches sont considérées comme des protections pour augmenter la survie des recrues (Lubchenco 1983) en fournissant un refuge contre le broutage (Hawkins 1981, Lubchenco 1983) mais l'hétérogénéité des surfaces peut aussi protéger les jeunes des stress de la chaleur et dessiccation (Jernakoff 1983, 1985, Johnson & Brawley 1998).

Considérant la dessiccation comme un facteur majeur de la mortalité des jeunes individus des algues, l'effet de la canopée, ou la protection offerte par les adultes aux jeunes individus, influence la survie des jeunes à un degré variable suivant les études (Menge 1976, Brawley & Johnson 1991, Johnson & Brawley 1998, Vadas et al. 1992). Les conditions environnementales optimales, physiques et biologiques, changent souvent si les juvéniles croissent sous les adultes. Les jeunes fucoïdes sont plus nombreuses et denses sur les balanes que sur les surfaces lisses des rochers (Choat 1977, Lubchenco 1980, Hawkins, 1981, Farrell 1991). Les adultes peuvent réduire le stress de dessiccation en fournissant une atmosphère humide pour les jeunes recrues des fucales. Jernakoff (1985) suggère que les rhizoïdes puissent augmenter la survie des algues en faisant décroître la dessiccation à certain moment de l'année. Les herbivores évitent d'aller sous la canopée à cause du délogement. La survie d'un embryon d'*A. nodosum* âgé d'une journée et celle de *F.*

vesiculosus ont été mesurées avec et sans canopée, la canopée des adultes réduit la survie d'un facteur 5 (Vadas et al. 1992). Mais l'effet négatif des canopées sur la survie des jeunes individus n'est pas vérifié dans toutes les études. L'étude menée par Brawley & Johnson (1991) mesure la survie des jeunes embryons de *Pelvetia fastigiata* âgés de 6 heures- une semaine avec et sans canopée et pour tous les âges, il n'y avait pas de survie des jeunes sans canopée. Les adultes protègent clairement les juvéniles des stress de dessiccation.

Toutefois malgré l'importance des jeunes stades, les études physiologiques portant sur la croissance ont reçu peu d'attention. Généralement l'évaluation de la performance photosynthétique de jeunes stades d'algues benthiques a lieu la plupart du temps en laboratoire car les stades sont microscopiques et donc impossible à suivre dans le milieu liquide et souvent si les études sont faites en milieu naturel, les méthodes employées requièrent de longues expériences sous des niveaux de lumière qui augmentent. L'établissement des courbes de saturation lumineuse de la photosynthèse (P vs E) basées sur la mesure du dégagement d'oxygène ou l'incorporation de substances radioactives comme l'isotope radioactif ^{14}C ont donné des informations utiles sur le métabolisme des producteurs primaires tels que la photosynthèse maximale (Pmax), la respiration et l'efficacité quantique, la saturation et l'inhibition. Pour obtenir toutes ces informations, les études doivent supporter des méthodes lourdes dont l'incubation dure une heure ou deux.

1.5 La mesure de la fluorescence

Les algues ont développé des mécanismes pour s'adapter aux changements des conditions lumineuses. L'énergie d'excitation des pigments photosynthétiques n'est pas seulement utilisée pour la photochimie mais aussi ré-émise en fluorescence ou thermiquement dissipée (Figure 1.2).

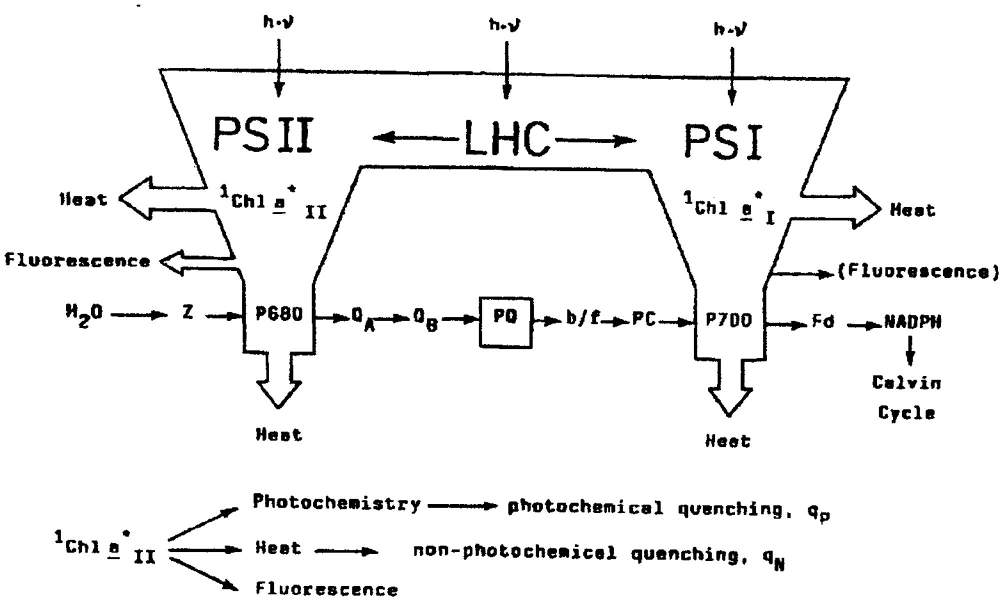


Figure 1.2 : Principe de la fluorescence de la chlorophylle (Walz 1993)

Légende

Chl *a* : chlorophylle *a*
PSII: photosystem II
LHC: Light-harvesting complex, collecteur d'électrons
PSI : photosystème I
Z : Résidu de Tyrosine
P680 : coeur de chlorophylle *a* du PSII
Qa : quinone a
Qb: quinone b
PQ : plastoquinone
b/f : cytochrome b/f
PC : plastocyanine
P700 : coeur de chlorophylle *a* du PSI
Fd : ferredoxine
NADPH : Nicotinamide adénine dinucléotide-phosphate hydrogènase

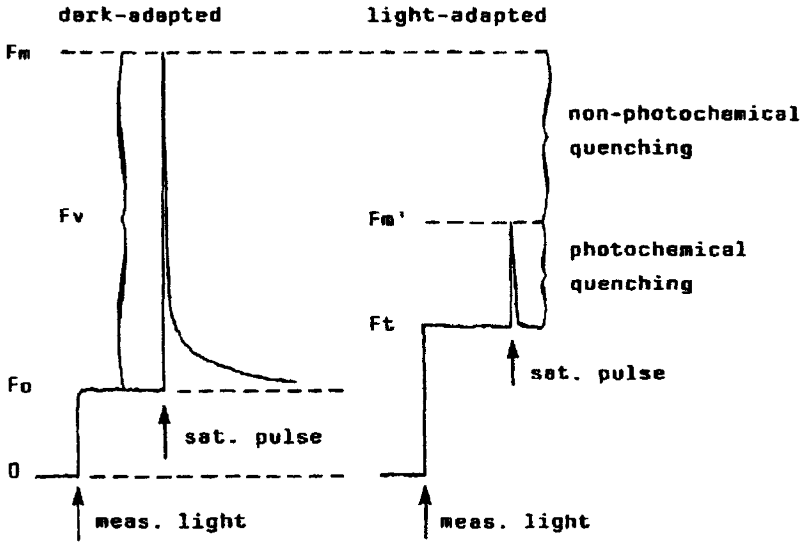
Cette dissipation d'énergie sous forme de chaleur aussi bien que la redistribution de l'énergie sont des mécanismes d'adaptation à court terme protégeant alors l'appareil photosynthétique des dommages causés par l'excès d'énergie lumineuse. Ces phénomènes de dissipation sont liés et complémentaires. Le changement d'ampleur de l'un de ces procédés influe sur les autres et provoque alors une variation changement des autres mécanismes de dissipation. A forte intensité, il y a une réduction du rendement de fluorescence engendrée par la photoinhibition pouvant être mise en évidence par des appareils capables de mesurer ces variations de fluorescence. Ainsi la mesure de la fluorescence peut être utilisée pour détecter les stress dans les algues, l'effet indirect ou direct sur la photosynthèse défini comme une conséquence des conditions de stress que subissent chacune du végétal.

De nos jours, il existe une méthode basée sur la mesure de la fluorescence chlorophyllienne pour évaluer l'activité photosynthétique directement *in situ* et immédiatement (Figure 1.3) (Walker 1987).

Les photosystèmes II existent sous quatre formes, centres ouverts (prêt à capturer l'énergie d'un photon) ou fermés (ayant capté l'énergie d'un photon et prêt à céder un électron). L'analyse des variations du rendement de fluorescence permet de caractériser ces quatres formes. En effet, placés à l'obscurité pendant une période de durée variable suivant le type de végétal, tous les centres photochimiques ont leurs molécules-pièges ouvertes, prêtes à capter l'énergie des photons. Sous l'action d'une lumière d'excitation de faible intensité, la plus grande partie des centres photochimiques resteront ouverts, le rendement de fluorescence restera faible et on peut mesurer le niveau Fo. En réponse à un flash lumineux d'énergie (qui fait fonctionner la photosynthèse) saturante, très court qui provoque une fermeture progressive des centres photochimiques sur l'énergie d'un photon capturé, le rendement de fluorescence augmentera et se maintiendra au niveau maximum Fm. Il est ainsi possible de caractériser le pourcentage d'ouverture des centres par la mesure de fluorescence. La mesure de la fluorescence sera déterminée par l'efficacité quantique maximum qui est calculée par l'équation (Fm-Fo)/Fm. Soit Ft le rendement de fluorescence à l'instant t, on définit le quenching photochimique qP : qP=(Fm-Ft)/(Fm-Fo). La valeur de qP permet d'estimer le pourcentage de centres ouverts.

Soumis à un éclairement continu, les photosystèmes II évoluent de façon à optimiser leur fonctionnement. Les photosystèmes II voient une partie de leurs antennes se déconnecter de l'ensemble antennaire. La quantité d'énergie collectée diminue et cause une diminution de la quantité d'électrons transférés et de la quantité de photons réémis par la fluorescence. L'intensité de l'émission de fluorescence diminuera passant ainsi de Fo à Fo' avec Fo'<Fo. Si par contre le centre photochimique est à l'état fermé, l'intensité de l'émission de fluorescence diminuera aussi mais en passant cette fois de Fm à Fm' avec Fm'<Fm. Le rendement de fluorescence de la plante adaptée à la lumière sera donc calculée par l'efficacité effective de fluorescence (ΦPSII) et donnée par l'équation suivante, Fm'-Fo'/Fm'. Ici, on définit le quenching non photochimique dû principalement aux modifications des antennes. L'amplitude du rapport est qN= (Fm-Fm')/(Fm-Fo). Ainsi pour estimer les quenchings photochimiques et non photochimiques, il faut donc estimer Ft et de plus connaître Fo et Fm, minimum et maximum mesurés au repos (à l'obscurité) ainsi que les valeurs Fo' et Fm', minimum et maximum instantanés mesurés lorsque la plante est adaptée à la lumière.

De plus, les paramètres de fluorescence permettent le calcul du transfert d'électrons exprimés par l'équation, rETR (electron transport rate) = Φ x Energie lumineuse.



Coéfficients pertinents à mesurer

Fv/Fm = Fm-Fo/Fm

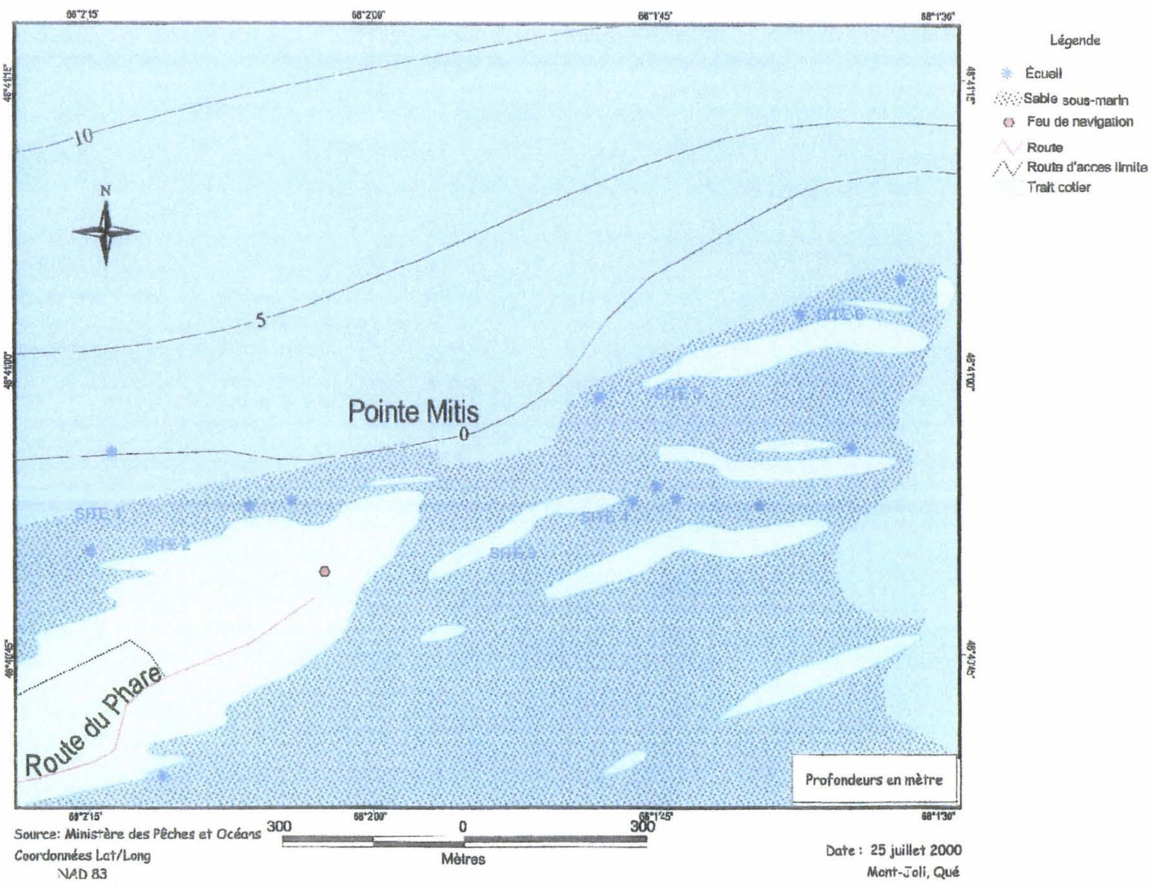
Φ_{PSII} = Fm'-Fo'/F'm
NPQ = Fm-Fm'/Fm'
rETR = Φ_{PSII} x E

Figure 1.3 : Illustration du pulse amplitude (PAM) – méthode (Walz 1993)

L'utilisation de la fluorescence permet de vérifier avec succès le fonctionnement de l'appareil photosynthétique, elle montre ses propriétés dynamiques spécialement sur le potentiel de changement dans la composition et la fonction (acclimatation, photoadaptation) et la capacité de supporter la photoinhibition, dépendante des facteurs environnementaux quand des changements brusques excèdent le taux d'acclimatation (Bradbury & Baker 1984, Schreiber et al. 1986, Schreiber & Bilger 1987). Cette méthode a déjà été employée avec succès et montre que les algues peuvent subir les effets des stress environnementaux (macro-algues rouges, des algues unicellulaires et des algues brunes, (Leverenz et al. 1990, Henley et al. 1991, Hanelt 1992, Häder et al. 1996). Durant les dix dernières années, les mesures de fluorescence ont été utilisées avec succès mais ces études portent essentiellement sur des algues adultes subtidales. Malgré certaines controverses sur la validité des informations déduites des mesures de la fluorescence chlorophyllienne, son utilisation est simple, non-destructrice et une des plus pratiques parmi les procédés actuels de mesure de la photosynthèse.

1.6 Objectifs et approche méthodologique

L'objet de cette étude est d'examiner les aspects écophysiologiques de jeunes individus d'algues brunes ; ***Ascophyllum nodosum*** (L.) Le Jolis, ***Fucus vesiculosus*** L. et ***Fucus distichus*** ssp. ***edentatus*** dans l'estuaire du Saint-Laurent (Figure 1.4), et une étude physiologique concernant les embryons de ***Fucus vesiculosus*** qui colonise les côtes du Nord de la France.



Source: Ministère des Pêches et Océans
Coordonnées Lat/Long
NAD 83

Date : 25 juillet 2000
Mont-Joli, Qué

Figure 1.4 : Pointe Mitis, Québec, Canada (48°41'N, 68°2'O). Les sites de travail sont indiqués sur la carte du numéro 1 au site 5. Le site 6 n'est pas mentionné dans le texte.

Le deuxième chapitre consiste à mesurer grâce à l'utilisation de substrats artificiels (Figure 1.5) le recrutement des jeunes embryons afin d'avoir une bonne connaissance de la dynamique des populations des algues brunes présentes dans notre site de travail.

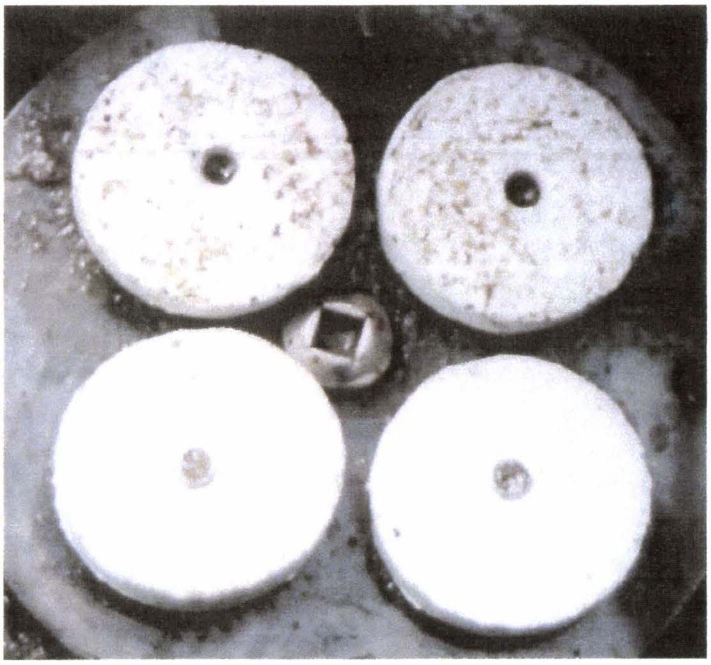


Figure 1.5: Photographie de quatre substrats artificiels constitués de quatre disques (4 mm d'épaisseur; 20 mm de diamètre) composé d'époxy-filled putty fabriqué de Sea Goin' Poxy Putty™ ,Permalite Industries, Newport Beach, CA, USA.

Celui-ci est pris par les glaces, les organsimes sont recouverts de neige durant les mois d'hiver rigoureux et lorsque la fonte des glaces survient, elle laisse place à de grandes étendues vierges propices à la colonisation par les algues (Figure 1.6-Figure 1.7). Pendant trois ans, lors des mois de recrutement des espèces, j'ai mesuré selon différentes fréquences le taux de recrutement des propagules dans différents sites et sous différents microhabitats; sous la canopée, sur des surfaces exposées et dans les cuvettes. De plus, des dénombrements de plantes matures et de conceptacles (organes qui contiennent les gamètes) ont été faits afin de déterminer une éventuelle action sur le taux de recrutement ; des données météorologiques fournies par l'aéroport de Mont-Joli (15 km de notre site de travail) ont été intégrées afin d'estimer s'il existe une relation avec le recrutement. Enfin, par fécondation artificielle, j'ai mesuré des pourcentages de mortalité des embryons de *Fucus* sous trois types de conditions, sous la canopée, dans des conditions exposées et dans des cuvettes.

Figure 1.6 : Pointe Mitis, Québec, Canada (48°41'N, 68°2'O) sous la glace

Figure 1.7 : Pointe Mitis, Québec, Canada (48°41'N, 68°2'O) au printemps, après la fonte des glaces laissant de nombreuses surfaces vierges propices à une nouvelle colonisation par les algues.

Le troisième chapitre est une étude de la mise en place de l'appareil photosynthétique lors du développement des embryons de *Fucus* sous des conditions naturelles (Figure 1.8). Cette étude est réalisée en laboratoire par des expériences d'émission et d'excitation de fluorescence. Ces expériences sont accompagnées d'un suivi du contenu pigmentaire dans les embryons de *Fucus* au cours de la première semaine de croissance.

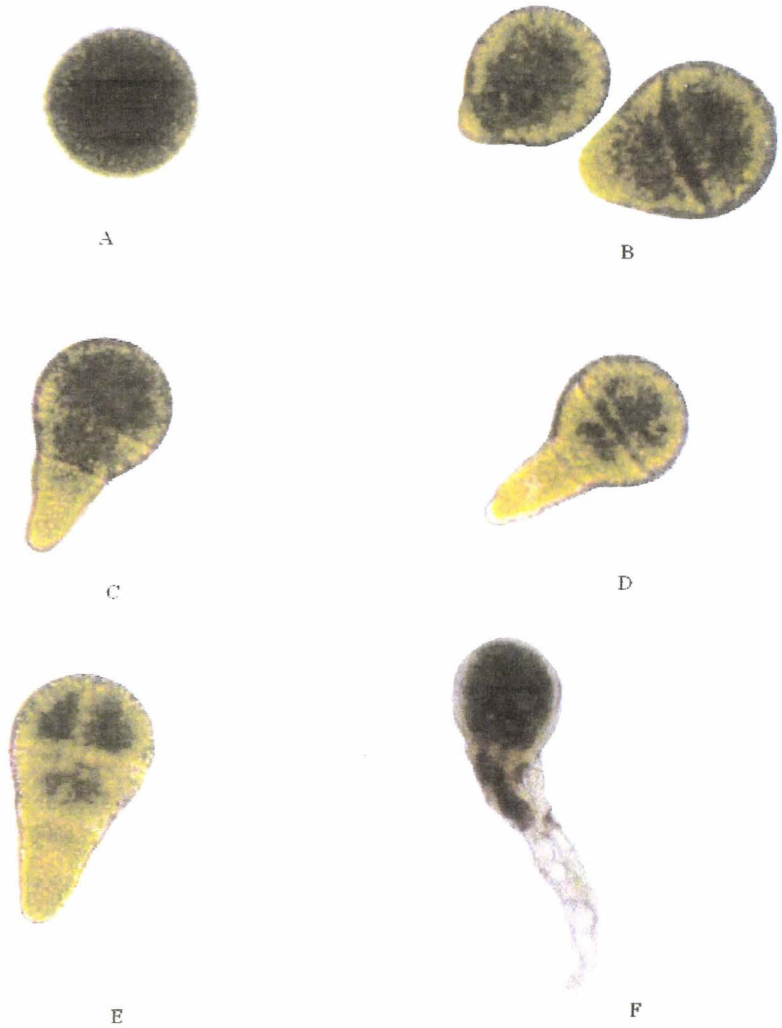


Figure 1.8: Profils de Division des embryons de *Fucus* ssp ; A, Fécondation, B, 24 heures, C, 48 heures, D, 72 heures, E, 96 heures, F, 1 semaine après la fécondation (G x 10). Germination sous 10°C.

Les chapitres 4 et 5 sont des études de la photosynthèse de jeunes stades de fucales directement sur le terrain en utilisant les mesures de fluorescence selon quatre conditions météorologiques déterminées par l'expérimentateur. Ces conditions météorologiques ont été définies par les données recueillies à l'aéroport : ensoleillées et très venteuses, nuageuses, très venteuses et plutôt nuageuses, et ensoleillées.

Le chapitre 4 est basée sur l'utilisation des mesures de fluorescence ; trois espèces dominantes de Fucales existent sur notre site de travail qui sont implantées suivant un gradient horizontal ; après avoir obtenu par fécondation artificielle, les jeunes embryons de ces trois espèces ont été posés dans leur niveau propre. J'ai suivi leur profil d'efficacité photosynthétique durant différentes marées basses et leur profil de redémarrage de la photosynthèse par la fluorométrie. De plus, j'ai déterminé deux microhabitats qui dominent sur les côtes rocheuses ; sous la canopée avec la protection offerte par des adultes et sur des surfaces exposées. Les mesures de fluorescence ont été réalisées sous ces deux types de microhabitats afin de les comparer et d'identifier le meilleur endroit où s'installer et croître. Cette étude différente de la première est une étude écophysiologique horizontale basée sur de plus grandes distances.

Le chapitre 5 concerne des jeunes embryons qui ont colonisé un mur rocheux vertical (Figure 1.9). Les mesures de fluorescence en lumière modulée donnent une assez bonne estimation de la photosynthèse de ces jeunes embryons suivant différents niveaux (hauteurs) choisis selon une micro échelle. En parallèle, des mesures de déshydratation et de lumière ont été réalisées afin d'estimer l'action possible de la dessiccation et de la lumière sur organismes juvéniles. Finalement, des données de densités de peuplement et de croissance mensuelles ont été prises lors des mesures de fluorescence suivant les cinq niveaux choisis sur le mur. Cette partie termine le travail expérimental.

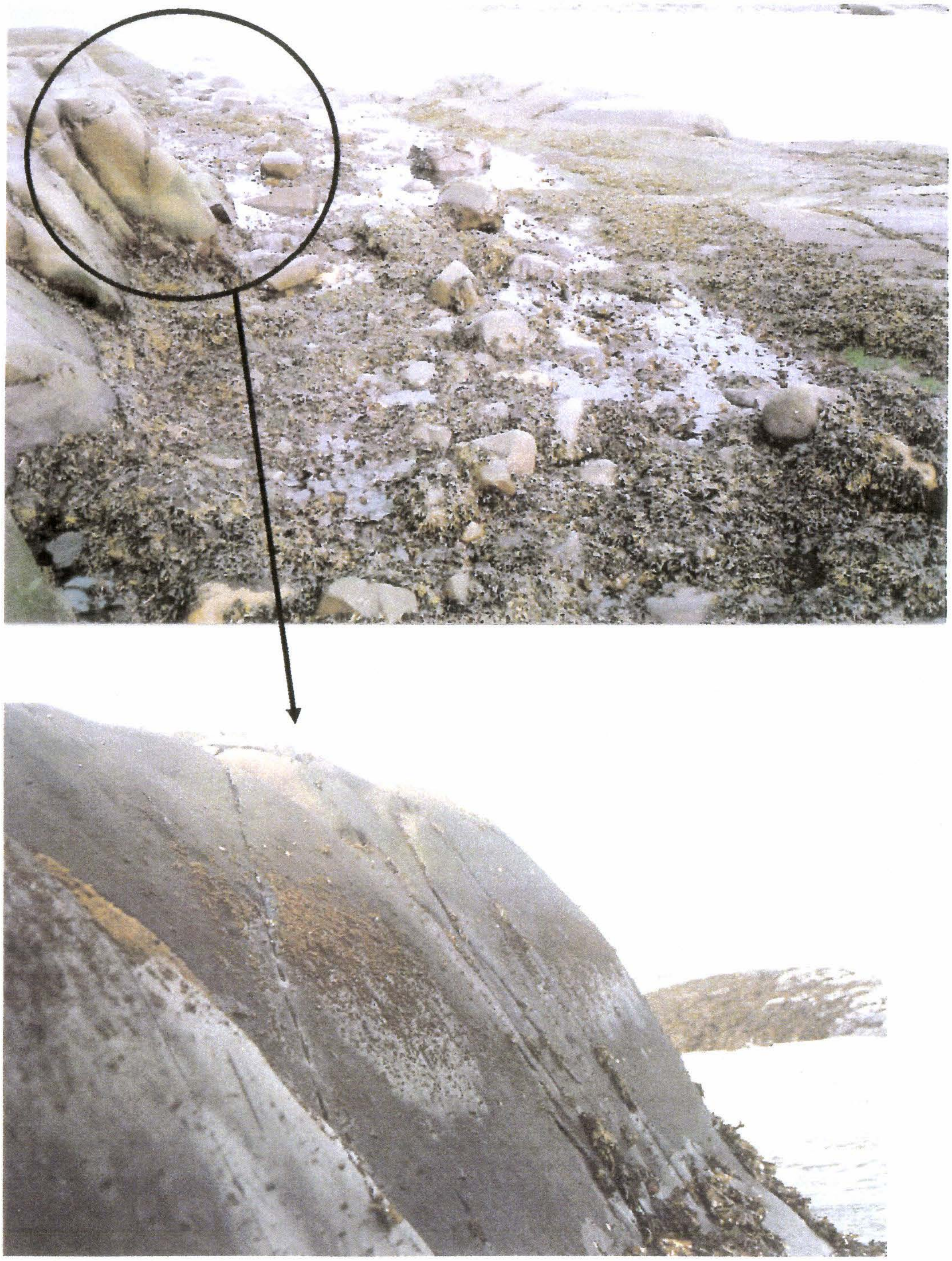


Figure 1.9: Le Mur (chapitre 5)

Ce travail s'inscrit dans le cadre d'une co-tutelle qui conjugue une étude en laboratoire et une étude dans le milieu du développement d'embryons de fucales. La co-tutelle associe le laboratoire de cytophysiologie et phycologie de l'université de Lille I sous la direction du Professeur Yves Lemoine et le laboratoire en écologie benthique de l'université Laval sous la direction du Professeur Ladd Johnson. Le laboratoire de Lille est un laboratoire dans lequel on étudie surtout la photosynthèse ; les méthodes utilisées étaient les mesures de fluorescence chlorophyllienne et de dégagement d'oxygène et l'analyse des pigments par chromatographie. Le laboratoire de l'université Laval est un laboratoire concerné par l'écologie du littoral et surtout par l'action des facteurs environnementaux sur les organismes qui vivent dans cette zone. De plus, le travail sur des jeunes stades est plus valorisant car après tout, ces jeunes sont l'avenir de la population. L'étude écologique combinée à une étude physiologique essaie de cerner dans sa globalité le problème du développement d'un jeune embryon dans le milieu. Ainsi le sujet de cette thèse entre parfaitement dans l'optique suivie par ces deux laboratoires ; l'alliance d'une étude physiologique approfondie du développement de l'embryon avec une étude plus réaliste de l'influence des facteurs environnementaux sur celui-ci.

CHAPITRE 2

Temporal and spatial variation in the settlement and recruitment of fucoid algae: the role of microhabitats and temporal scales

2.1 Résumé

L'installation et le recrutement d'un assemblage de multiples espèces de Fucales (*Ascophyllum nodosum* (L.) Le Jolis, *Fucus vesiculosus* L. et *Fucus distichus* ssp. *edentatus*) sur des substrats artificiels dans différents microhabitats et sur des périodes d'échelles de temps différentes ont été examinés sur des côtes rocheuses semi-exposées à Québec (Canada). Les mesures ont été effectuées à des fréquences journalières, bimensuelles et tous les 28 jours dans trois microhabitats (surfaces rocheuses exposées, sous la canopée des adultes de Fucales et dans les cuvettes) de juillet à septembre durant trois années consécutives (1998-2000). La colonisation était spatialement et temporellement variable suivant les mois et les années avec moins d'installation en 1998. Les taux d'installation étaient constamment plus faibles en septembre. La densité d'adultes reproducteurs n'affectait pas le taux d'installation, mais celui-ci était négativement corrélé avec la vitesse du vent, un indicateur de mouvement des vagues. Suivant les microhabitats, le taux d'installation était 10 à 50 fois plus fort sous la canopée qu'en surfaces exposées ou dans les cuvettes mais la mortalité des embryons transplantés démontrait que les taux de mortalité étaient similaires pour les microhabitats. Ces résultats suggèrent que la dispersion des popragules est limitée. Il n'y avait pas de différences dans le nombre de recrues mesuré dans l'échelle de temps entre 1 et 30 jours suggérant que le taux de mortalité post-installation était extrêmement important, spécialement dans les microhabitats exposés.

2.2 Abstract

The settlement and recruitment of a multi-species assemblage of fucoid algae (*Ascophyllum nodosum* (L.) Le Jolis, *Fucus vesiculosus* L. and *Fucus distichus* ssp. *edentatus*) onto artificial substrata in different microhabitats and over different temporal scales was examined on a semi-exposed rocky shore in Québec (Canada). Measurements were taken at daily, fortnightly and monthly frequencies in three microhabitats (exposed rock surfaces, under a canopy of adult fucoids, and in tide pools) from July through September in three consecutive years (1998-2000). Settlement was spatially and temporally variable among months and years with much less settlement in 1998. Settlement was consistently lowest in September. The density of reproductive adults did not generally affect the rate of settlement, but the rate of settlement was negatively correlated with wind speed, a possible indicator of wave action. Among microhabitats, the settlement was over 10 to 50 times greater under the canopy relative to exposed surfaces and tide pools, but mortality of outplanted embryos demonstrated that mortality rates were similar among the microhabitats. These results suggest that the dispersal of propagules is limited. There were no differences in the numbers of recruits measured over time scales ranging from 1 to 30 days suggesting that post-settlement mortality rates are extremely high.

2.3 Introduction

Demography is the study of populations and to understand populations, one must not just study the state of the population (i.e., abundance) but must also consider the dynamics (i.e., birth and death rates). This latter depends critically on the rate at which new individuals are added to the population, i.e., the process of recruitment. In benthic marine algae, this is typically accomplished by the dispersal and settlement of propagules (i.e., spores or zygotes) and their subsequent survival. Settlement is the initial post-sinking attachment of propagules to the substratum after their release from adult plants or after the fertilization of gametes released by adults.

The dynamics of recruitment will depend on the temporal and spatial variation in the availability of propagules (Roughgarden 1975), but the small size of most algal propagules makes it difficult to assess this variation. Often, the entire process has been inferred from the distribution of juvenile stages (Menge 1976, Reed et al. 1988, Bingham 1992, Gaines & Bertness 1992, Miron et al. 1995, Wildish & Kristmanson 1997, Archambault & Bourget 1999, Ellien et al. 2000, Olivier et al. 2000), which can lead to incorrect interpretation of the true patterns of settlement (Keough & Downes 1982). It is now apparent that the time scale at which observations are made can affect the interpretation of different ecological processes. While this concern has been raised in studies of invertebrates and fish (Choat et al. 1988, Wilson & Osenberg 2002), very few studies of marine algae have closely examined the early events surrounding settlement and early survival (e.g., Bird and Mc Lachlan 1973, Edelstein & McLachlan 1975, Gunnill 1980, Reed & Foster 1984, Pearson & Brawley 1996, Johnson & Brawley 1998; reviewed by Vadas et al. 1992). During the period from the initial settlement of propagules to the first appearance in the population, these algae are likely to experience substantial mortality (Brawley & Johnson 1991), that varies greatly in time and space (Ang 1991). Given that the survival and development of the early post-settlement stages is required for the eventual establishment of adults, it is critical to understand this process if we are to understand the dynamics of adult populations (Vadas et al. 1992).

Recruitment can function in two very different modes, the first being the continual replacement of individuals lost from the population and the second being the massive reestablishment of disturbed areas. The dynamics of these two situations are likely to vary considerably for two reasons: (1) the availability of propagules may differ as dispersal in other brown algae has been shown to be very limited (Johnson & Brawley 1998); (2) the microenvironment into which the propagules settle is vastly different – moist but with limited light under the adult canopy and dry and bright on recently scoured surfaces. Survival among such microhabitats can vary from 0 to 100% over spatial scales as small as 10 cm (Brawley & Johnson 1991) with those under adult canopies being the most benign, at least in the short term.

The rocky intertidal communities of Gaspé (Québec) are severely affected by ice scour (Archambault & Bourget 1983), and sessile invertebrates and perennial algae are generally restricted to low-lying areas between topographical high points. However, interannual variation in this disturbance regime can also create patches within established beds of fucoids. This situation thus provides an excellent opportunity to study these two poles of recruitment in a perennial intertidal algal community. In this study we examined the temporal and spatial dynamics of settlement and early recruitment of a complex of fucoid algae over a 3-yr period. By examining this process at different time scales and within different microhabitats, we determined that there is substantial variation in settlement rates at all temporal and spatial scales and that extremely high rates of mortality prevent a measurable accumulation of recruits at temporal scales of less than a month.

2.4 Methods

2.4.1 Study site

The study was conducted in the intertidal zone at Pointe-Mitis near Mont Joli, Québec, Canada (48°41'N, 68°2'W), a semi-sheltered shore (Bourget et al. 1985) consisting of a series of rocky ridges interspersed with consolidated boulder fields. These boulder fields and the lower portions of adjacent ridges were dominated by three species of fucoid algae (*Ascophyllum nodosum* (L.) Le Jolis, *Fucus vesiculosus* L., and *Fucus distichus* (L.) ssp.

edentatus; Figure 2.1) that formed dense canopies covering up to 80% of the substratum. These perennial algae are not able to colonize the more exposed rocky surfaces (e.g., the top portions of the ridges) due to ice scour and only occur in areas protected from abrasion by ice (Archambault & Bourget 1983). Tides are semi-diurnal at this site with a tidal range of 4.0 m.

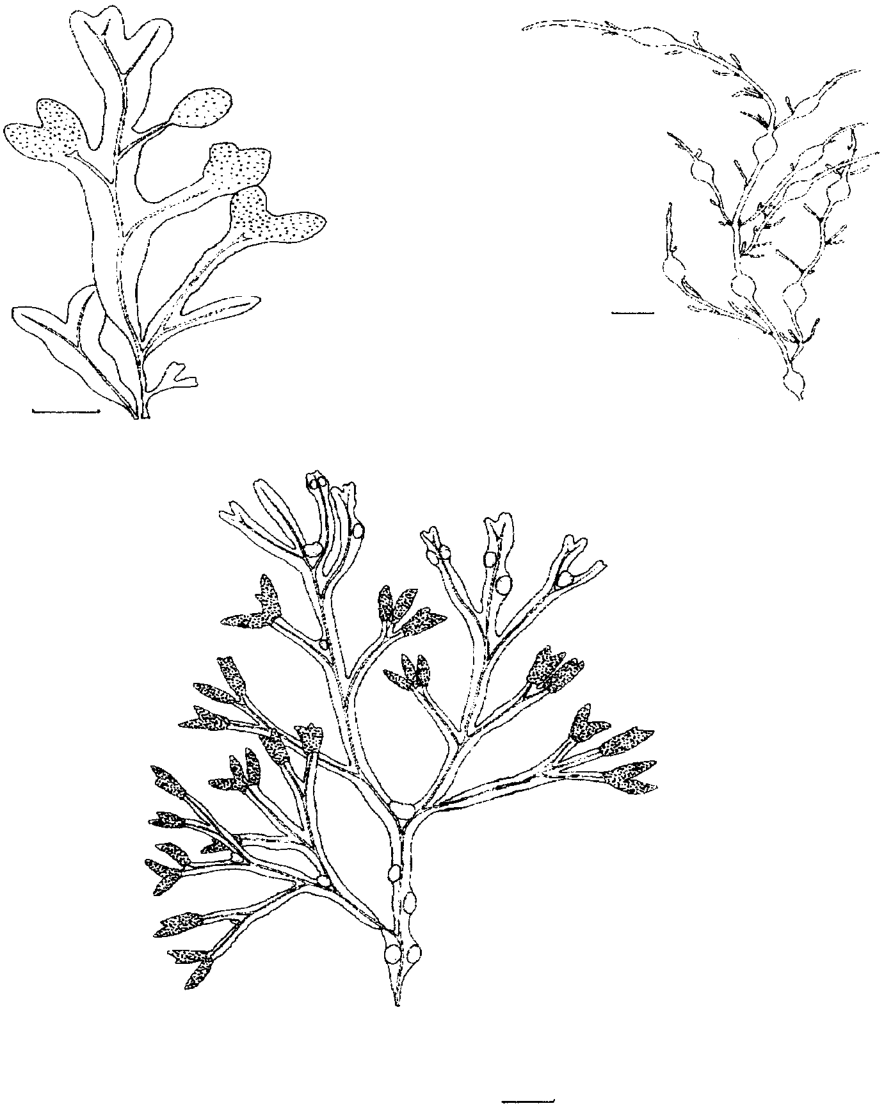
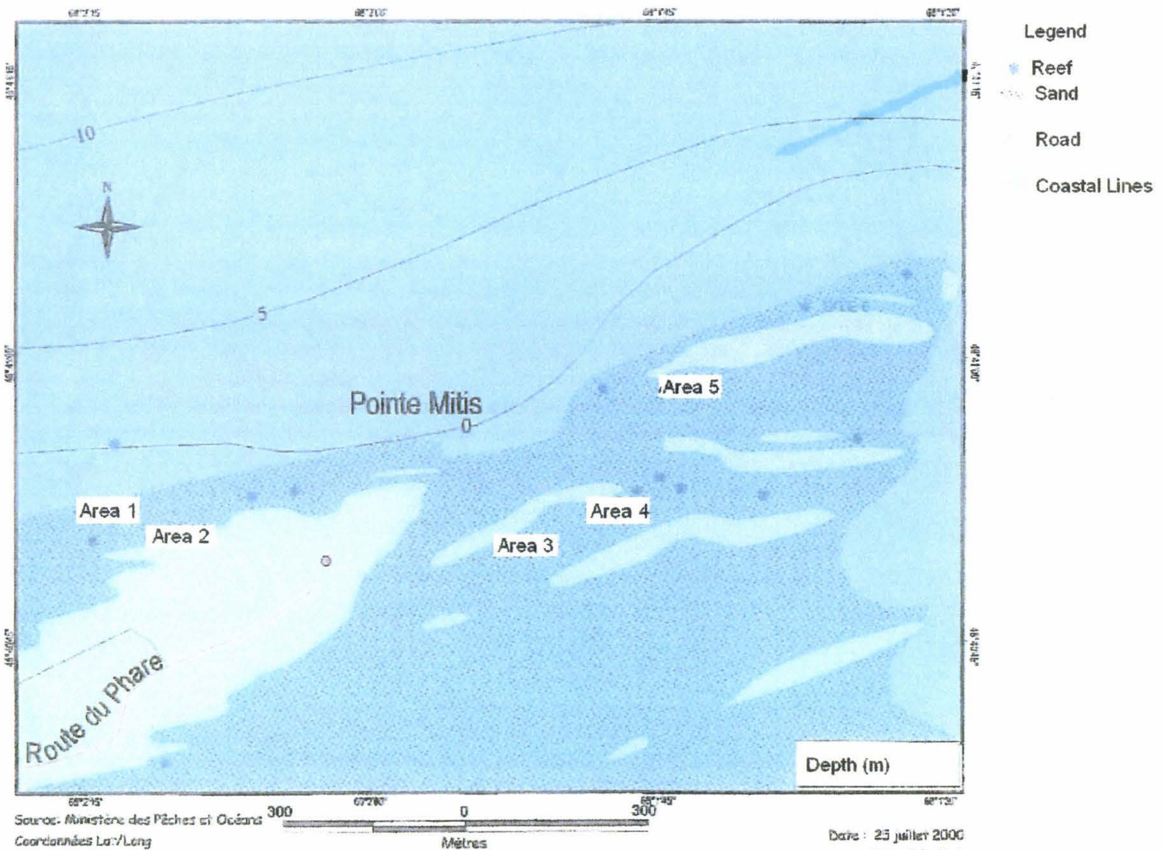


Figure 2.1: *Fucus distichus* ssp., *Ascophyllum nodosum* (L.) Le Jolis and *Fucus vesiculosus* L. (1 x 20 cm).

2.4.2 Selection of study areas

Five study areas were haphazardly selected along a 150 m stretch of the mid-intertidal zone (Figure 2.2). Areas were separated by at least 20 m and were naturally isolated by the rock ridges that dominate this shoreline. Fucoid cover at the time of selection ranged from 25 to 80 % cover. Although individuals of all three species were usually present, most areas were dominated by a single species at any given time.



Source: Ministère des Pêches et Océans
Coordonnées Lat/Long
NAD 83

Date : 25 juillet 2000
Mont-Joli, Qué

Figure 2.2 : Study Site, Pointe Mitis, Québec, Canada (48°41'N, 68°2'W) indicating the location of the Five areas.

Within each area the settlement and early recruitment of fucoid propagules was examined in three distinct microhabitats: emergent rock surfaces covered by a canopy of adult fucoids, emergent rock surfaces not covered by a canopy (representing scoured areas), and submergent rock surfaces without a canopy, henceforth "canopy", "exposed", and "tide pool" microhabitats, respectively. This latter microhabitat was selected to control for effects of desiccation that were expected to be higher outside the adult canopy (Brawley & Johnson 1993). Eight locations were haphazardly selected to represent the canopy microhabitat and two each for the other two microhabitats.

2.4.3 Artificial substrata

Recruitment was estimated by the use of artificial substrata placed in each location. These substrata were identical to those used in earlier studies (Brawley and Johnson 1991, Johnson 1994, Johnson & Brawley 1998) and consisted of small, white disks (4 mm thick, 20 mm in diameter) fabricated by a molding and casting process from Sea Goin' Poxy Putty $^{\mathrm{TM}}$, a filled epoxy putty (Permalite Industries, Newport Beach, CA, USA), by which the top surface was given the rough texture of sandpaper (Johnson 1994). Holes were drilled and tapped so that they could be attached with small stainless steel machine screws to small, polycarbonate base plates (3-mm thick, 85-mm diameter, chapter 1, Figure 1.5). These plates were then attached to the substratum using stainless steel screws driven into plastic wall anchors placed in holes drilled into the rock surface at each location (Johnson 1994).

2.4.4 Temporal scales

Measurements were made over three distinct time scales: daily, fortnightly, and monthly. For daily measurements, two disks were set out at each location at the beginning of the ebbing tide period and recovered at the same time the following day. Because of number of locations and the distance between areas, the collection and replacement of disks required a period of 4 h and thus could not be done at a quasi-simultaneous moment as in earlier studies (Johnson & Brawley 1998, Pearson & Brawley 1996). Areas were visited in the same order, but individual locations within a given area were done as they were found. After collection, disks were transported to the laboratory where all attached fucoid zygotes were counted within hours using a stereomicroscope. This protocol was repeated for 5 consecutive days during each spring tide series (i.e., twice each lunar month) for the three-month period in which fucoid algae are reproductive at this site (July to September). Observations could not be done for additional days because the period of low tide was only long enough during spring tides for the time required to visit all areas. For fortnightly and monthly measurements, a similar procedure was followed but with disks recovered and replaced after 14-d and 28-d periods, respectively. All the disks resided on the same base plate at a given location (i.e., 6 disks per base plate). Disks were reused in all cases, but scrubbed with a toothbrush in tap water to remove any embryos before reuse. Although in the purest sense settlement is an instantaneous process and cannot be measured as the accumulation of settlers over any period of time, we refer to the daily accumulation as "settlement" to differentiate it clearly from the longer time scales that we examined which we refer to as "recruitment", either fortnightly or monthly.

2.4.5 Survival of embryos

To estimate mortality rates, we outplanted fucoid embryos of the three species on putty disks during 1999 and 2000. Embryos were obtained by osmotically shocking receptacles (reproduction organs in life cycle, chapter 1, Figure 1.1) collected from the field. Care was taken to avoid algae with obvious epiphytes and to store the receptacles at 10 C° in dark conditions. Before use, the receptacles were scrubbed with a toothbrush and rinsed with seawater to remove epiphytes (e.g., diatoms) from the receptacle surface. The receptacles were then osmotically shocked to induce gamete release by air drying for 20 min followed by 5 min in cold tap water. Receptacles were then placed into filtered (5 μm) seawater. After the release of gametes, but before attachment began (which typically occurs 4-5 hr after fertilization [Kropf 1989]), the "solution" of seawater and zygotes was gently poured over putty disks and then left overnight in the dark at 4 C° to allow attachment. The number of attached zygotes was determined by direct counts the following morning, and then the developing embryos (now 16-20 hr-old) were outplanted into field at the beginning of the low tide period. Disks were attached to base plates located in three different microhabitats: exposed rock, under a canopy of adults, and in a tide pool with no algal canopy. Six disks were attached to two replicate base plates in each microhabitat in all five study areas (i.e., 180 disks for each experiment). Disks were recovered the following day at the same tidal time, and the number of embryos again counted (the attachment of zygotes was strong enough to avoid displacement by waves). This experiment was repeated every two weeks during the reproductive season of *F. vesiculosus* as its reproductive season was the longest.

2.4.6 Abundance of mature adults

The abundance of reproductive fucoid algae was determined twice monthly during the periods when recruitment measurements were taken. The density of mature adults was estimated using three 625-cm^2 quadrats haphazardly located within each study area. The number of receptacles was also determined for each mature adult alga found within the quadrat.

2.4.7 Environmental parameters

Hourly wind speed and air temperature were obtained from the Mont Joli airport, located 15 km from the field site, and thus represented an approximation of the weather at the field site.

2.4.8 Analysis

A mixed ANOVA model (SAS-Institute, 2000) was used to analyse log-transformed data on the rate of settlement/recruitment at different frequencies (daily, fortnightly, monthly), months (July, August, and September) and years (1998, 1999, and 2000) in different areas (1-5). Temperature, wind speed, density of mature plants, and density of receptacles were used like covariable to assess the effect on recruitment. Linear regressions were performed using Statistica (Statistica 1999). The contrasts were performed using Scheffe Tests.

2.5 Results

2.5.1 Rate of settlement in different areas

The daily settlement of fucoid propagules varied significantly with respect to years, months, and areas (Tables 2.1-2.2; Figure 2.3).

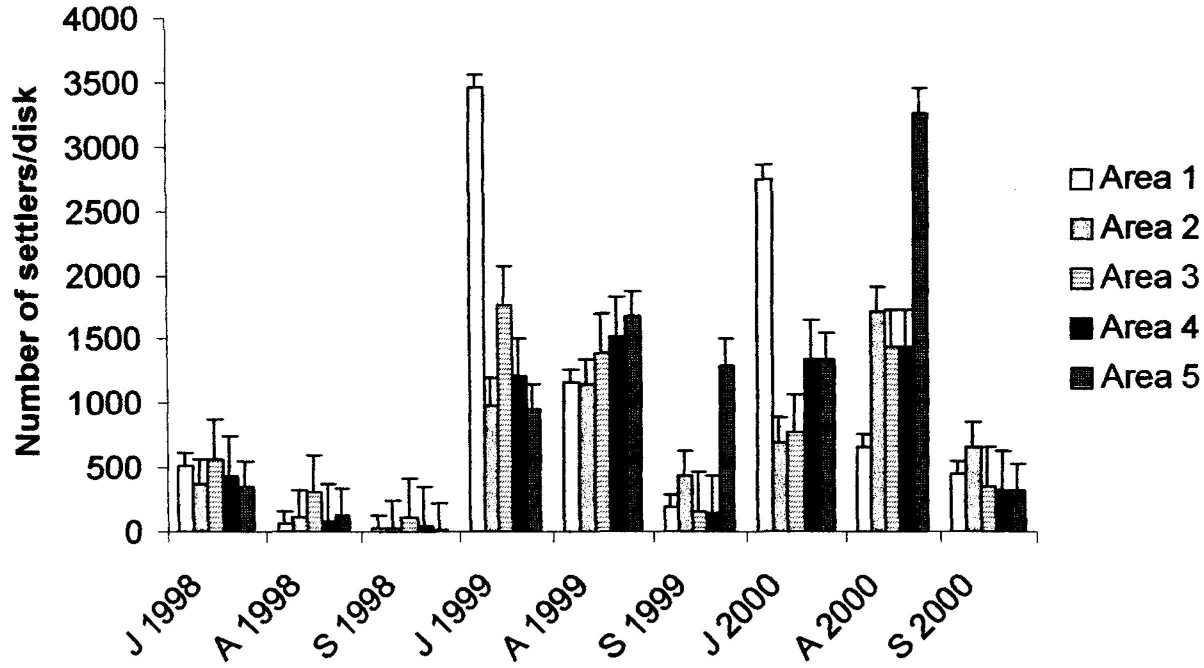


Figure 2.3: The daily rate of recruitment of fucoid propagules onto epoxy disks under an adult canopy in different areas of the shore at Pointe-Mitis in July (J), August (A) and September (S) over a 3-y period. Bars represent the averages of all days sampled within a given month (n=160) with 95% confidence intervals. Each daily value was the mean of 8 disks.

Settlement was much greater in 1999 and 2000 relative to 1998, and among the three months was consistently lowest in the month of September. In 1998, settlement was highest in July (Table 2.2), but in the subsequent years of higher settlement, July and August levels were similar overall. Within a given month, settlement was generally similar among the different areas, except for extremely high values (2500 to 3500 zygotes/disk) for single areas during some months (i.e., Area 1 in July of both 1999 and 2000 and Area 5 in August of 2000).

Patterns of fortnightly and monthly recruitment of fucoid propagules generally paralleled those of daily settlement, but recruitment was higher in 1998 (especially in July) and lower in 2000 (especially in August and September relative to settlement patterns (Figures 2.4 and 2.5).

Table 2.1 : ANOVA (Proc Mixed) showing the variance of settlement following Areas, Month, Year, and Frequency.

Source of Variation	df	F Value	p
Area	4	44.39	<0.001*
Year	2	6.82	0.0037*
Year x Area	8	16.36	<0.001*
Month	2	54.56	<0.001*
Month x Area	8	0.53	0.8266
Year x Month	4	5.41	0.0022*
Year x Month x Area	16	0.66	0.8075
Frequency	2	0.1163	0.5523
Area x Frequency	8	2.58	0.029*
Year x Frequency	4	1.08	0.3845
Year x Area x Frequency	16	1.92	0.0611
Month x Frequency	4	1.16	0.3476
Month x Area x Frequency	16	1.32	0.2511
Year x Month x Frequency	8	1.07	0.4122

Table 2.2: Conditional Effect of Month for each year and conditional Effect of Year for each Month.

Source of Variation	Month	Year	df	F Value	p
Year x Month		1998	2	10.28	<0.001*
Year x Month		1999	2	14.37	<0.001*
Year x Month		2000	2	13.99	<0.001*
Year x Month	July		2	6.22	0.0028*
Year x Month	August		2	2.16	0.12
Year x Month	September		2	0.59	0.55

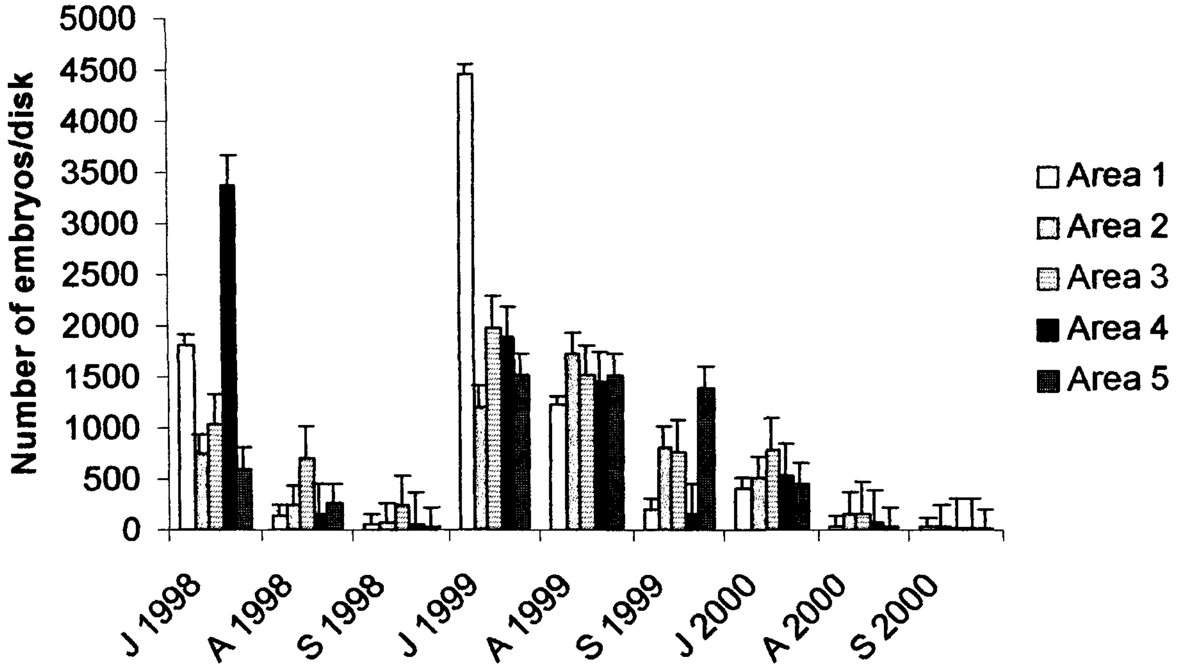


Figure 2.4: The fortnightly rate of recruitment of fucoid propagules onto epoxy disks under an adult canopy in different areas of the shore at Pointe-Mitis in July (J), August (A) and September (S) over a 3-y period. Bars represent the averages of all days sampled within a given month (n=32) with 95% confidence intervals.

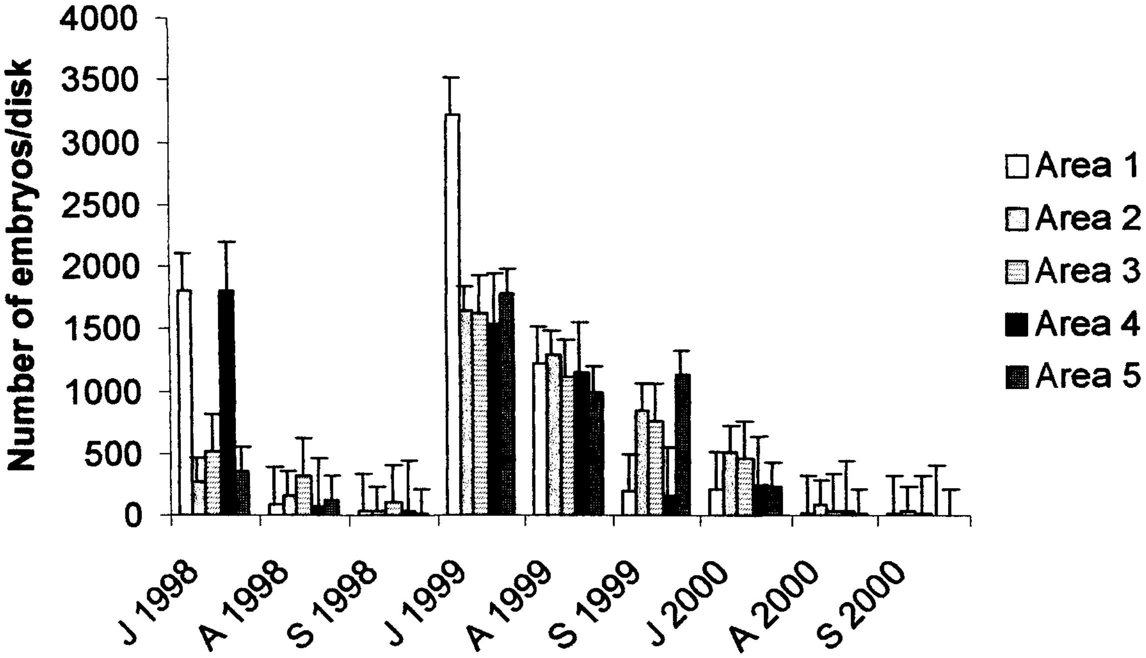


Figure 2.5: The monthly rate of settlement of fucoid propagules onto epoxy disks under an adult canopy in different areas of the shore at Pointe-Mitis in July (J), August (A) and September (S) over a 3-y period. Bars represent the averages of all days sampled within a given month (n=16) with 95% confidence intervals.

The number of recruits that accumulated over the entire month period was generally equal to or less than the daily average settlement (Figure 2.6) except in August and September 2000 when daily settlement far exceeded monthly recruitment.

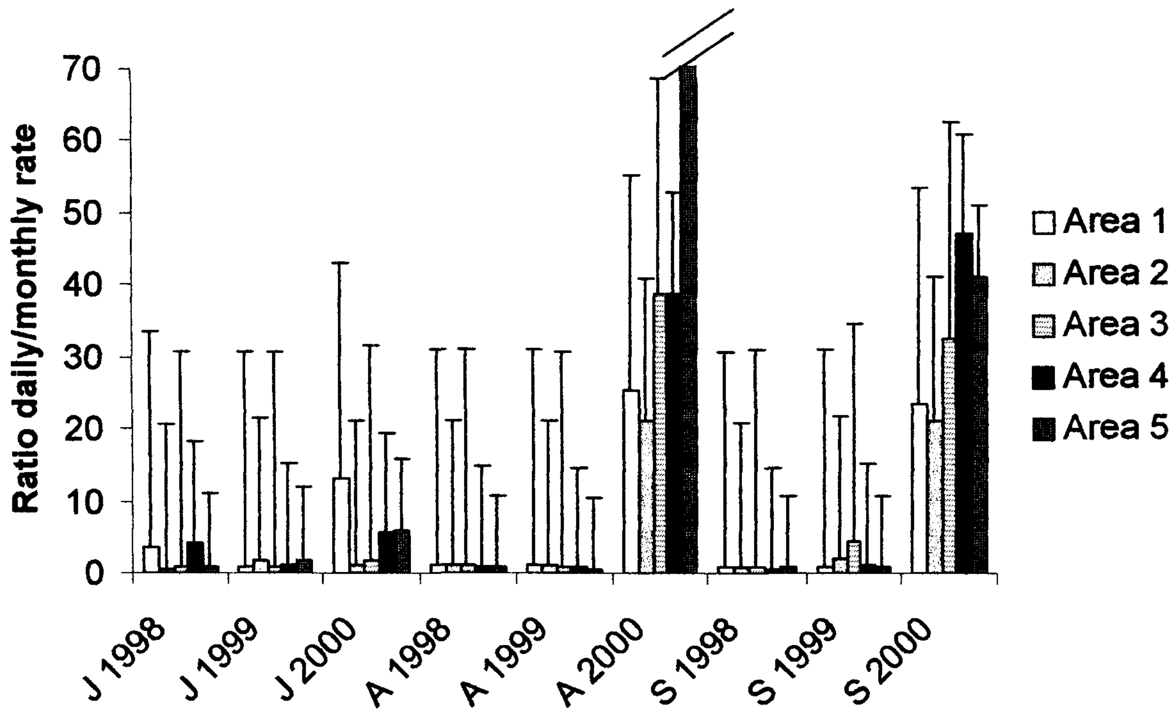


Figure 2.6: The ratio daily rate/monthly rate settlement of fucoid propagules on epoxy discs on different areas of the shore at Pointe-Mitis in July (J), August (A) and September (S) over a 3-y period. Results are monthly means (n=6). Bars represent 95% confidence intervals (n=18). The scale was defined to show the smaller data, the data in August 2000 had a value of 250.

There was a similar lack of accumulation over a fortnightly time scale as well (data not shown). The similarity of daily settlement to 14-d and 28-d recruitment suggests that very few zygotes were surviving and accumulating on the disks and that the majority of the propagules observed on the 14-d and 28-d recruitment disks were simply embryos that had settled the previous day. Unfortunately, embryos were not distinguished from zygotes in these counts, and thus we cannot quantitatively assess if the algae on the 14-d or 28-d disks were zygotes (i.e., just settled propagules) or embryos (established individuals). Overall there was a reasonably strong correlation between the average daily settlement and the monthly settlement (Figure 2.7; r = 0.43; p = 0.029; n = 45).

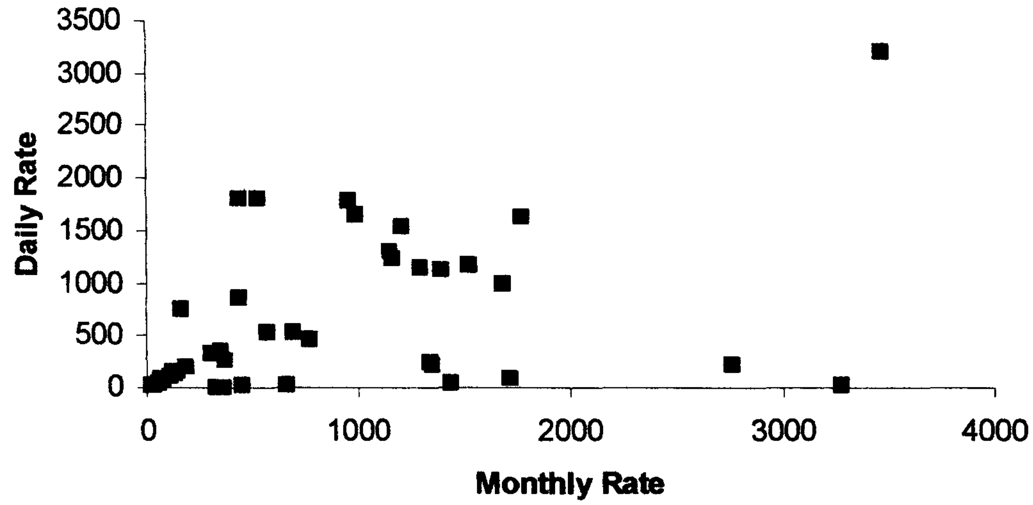


Figure 2.7: Relationship between daily and monthly settlement rates (n=45; p<0.05)

This relationship was the weakest in 2000 when daily rates remained high, but monthly rates were even lower than those in 1998. The correlation was much stronger between fortnightly and monthly data (Figure 2.8, r = 0.92; p = 0.08; n = 45), but this again could be due to a disproportionately large effect of settlement just before the moment of collection.

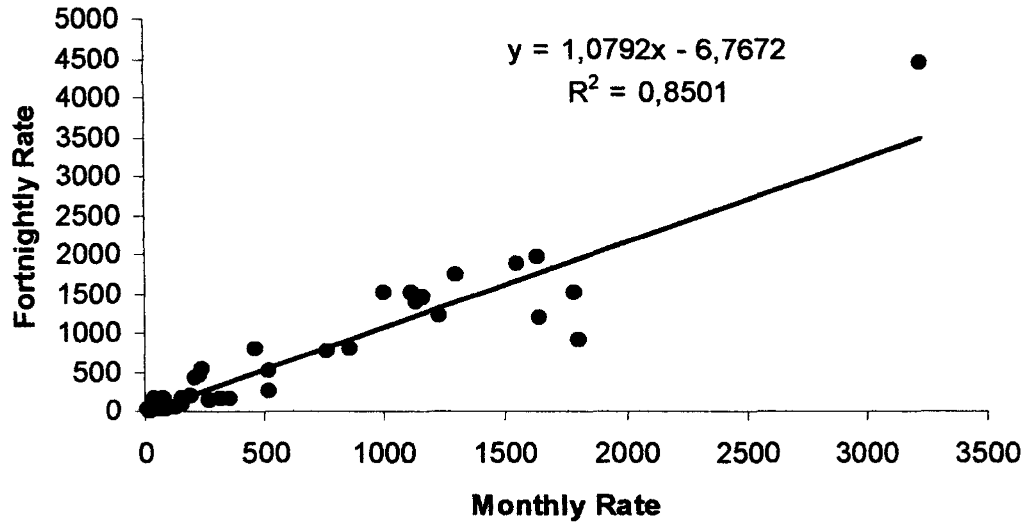


Figure 2.8: Relationship between fortnightly and monthly settlement rates (n=45; p=0.08).

2.5.2 Rates of settlement in different microhabitats

When examined by different microhabitats, the daily settlement of fucoid zygotes was 10 to 50 fold higher under the adult canopy than in tidepools or exposed rock surfaces except during months of low settlement, e.g. September (Figure 2.9).

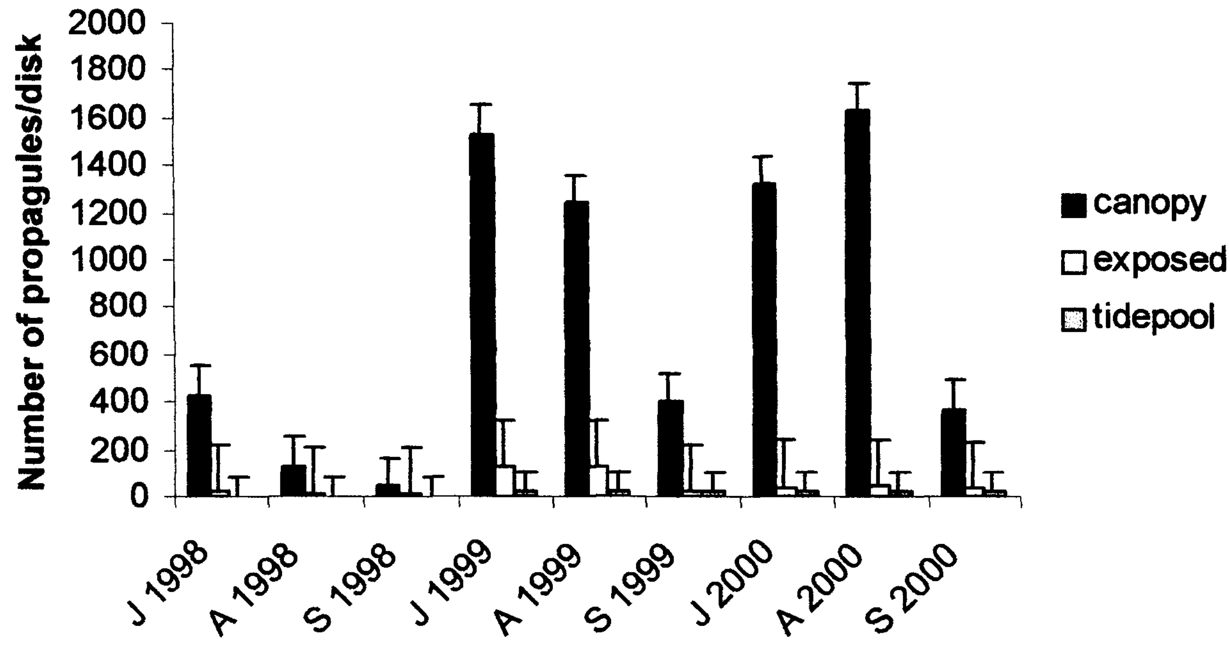


Figure 2.9: The rate of daily recruitment of fucoid propagules on epoxy discs on different microhabitats of the shore at Pointe-Mitis in July (J), August (A) and September (S). (■) canopy, (□) exposed conditions, and ( ) tidepools. Bars represent 95% confidence intervals (n= 10).

2.5.3 Rates of mortality in different microhabitats

Mortality of outplanted 16 to 20-hr-old embryos was remarkably stable and averaged between 40-50% across all years and all months (Figure 2.10). Except for two months (August 1999 and 2000), mortality was always the lowest under the adult canopy than other microhabitats. Mortality rates in other two microhabitats were at most double and usually only 10-25% more than that in the adult canopy microhabitat. No differences were seen between the exposed rock surface and the tide pool microhabitats.

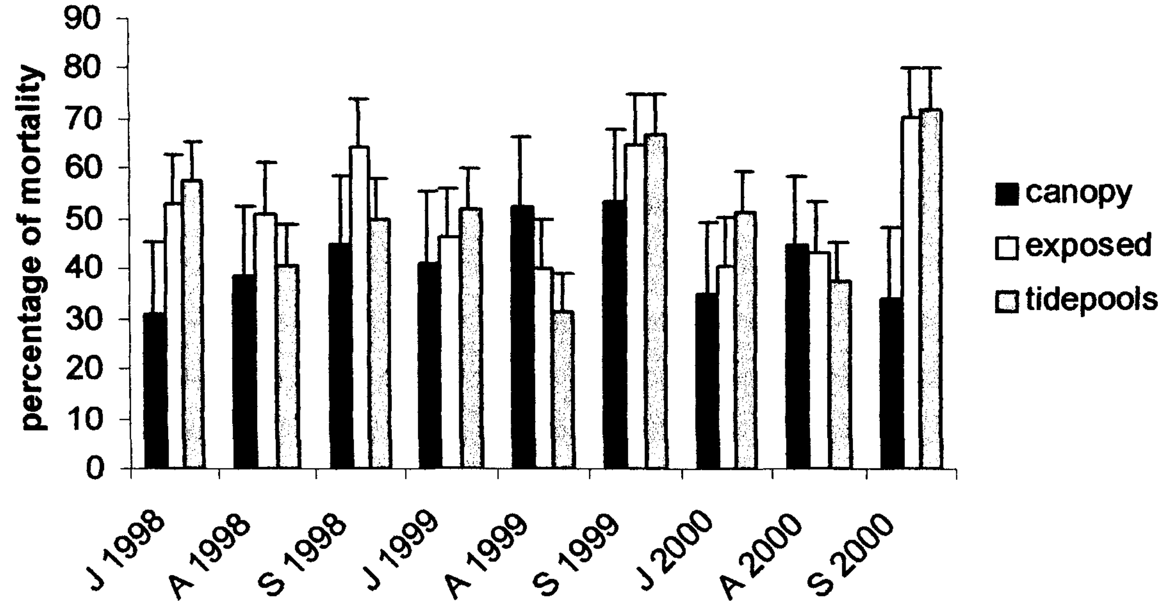


Figure 2.10 : Mortality of outplanted 16-20-h-old zygotes in July (J), August (A) and September (S) during 3 summers, 1998-2000; (■) canopy, (□) exposed conditions, and ( ) tidepools. Bars represent averages with standard errors from 9 disks each month. Data from two experiments each month are pooled for presentation and analysis.

2.5.4 Relationship to adult abundance

The abundance of mature algae varied consistently across the seasons (Figure 2.11). *Ascophyllum nodosum* was most abundant in July with some mature plants found in August. The abundance of *Fucus vesiculosus* was high in July and August but then declined during September. Finally, *F. distichus* ssp. *edentatus* had the reverse pattern – low abundance in July but then high abundance during August and September. Interannual variation did occur but mostly in the months of low abundance. An exception was the low abundance of *F.*

distichus ssp. ***edentatus*** in August 2000, 25% of the average abundance of the two previous years.

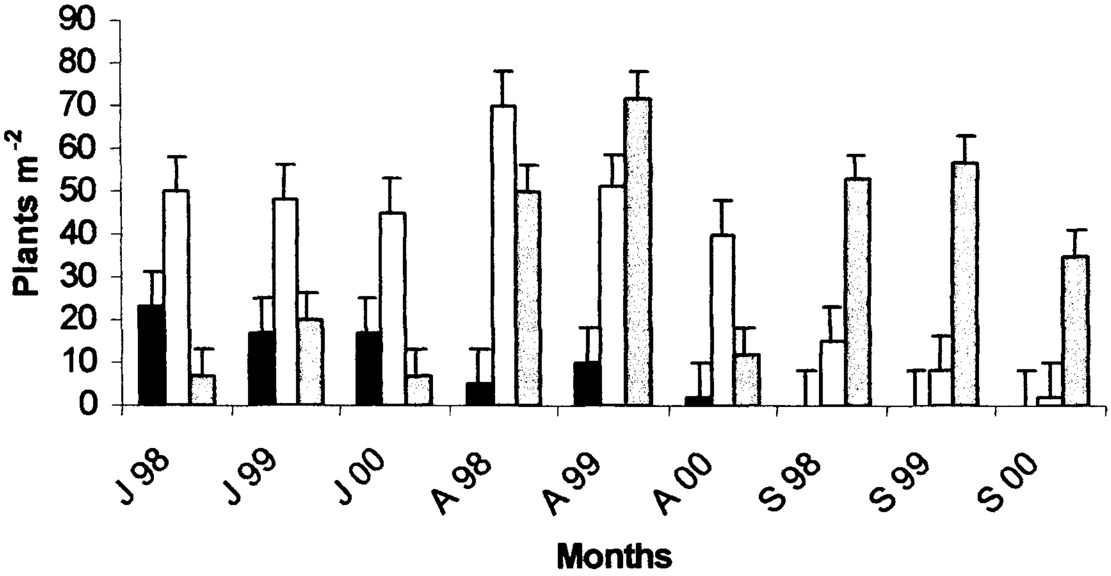


Figure 2.11: Density of plants of the three species during 3 summers in July (J), August (A) and September (S). (■) *A. nodosum* (L.) Le Jolis, (□) *F. vesiculosus* L. and ( ) *F. distichus* L. Bars represent 95% confidence intervals (n=15, twice a month).

The total number of receptacles (pooled for all species) peaked in August for the first two years (Figure 2.11) when both *Fucus* species were abundant (Figure 2.12). August receptacle abundance was substantially lower in 2000, essentially at the same level of the other months. No effect of the density or the receptacles on settlement was shown (Table 2.3).

Table 2.3 : ANCOVA (Proc Mixed) showing the effect of receptacles and density of mature adults on rate of settlement. Effect of receptacles, density of mature adults were used as covariables.

Source of Variation	df	F Value	p
Year	4	39.04	<0.001*
Month	2	40.41	<0.001*
Year x Month	8	21.73	0.001*
Frequency	2	0.63	0.5326
Year x Frequency	8	0.44	0.7767
Month x Frequency	4	0.21	0.9348
Year x Month x Frequency	16	0.18	0.9925
Receptacles	1	0.17	0.6832
Density of Mature Adults	1	0.72	0.3982

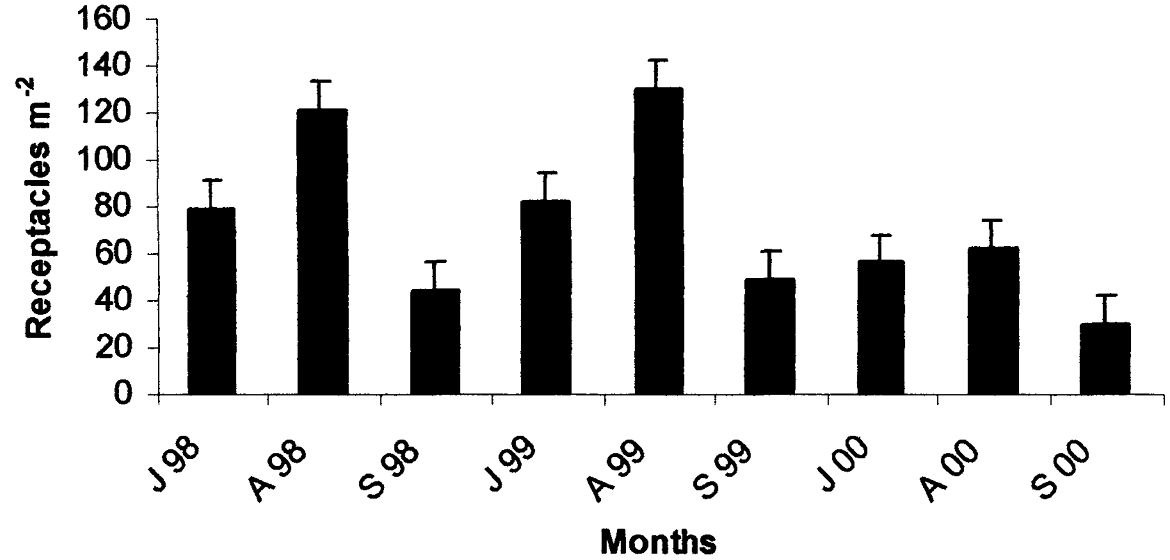


Figure 2.12 : Density of receptacles over the 3 summers in July (J), August (A) and September (S) (n=15). Bars represent 95% confidence intervals.

Correlations between the mature adults and the daily settlement yielded inconsistent results (Figures 2.13-2.16). For *Ascophyllum nodosum*, there was a negative relationship between these two variables (Figure 2.13). No relationship was seen for *F. vesiculosus* (Figure 2.14), a positive relationship was seen for *F. distichus* ssp. *edentatus* (Figure 2.15). For all species, a similar relationship was seen (Figure 2.16).

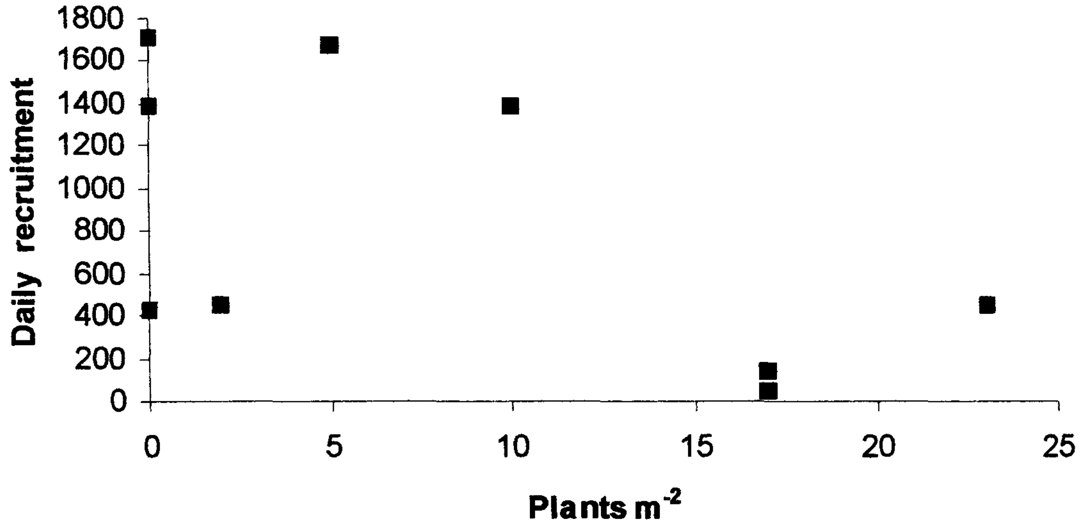


Figure 2.13: Relationship between recruitment (daily number of embryos per disk) and the density of mature plants of *A. nodosum* (L.) Le Jolis during a 3 yr-period (n= 9; p=0.7).

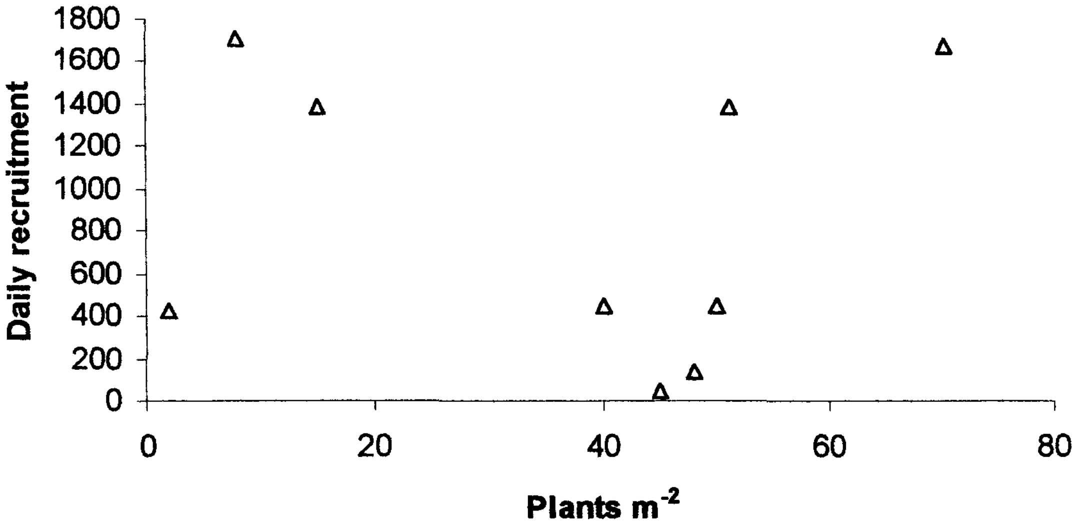


Figure 2.14: Relationship between recruitment (daily number of embryos per disk) and the density of mature plants of *F. vesiculosus* (L.) during a 3yr-period (n= 9; p = 0.8).

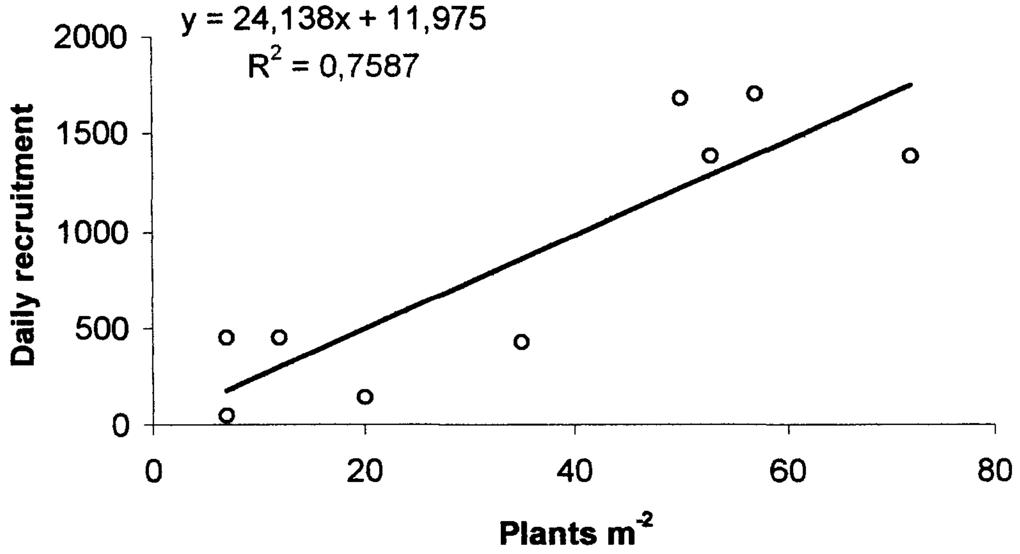


Figure 2.15: Relationship between recruitment (daily number of embryos per disk) and the density of mature plants of *F. distichus* (L.) during a 3yr-period (n= 9; p=0.06).

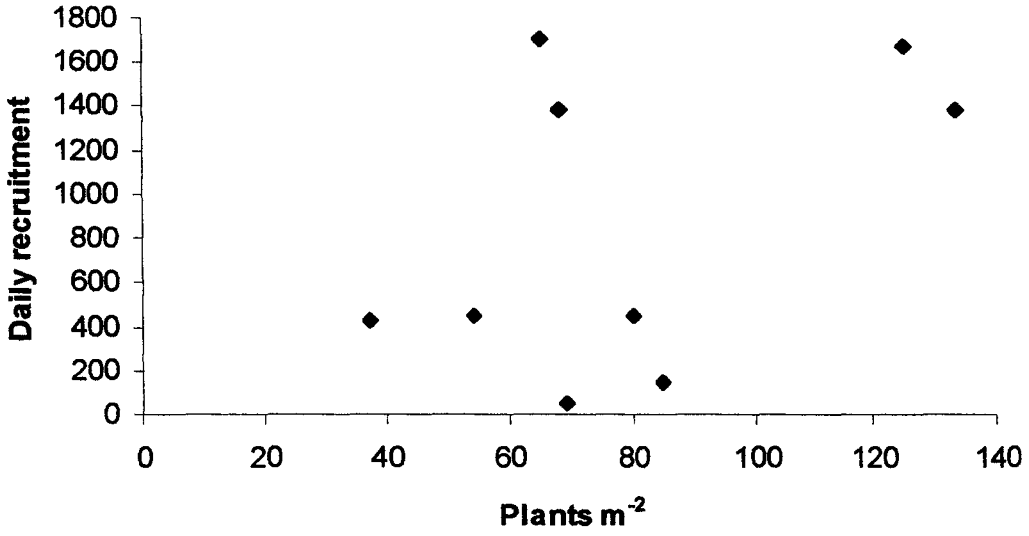


Figure 2.16: Relationship between recruitment (daily number of embryos per disk) and the density of mature plants (all species) during a 3yr-period (n= 9; $p<0.05$).

2.5.5 Effect of environmental parameters

Using the meteorological data of the Airport, there was significant effect of wind speed on settlement (Table 2.4) with a pattern of daily settlement's diminution with increasing wind speed. There were no significant correlations with temperature.

Table 2.4 : ANCOVA (Proc Mixed) showing the effect of the wind speed on rate of settlement. Wind Speed was used as covariable.

Source of Variation	df	F Value	p
Year	4	43.8	<0.001*
Month	2	51.72	<0.001*
Year x Month	8	22.25	0.001*
Frequency	2	0.60	0.5523
Year x Frequency	8	0.48	0.7487
Month x Frequency	4	0.16	0.9579
Year x Month x Frequency	16	0.19	0.9924
Wind speed	1	4.74	0.003*

2.6 Discussion

2.6.1 Spatial and temporal patterns

Spatial and temporal patterns of recruitment depend firstly on the reproductive patterns of adults (i.e., when and where fertile plants are found), secondly on the release and dispersal of the gametes, and finally on the settlement and early survival of these propagules. In this study we did not generally find strong relationships between the abundance of adults and rates of settlement (Table 2.3). There were no large differences in adult or receptacle abundance between the 3 years of the study (Figures 2.11 and 2.12) whereas settlement varied substantially among the years (Figure 2.3). There was a better correspondence at the monthly level: receptacles were most abundant in July and August which were also the months in which the most settlement occurred. A general correlation was seen between the abundance of mature plants and daily settlement (averaged over the month), but when examined at the species level, this pattern was only found for one species (especially for *Fucus distichus*).

This lack of a strong relationship may have several explanations. First is that our sampling effort to quantify adult or receptacle abundance (three quadrats) may have not been sufficient to estimate these parameters with enough precision. Second is that we were examining settlement and recruitment in a multi-species assemblage, and differences among species in the production of gametes or the rates of fertilization would have added variation to any pattern. Third is that we could not distinguish the embryos at the species level; thus the specific contributions of individual species could not be determined. Last is that propagules (zygotes in this case) may have been widely dispersed, thereby decoupling patterns of gamete production and settlement. Given our knowledge of the limited dispersal in other fucoid algae (Santelices 1990, Norton 1992, Johnson & Brawley 1998) and the evidence of limited dispersal in this system (see below), this last explanation is rather unlikely.

2.6.2 Dispersal

Dispersal of propagules is the next key determinant of recruitment patterns. Although we have no direct measures of dispersal in these species, we can infer it from the patterns of settlement onto disks placed within the fucoid canopy relative to those placed just outside the patches of adult plants (*sensu* Johnson & Brawley 1998). Settlement was more than an order of magnitude higher within the canopy than just outside, which is consistent with the results seen in the study of settlement patterns in *Pelvetia fastigiata*, another fucoid alga (Johnson & Brawley 1998), and suggests that, on average, propagules have extremely limited dispersal. Although we are not certain of how this limited dispersal is achieved, it is likely that, as is the case for some other fucoids, these species release gametes during low tide. This behaviour would not only permit high rates of fertilization as often seen in fucoids (Brawley 1992) but would also result in the deposition of zygotes directly below the adults. Attachment in fucoid algae generally can only occur 4-6 h after fertilization when adhesive polymers are formed. The disks were rarely exposed for more than 5-6 h, but the rugosity of the disk surface may have been sufficient to protect them from the immediate dislodgment by the returning tide.

Our inability to retrieve and replace disks simultaneously just as they were being exposed by the ebbing tide may have also contributed to the spatial variation that we observed in this study. If there was no substantial mortality of recently settled zygotes (an unlikely assumption – see below) and settlement was relatively constant from day-to-day (also unlikely; see Johnson & Brawley 1998), the time of collection would not influence the results because disks replaced late during the low tide (and thus with little remaining time to accumulated zygotes) would be compensated by having settlement during the earlier part of the low tide on the following day. If, however, there was substantial mortality during high tide, then those disks collected later during the low tide would have a greater accumulation of zygotes. We collected disks in the same order each day (at least at the "area" level; areas were visited in their numerical order, 1 to 5) and thus should have been able to detect such a pattern. The data show no clear pattern (Figure 2.3).

2.6.3 Post-settlement mortality

The final source of variation in recruitment patterns would be due to post-settlement mortality. Our experiments demonstrated that a substantial amount of mortality (30-70%) occurred over a single day for young stages. Although mortality was slightly lower in the canopy microhabitat, there were not large differences between the different microhabitats, which suggest that neither sweeping by adult fronds (canopy microhabitat) or desiccation (exposed microhabitat) were importance factors involved in the mortality of this stage. Thus another factor common to all three microhabitats (e.g., water motion, mobile grazers) must be responsible. These stages were, however, at least 16-hr old and thus had ample opportunity for attachment before being set out in the field. Thus, the mortality rates for younger stages are probably even higher.

The lack of any substantial accumulation of recruits on disks set out for fortnightly or monthly periods offers further evidence that mortality rates are extremely high for these propagules. In a world with no mortality of zygotes, one would predict the numbers of zygotes on disks left out for a fortnight and a lunar month to be 14x and 28x greater, respectively, than the average daily settlement that occurred during that period. While such results can hardly be expected, the numbers of zygotes on fortnightly and monthly disks were surprising low, indeed, not different from or below the average daily values for the period. These results suggest that there is no detectable accumulation (i.e., survival) of embryos on the disks. Indeed, being left out for multiple weeks appears to even reduce settlement, perhaps through the build up of a biofilm that prevents adhesion. We did not distinguish the age of the embryos growing on disks, but there were always a few older ones seen on the fortnightly and monthly disks which indicate that a low level of survival was occurring.

We cannot say, however, whether the rates of settlement or survival were the same on natural surfaces. However, this was not our goal – we were instead interested in the maximal levels of settlement and recruitment occurring in this environment. The disks had a rougher texture than the natural rock surface at this site, and this was possibly better at capturing and retaining zygotes. Longer term observations of fucoid recruitment do suggest that this epoxy putty is less suitable for long-term colonization than natural surfaces (M. Lamote, personal observation). Regardless, these results clearly show that shorter term measurements are needed for estimating settlement rates of these fucoid algae as there is only a negligible accumulation of recruits over time relative to rates of daily settlement.

2.6.4 Settlement

The daily estimates of settlement allow us to estimate the annual rate of settlement for these species at these sites. To do so requires several assumptions including that the 3-month period examined each year encompasses the entire reproductive season for fucoid algae at this site and that our 5 days of daily samples were representative of each 2-wk period, i.e., that the total production was the 5-d mean multiplied by 14. The former is quite reasonable as reproductive fucoids were rarely seen outside of this period, except for the high tide pool species, *Fucus distichus* ssp. *distichus*. The latter assumption is less certain. In fucoid species, gametes may be released on a lunar or semi-lunar cycle (Brawley & Johnson 1992, Pearson and Brawley 1996, Serrao et al. 1996, Berndt et al. 2002), giving rise to 2-wk and 4-wk peaks in gamete production or settlement (Andersson et al. 1994, Creed et al. 1996). If our 5-d sampling fortnightly sampling period coincided with such peaks in gamete production, then we would overestimate annual production. The peaks in settlement do not, however, always fall during the time of the new or full moons (e.g., Johnson & Brawley 1998).

Based on these data and assumptions we estimate that the total settlement during the reproductive season was from 0.9 x 10^7 to 2.6 x 10^7 zygotes m^{-2} y^{-1} [= (daily average)(30 days)(3months)] depending the year. These levels of settlement are consistent with earlier studies of fucoid algae. Creed et al. (1996) estimated an annual rate of 1.2 x 10^7 propagules m^{-2}. From the number of eggs in receptacles, Ang (1991) estimated a potential production of 1.5 x 10 ^{7} zygotes.m^{-2}.y^{-1} for *F. distichus* ssp. *distichus* on the west coast of Canada.

The high spatial and temporal variation in settlement is consistent with previous studies of recruitment in algae (Wright & Steinberg 2001), invertebrates (Caffey 1985), and fishes (Choat et al. 1988, Wilson & Osenberg 2002). Such variation can be an important determinant of local population abundance, but other factors such as density-independent mortality after settlement also play a role, and the relative importance of these processes can vary in time and space (Wright & Steinberg 2001). Lazo et al. (1994) found that only 0.01% of *Ascophyllum nodosum* survived annually and that the mortality was largely due to molluscan herbivores. Our results are consistent with this idea and suggest that the majority of mortality is occurring shortly after settlement. The exact cause of this mortality remains unclear, and the possibilities include wave dislodgment (Vadas et al 1992, Serrao et al. 1996) or grazers (e.g., Gieselman & McConnell 1981, Lubchenco 1983, Hartnoll & Hawkins 1985, Barker & Chapman 1990, Benedetti-Cecchi & Cinelli 1992, McCook & Chapman 1993, Lazo et al. 1994). Other causes such as desiccation (Santelices 1990, Chapman 1995, Pearson 1996) or density-dependent competition (Ang & De Wreede 1992, Kendrick 1994) are less likely possibilities as explained above.

2.6.5 Seasonal periodicity

Because we examined settlement within a multi-species assemblage, it is difficult to distinguish the individual contributions of the different species. The reproduction of species of *Ascophyllum* and *Fucus* shows a seasonal periodicity (M. Lamote, personal observation), and the asynchronous phenology of the three species did allow some temporal separation of their relative contributions. *Ascophyllum nodosum* began receptacle initiation in April and conceptacle development by early summer with gamete liberation commences towards the end of June, followed by an abrupt loss of receptacles. This early reproduction combined with low abundance relative to the other species in these areas (Figure 2.11) suggests that this species made a relatively small contribution to the settlers we observed. *Fucus vesiculosus* initiated receptacles in early May, and maturation and gamete liberation started towards the end of July and continued into early autumn. This phenology combined with its higher abundance supports the idea that this species was the primary contributor to the high settlement observed in August. Finally, *Fucus distichus* ssp. *edentatus*, the last species to become reproductive, with gamete release beginning in August, was probably responsible for most of the settlement observed in September. Spatial variation in the abundance of these species supports these ideas (data not shown). For example, *F. distichus* was the dominant macroalga in Area 5, and a clear peak in settlement was observed in August 2000, the time in which this species was in peak reproduction.

2.6.6 Canopy vs. exposed

Settlement varied substantial among the three microhabitats with much greater settlement under the existing adult canopy (Figure 2.9). This pattern has been observed elsewhere (McCook & Chapman 1991, Worm & Chapman 1996, Johnson & Brawley 1998), and it is generally thought to provide the advantage that the juvenile stages are better sheltered from some potentially fatal conditions encountered either during low tide (e.g., desiccation; Brawley & Johnson 1991, 1993) or high tide (e.g., dislodgment by water motion; Vadas et al. 1991). Consistent with this idea are the observations that germlings of fucoid algae (i.e., juvenile stages visible to the unaided eye) recruit more abundantly into areas with an adult canopy than onto exposed rock surfaces (Menge 1976, Lubchenco 1983, Vadas et al. 1992, De Vogelaere 1994) because the canopy provides a refuge from herbivory (Hawkins 1981, Lubchenco 1983) or from physically stressful conditions (Jernakoff 1983, 1985, Brawley & Johnson 1991). On the other hand, disadvantages also exist from living under an adult canopy as light levels could be sub-optimal and other sources of mortality could be encountered (e.g., disturbance from sweeping adult fronds). Whether the short-distance dispersal we observed and its concomitant "protection" by adults is a specific strategy or simply a consequence of maximizing fertilization rates remains unclear (Brawley et al. 1999), and whether any advantages outweigh any disadvantages may depend on the specific environmental conditions at the time. Our short-term outplant experiments did not show any substantial differences in mortality between the habitats, but previous studies have shown that under certain conditions, mortality due to the combination of high desiccation, high temperatures and high irradiance can be quite high in open surfaces (Brawley & Johnson 1991, 1993). Thus, under different conditions or for longer term experiments, greater differences might have been observed.

2.7 Conclusion

The results of this study demonstrate the large variability in the processes of recruitment, the fundamental step in maintaining populations of fucoids. Spatial and temporal variation appear to be a consistent feature of marine populations, but this variation is often attributed to the decoupling of local population processes due to the open nature of many marine species. Here, however, there should have been little decoupling of the reproductive effort of the adults and the settlement of zygotes and the recruitment of juveniles. Still, substantial variation occurred, suggesting that there are both high annual differences in reproductive effort and high variability in the ecological processes affecting the survival of the early stages.

CHAPITRE 3

Assembly of the photosynthetic apparatus in embryos from *Fucus serratus* L.

Lamote, M., Scheofs, B., Darko, E. et Y. Lemoine (2002)

3.1 Résumé

La biogénèse de l'appareil photosynthétique a été étudiée durant les six premiers jours de développement des embryons de *Fucus serratus* L. Les analyses pigmentaires par chromatographie liquide à haute performance (HPLC) ont mis en évidence que les oosphères et les zygotes contiennent les mêmes pigments photosynthétiques (*ex.*, chlorophylle *a*, chlorophylle c, fucoxanthine, violaxanthine, et β-carotène) que ceux des thalles adultes. Le contenu total en pigment augmente après la fécondation, principalement lié à une synthèse active de la Chl *a* et de la fucoxanthine. Les modifications spectrales démontrant l'intégration progressive des Chl *a* et Chl c dans les unités photosynthétiques sont décrites. En particulier, une émission distincte à 705 nm, reflétant l'accumulation de l'antenne LHCI, a été clairement détectée. Les bandes d'émission à 705 nm et 725 nm ont été caractérisées par des mesures de fluorescence à 77 K. Leurs spectres diffèrent par la présence d'une large bande à approximativement 550 nm due à la fucoxanthine dans le spectre d'excitation F705 nm. La fluorescence variable à température ambiante est observée 30 heures après la fécondation indiquant un transfert fonctionnel d'électrons à ce stade de développement.

3.2 Abstract

The assembly of the photosynthetic apparatus has been studied during the first 6 days of development of *Fucus serratus* L. embryos. HPLC analysis revealed that oospheres and zygotes contain the same photosynthetic pigments (*i.e.*, chlorophyll *a*, chlorophyll c, fucoxanthin, violaxanthin, and β-carotene) as fully developed thalli. Total pigment amount increased after fertilization, mainly due to active synthesis of Chl *a* and fucoxanthin. Spectral modifications revealing the progressive integration of Chl *a* and Chl c in the photosynthetic units are described. In particular, a distinct emission at 705 nm, reflecting the accumulation of LHCI, was clearly detected. The emission bands at 705 nm and 725 nm were characterized by 77 K excitation fluorescence measurements. Their spectra differed by the presence of a large band at approximately 550 nm due to fucoxanthin in the excitation spectrum of F705 nm. Room temperature variable fluorescence was first observed 30 hours after fertilization indicating a functional photosystem II electron transfer at this developmental stage.

3.3 Introduction

The biogenesis of the photosynthetic apparatus has served as a framework for numerous studies (reviewed by Sundqvist & Ryberg 1993, Hoober et al. 1994), and the preferred model organisms to date have been angiosperms and green microalgae. As angiosperms are only able to synthesize chlorophyll in the light, the biogenesis of the photosynthetic apparatus is triggered by an illumination (reviewed by Schoefs 1999, Schoefs 2001). So far, it has been found that the other groups of photosynthetic organisms - gymnosperms with exceptions, lower plants and cyanobacteria - are capable of chlorophyll synthesis in the absence of light. Therefore, the assembly of the pigment-protein complexes can proceed in the dark (*e.g.*, Raskin & Marder 1997, Schoefs & Franck 1998). This makes the use of xenobiotics or of the 'yellow-in-the-dark' mutants necessary to investigate the biogenesis of the photosynthetic apparatus in algae (*e.g.*, Chan et al. 1989). In addition, the green algae usually divide vegetatively and, consequently, there is no *de novo* biogenesis of the photosynthetic apparatus after cell division. Few studies on the chloroplast development in groups of photosynthetic organisms other than angiosperms and green microalgae have been carried out. This is especially true for the marine macrophytes, which reproduce sexually (see McCook & Chapman 1992) and in which embryo development should be accompanied by *de novo* synthesis of the photosynthetic apparatus.

In their natural environment, fucoids release eggs and motile sperm in the surrounding medium, where fertilization takes place successfully (Brawley 1992, Pearson & Brawley 1996, Serrao et al. 1996, Serrao et al. 1999, and Berndt et al. 2002). Then the dense aggregations of propagules settle within a short time (Knight & Parke 1950, Johnson & Brawley 1998). The embryos develop into a thallus and a rhizoid, which are morphologically (Quatrano 1980) and biochemically distinguishable (Evans et al. 1982, Berger et al. 1993). As fucoid gametes and embryos are easy to obtain and manipulate, they have constituted the first cellular model used in experimental biology (Thuret 1854, Rosevinge 1889, reviewed by Bouget et al. 2000). Today fucoid embryos still constitute a material of choice to study the biochemical and physiological basic processes involved in embryonic plant development (Jaffe 1968, Corellou et al. 2000), particularly those involved in cell wall formation and in the establishment of cellular polarity of zygotes (Kropf 1982, Bouget et al. 1996). The understanding of the embryo development as a whole and of the influence of external factors requires the knowledge about the development of the photosynthetic apparatus. So far the only studies entering in this frame concerned gas exchange and fluorescence kinetic measurements (Major & Davison 1998). The main aim of this paper was to examine the assembly of the photosynthetic apparatus during the first stages of development of *Fucus serratus* embryos using HPLC and *in situ* fluorescence measurements.

3.4 Methods

3.4.1 Harvesting and cultivation of the algae

Dioecious thalli of *Fucus serratus* (L.) Powell were collected in the intertidal zone near Boulogne sur Mer, at Wimereux (50° 45' N, 01° 36' E) at the French shores between January and June 1997. They were transported on ice to the laboratory and stored at 283 K in seawater in dark conditions. Before use, the sexual parts were cleaned by a gentle sonication (three minutes at room temperature) to remove diatoms from the thallus surface. The receptacles were placed in cold water for 5 minutes and then into filtered natural seawater. The release of the gametes was induced by osmotic shocks. Fusion of sperm and eggs occurred on filters (Millipore AP 20, Bedford, USA) in Petri dishes containing filtered natural seawater. The fusion of gametes and germination was performed at constant temperature (283 K) under a 30 μmol photon m^{-2} s^{-1} polychromatic light.

3.4.2 *In situ* 77 K fluorescence emission and excitation measurements

Fluorescence emission and excitation spectra were recorded at 77 K using a Hitachi F-3010 spectrofluorimeter (Tokyo, Japan). Both the excitation (at 440 nm) and emission slits were set at 5 nm. The filters supporting the embryos were immersed directly into liquid nitrogen. The spectra were corrected for the sample baseline and the photomultiplier response.

3.4.3 HPLC set-up and pigment analysis

Pigments were prepared according to Bertrand & Schoefs (1997) by grinding the embryos in methanol at 273 K. The homogenate was centrifuged at 10000 x *g* for 5 min and filtered through a 0.45 µm PTFE filter (diameter 13 mm, Millipore, France). The pigment solution was dried using a rotary evaporator. In order to eliminate salt traces, the residue was solubilized with methylene chloride and washed three times with distilled water. The organic phase was finally dried under a stream of nitrogen gas and stored at 243 K until use.

All pigment separations were carried out with an original reversed-phase ODS column (C-18, particle size of the packing: 4.65 µm; 250 mm X 4.6 mm i.d.; Zorbax, Hewlett Packard). The detector was a UV-VIS diode array detector (190-800 nm, model 991-25, Waters, S^t Quentin-en-Yvelines, France). Pigments were eluted according to Arsalane et al. (1994). Solvent A (methanol, acetonitrile, water, 51:36:13, V/V/V + 0.3 M ammonium acetate) was continuously mixed with solvent B (ethyl acetate, acetonitrile, 70:30, V/V). During the first 23 minutes, the proportion of solvent B was linearly increased from 0 to 90 %. This elution mixture was then run isocratically during the next 7 minutes. Between two analyses the column was equilibrated with solvent A for at least 15 minutes. All the solvents used were HPLC grade and purchased from Merck (Darmstadt, Germany).

Pigments were quantified using the external standard method. Chl *a*, Chl c, β-carotene, fucoxanthin and violaxanthin were quantified using the extinction coefficients published by Ziegler & Egle (1965), Jeffrey (1968), Jeffrey (1972), Johansson et al. (1974) & Britton (1985), respectively.

3.4.4 Variable fluorescence measurements

Variable fluorescence of zygotes and embryos was measured at room temperature using a Pulse Amplitude Modulation Fluorometer (PAM-2000, Walz GmbH, Effeltrich, Germany). Prior the measurements, the samples were dark-adapted for 10 min. The initial fluorescence level (Fo) was excited by a weak 650 nm light beam modulated at 1.6 kHz. The maximum fluorescence level (Fm) was induced by one polychromatic saturating light pulse (light intensity: 600 µmol photon m^{-2} s^{-1}) of 1 s duration, which was provided by a halogen lamp (Schott, Germany). The pulses did not trigger photoinhibition. The maximum quantum yield of PSII (Fv/Fm) was calculated as (Fm - Fo)/Fm, according to Genty et al. (1989).

3.5 Results and Discussion

3.5.1 Modifications of the content in photosynthetic pigments during the first 6 days of development of *Fucus serratus* embryos

A typical pigment composition for brown seaweeds (*i.e.*, Chl *a*, Chl c, fucoxanthin, violaxanthin and β-carotene) was found in the eggs and at all the developmental stages of the embryos (data not shown). This result is consistent with ultrastructural studies of oospheres and embryos which have demonstrated the presence of chloroplasts with appressed membranes (Evans et al. 1982). During the first 6 days of growth an active pigment synthesis occured. No lag phase in the Chl and Car accumulations could be observed (Figure 3.1).

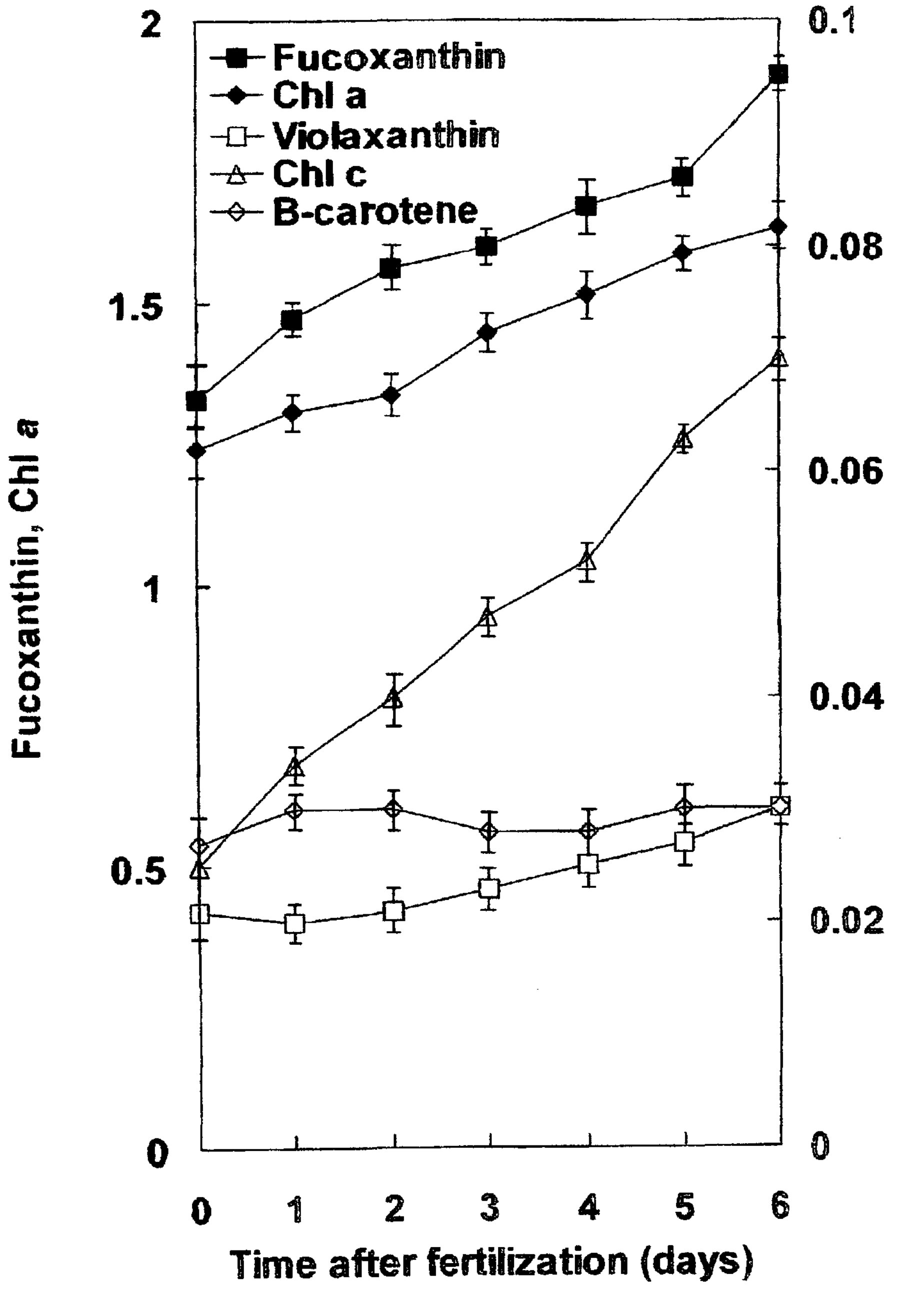


Figure 3.1 : Variations of the individual pigment amount the first 6-days of *Fucus serratus* embryos development. The amount of pigment is in µg per embryo. Error bars represent 95% confidence intervals.

This leads to the suggestion that the coordination of their synthesis is similar to that in angiosperms (*e.g.*, Schoefs et al. 1998, Schoefs et al. 2001).

During the first 6-d of development, the amount of violaxanthin and fucoxanthin increased in parallel (Figure 3.1). As fucoxanthin was approximately 50 times more abundant than violaxanthin (and β-carotene), the main increase in carotenoid was due to fucoxanthin accumulation. During the same period of development, the amount of β-carotene remained approximatively at the same level. This does not actually reflect the production of β-carotene as it was continuously transformed to fucoxanthin and violaxanthin, which accumulated (Figure 3.1).

The increase of the Chl level was associated with the synthesis of both Chl *a* and Chl c (Figure 3.2) with faster rates of accumulation of the latter. This resulted in decrease of the Chl *a* to Chl c ratio (Figure 3.2), similar to the decrease of the Chl *a* to Chl b ratio that has been observed during the first stages of the greening of angiosperm tissues (Akoyunoglou et al. 1966, Schoefs et al. 1998).

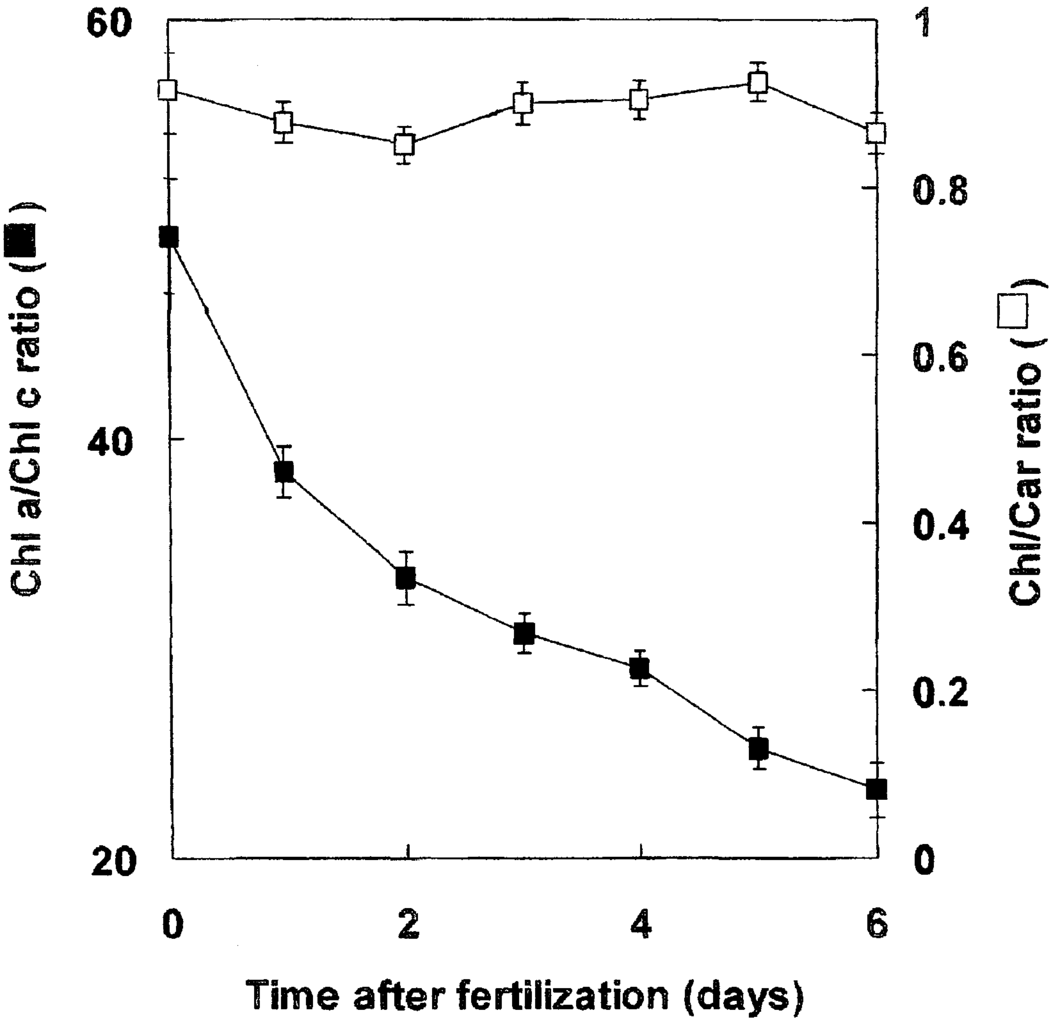


Figure 3.2 : Evolution of the Chl *a* to Chl c (■) and Chl to Car (□) ratios during the first 6 days of development *of Fucus serratus* embryos. Error bars represent 95% confidence intervals.

As Chl c has the same role than Chl b, we assumed that the decrease of the Chl *a* to Chl c ratio reflects the integration of the light-harvesting complexes in the photosystem units. The fact that after 6 days of development, the value of the ratio was close to the typical value of fully developed *Fucus* thallus (a constant value of approximately 12-17; Caron et al. 1985, Berkaloff et al. 1990, De Martino et al. 1997 suggests that under our culture conditions, the assembly of the photosynthetic apparatus was brought to near completion).

3.5.2 Changes in the *in situ* 77 K emission and excitation fluorescence spectrum during the development of *Fucus serratus* L. embryos

In order to further document of the biogenesis of the photosynthetic apparatus, *in situ* 77 K fluorescence emission spectra during development of embryos were recorded (Figure 3.3).

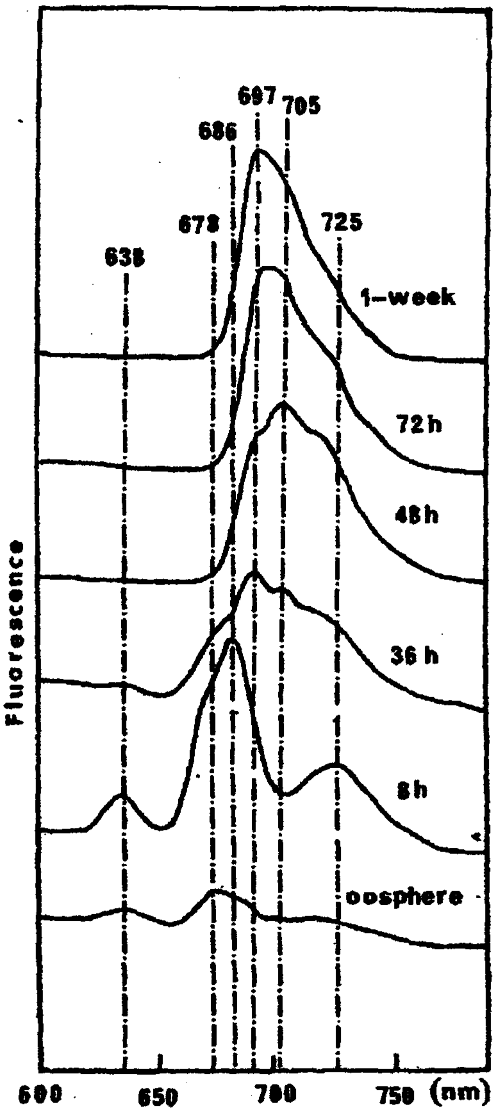


Figure 3.3. *In situ* 77 K fluorescence emission spectra during the biogenesis of the photosynthetic apparatus of *Fucus serratus* embryos. From the bottom to the top: spectrum recorded with oosphere, 8 h, ,36 h, 48 h, 72 h and one week-old embryos. The excitation was at 440 nm.

The bottom curve shows the fluorescence spectrum of oospheres and shows one distinct broad band at 678 nm due to Chl *a*. This is unlikely to be due to free Chl *a* which emits at shorter wavelengths (maximum at 675 nm) (Franck et al. 1995). To identify the pigments responsible for the emission at 678 nm, excitation spectra at 678 nm were recorded. A typical spectrum is presented in Figure 3.4. The bands at 420 and 437 nm reflected the contribution of Chl *a* whereas that at 450 and 465 nm are due to Chl c and Cars, respectively. The absence of the typical band of fucoxanthin, at 544 nm, should be noted. These results, together with the finding that isolated LHCII complexes have an emission maximum at 677 nm (Douady et al. 1993, De Martino et al. 1997), indicate that the band at 678 nm observed in the oospheres reflects the presence of uncoupled LHCII. Interestingly a very similar band was also found at the very early stages of photosynthetic apparatus pine tissues (Raskin & Marder 1997, Schoefs & Franck 1998). The contribution of the band at 678 nm remained important during the first 24 h of development and then decreased rapidly (data not shown). The emission spectrum of the oospheres also show two minor bands at 638 and 725 nm due to Chl c and Chl *a*, respectively (Figure 3.3, oospheres).

Eight hours after fertilization, a new band at 686 nm appeared and became predominant after 24 h of growth (data not shown). On the basis of the characterization of pigment-protein complexes isolated from higher plants, this band was attributed to CP43 (Zucchelli et al. 1992, Wang et al. 2000). This conclusion is further supported by the fact that isolated PSII particles from *Laminaria saccharina* present a maximum at 686 nm (Caron et al. 1985, Douady et al. 1993). During this period, the band at 638 nm and the shoulder at 678 nm were still clearly detected (Figure 3.3). The relative amplitude of these bands progressively decreased and was no longer detected after 48 h of growth (Figure 3.3, spectra from 48 to 1 week). Between 8 and 48 hours of growth, a new maximum at 705 nm appeared (Figure 3.3, spectra from 8 to 48 h). A similar band has been observed in the 77 K fluorescence spectra of isolated PSI from *Laminaria saccharina*. The presence of a shoulder at approximately 705 nm in the corresponding spectrum of *Fucus serratus*, however, is less clear (Caron et al. 1985, Berkaloff et al. 1990). It probably arises from uncoupled LHCI (emission at 707 nm, Wollman & Bennoun 1982). Both intensities at 697 and 705 nm increased more rapidly than that at 725 nm (Figure 3.3, spectra 36 h → 1 week), which however became larger than in the oosphere.

The band at 697 nm could be due to the CP47 (Nakatani 1983, Zucchelli et al. 1992). The increase at 725 nm could reflect the early assembly of PSI as observed during higher plant greening (Bertrand et al. 1987). This interpretation is supported by the fact that isolated PSI particles from *Fucus serratus* have an emission maximum at 724 nm (Duval et al. 1983, Caron et al. 1985; Berkaloff et al. 1990). The 77 K emission spectra of 1-week-old embryos presented a main broad band at 697 nm and two shoulders at approximately 705 and 725 nm. Interestingly, this spectrum is the same as that of *Laminaria* thalli, but differs from the spectrum of mature chloroplasts of *Fucus serratus,* which consists of a broad band at 720 nm (Goedheer 1970, Berkaloff et al. 1990). This observation suggests that the relative amount of individual pigment-protein complexes (LHC) is different in *Laminaria* and in *Fucus.*

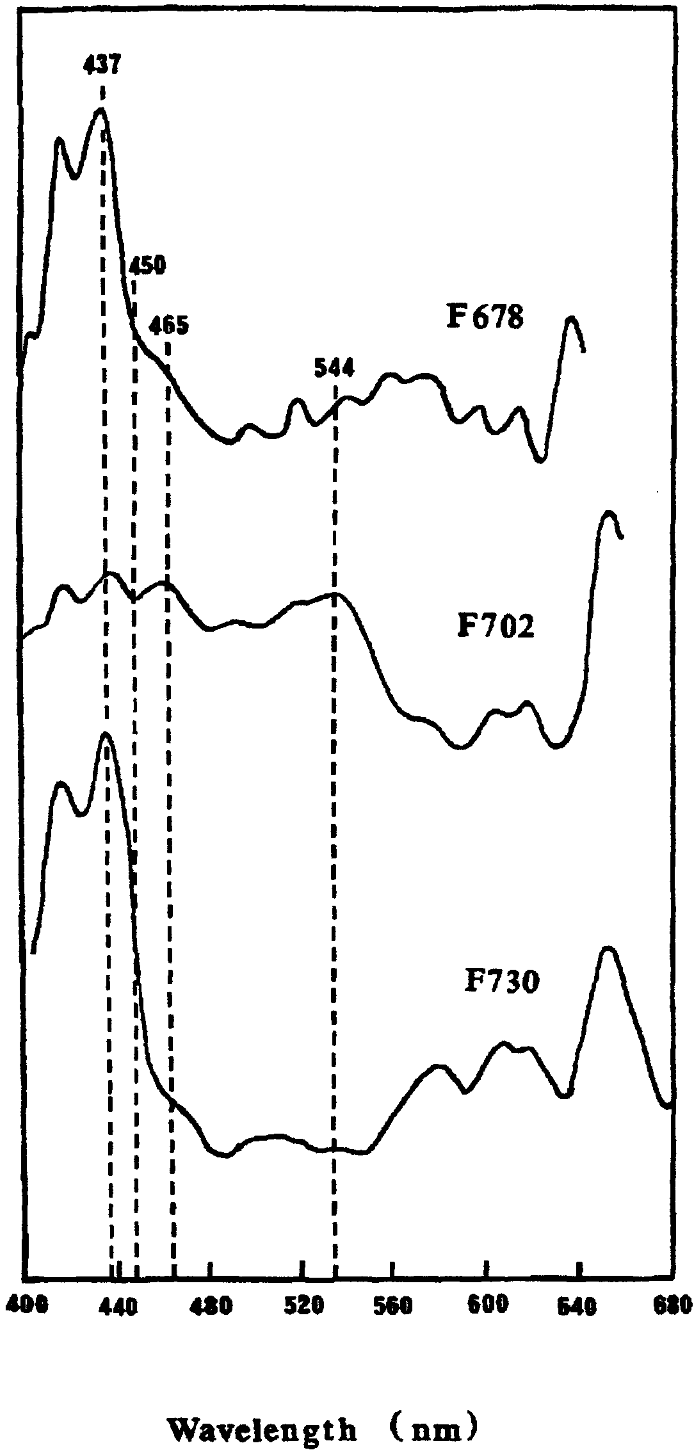


Figure 3.4. 77 K excitation spectra of the fluorescence at 678 nm, 702 nm, and 730 nm in eggs.

To better characterize the pigments, which are responsible for the emission at 705 and 725 nm, their excitation spectra were recorded at 702 and 730 nm, respectively. These wavelengths were chosen in order to decrease the possible contribution of the pigments from one of the bands to the excitation of the other. The excitation spectrum of the band at 705 nm differed clearly from that recorded at 725 nm (compare the spectra presented in Figures 3.4): the excitation band at 544 nm, due to fucoxanthin (Kirk 1977), is much larger in the excitation spectrum at 705 nm than at 730 nm. This finding brings an additional argument in favour of the heterogeneity of the LHC in brown marine seaweeds. The two excitation spectra are very similar to the 'band *a*' and 'band *b*' isolated from the main light-harvesting of the brown algae *Pelvetia canaliculata* by nondenaturing iso-electrofocusing (De Martino et al. 1997). Therefore, they could arise from similar pigment-protein complexes. Our study also demonstrates that the pigment-protein complexes emitting at 705 and 725 nm do not exchange energy. This fact together with the spectral heterogeneity of the LHC of photosystem I is reminiscent of the heterogeneity of the LHC of PSI of higher plants. In green plant chloroplasts, the LHCI is composed by dimers of LHCa2/LHCa3 (emission at 703-708 nm) and of LHCa1/LHCa4 (emission at approximately 730 nm) (Ihalainen et al. 2000). Altogether the fluorescence data reported in this contribution suggest that the organization of the light-harvesting complex in organisms containing Chl c is similar to those containing Chl b. This conclusion is supported by the fact that the secondary structures of the polypeptides are similar (Caron et al. 1996, Durnford et al. 1996, Grabowski et al. 2001), and can accomodate both pigments (Grabowski et al. 2001).

3.5.3 *In situ* room temperature variable fluorescence measurements

In order to determine whether the development of the 77 K fluorescence bands was connected to the appearance of a functional photosynthetic apparatus, variable fluorescence kinetics were recorded during the development of the *Fucus serratus* embryos. Figure 3.5 presents the modification of the maximum quantum yield of PSII photochemistry (*i.e.*, the Fv/Fm ratio; reviewed by Rohacek & Bartak 1999).

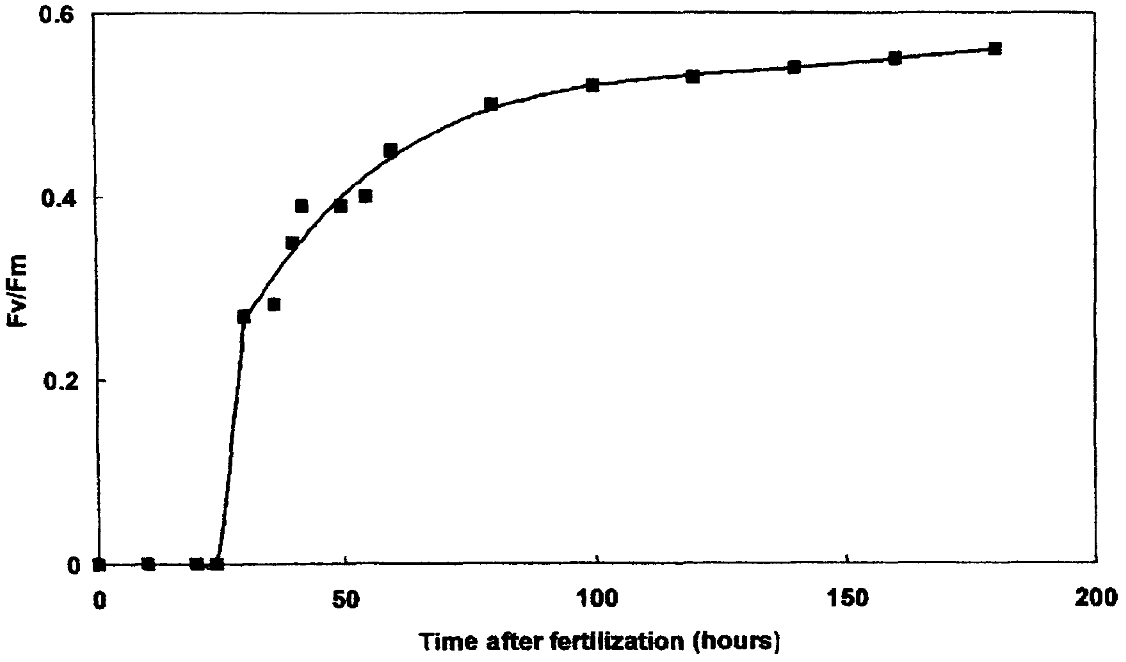
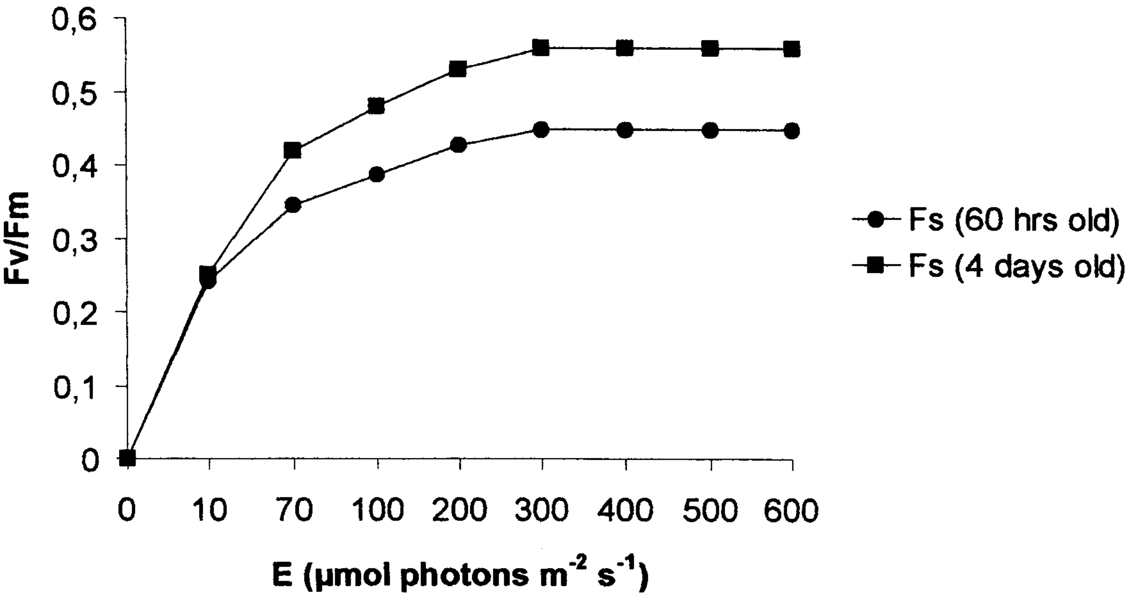


Figure 3.5 : Variations of the maximum quantum yield of PSII photochemistry during the first 6 days of development of *Fucus serratus* embryos.

As indicated by the absence of variable fluorescence (Fv/Fm = 0), the photosynthetic apparatus was not functioning during the first 24 h of development. When the variable fluorescence appeared, the Chl *a*/Chl c was still high (Figure 3.2). This observation indicates that the assembly of the PSII was not yet complete (Akoyunoglou et al. 1966). After this period, the Fv/Fm ratio increased rapidly until 75 h of growth. The development of the Fv/Fm ratio also coincides with the decrease of the relative fluorescence at 705 nm, which indicates the formation of functional PSI units. Then it continued to increase, however, at a much slower rate. At the end of the 1st week of development, the ratio amounted to approximatively 0.56 (Figure 3.5). A very similar value (0.54) was found in one-week-old *Fucus evanescens* embryos (Major & Davison 1998).

3.6 Conclusions

The pigment composition of oospheres is qualitatively identical to that of mature thallus. Accumulations of chlorophylls and carotenoids are coordinated during development. Fertilization leads to immediate qualitative and quantitative modifications of the oosphere fluorescence spectrum, reflecting the assembly of the photosystems and their accumulation. Particularly, this study of early development stages allows the detection of the uncoupled PSI antenna, which fluoresces at 705 nm. This band is difficult to observe in mature thalli, probably because it transfers efficiently the excitation energy to the PSI core. Variable fluorescence measurements indicate that PSII are already functioning 30 h after fertilization. The spectral data, together with the decrease of the Chl *a* / Chl c ratio, suggest that, before the increase of the Fv/Fm ratio, the PSII and PSI cores have started to integrate the antenna pigment-protein complexes. A similar observation was made in dark grown gymnosperm tissues where photosynthetic units can be assembled in the absence of an activated oxygen-evolving system (Lemoine 1981, Schoefs & Frank 1998). Thus, we propose that the absence of variable fluorescence at the first stages of the *Fucus* embryo development is probably due to a defect of the water-splitting system.



Variations of the maximum quantum yield of PSII photochemistry during the first 60hr of the development of the *Fucus serratus* embryos and 4-days old embryos of *Fucus serratus* embryos.

CHAPITRE 4

Interspecific differences in the response of juvenile stages to physical stress: fluorometric response of fucoid algae to variation in meteorological conditions

4.1 Résumé

La fluorescence à « pulse d'amplitude modulée » (PAM) a été utilisée afin de comparer les réponses photosynthétiques des embryons de trois Fucales (*Ascophyllum nodosum* (L.) Le Jolis, *Fucus vesiculosus* L., *Fucus distichus* L. ssp. *edentatus*) dans l'estuaire du Saint Laurent durant et immédiatement après la période d'émersion sous quatre conditions météorologiques définies. Ces trois espèces sont verticalement distribuées dans des zones qui se chevauchent et nous avons défini trois zones pour lesquelles chaque espèce est dominante. A partir de fécondations contrôlées en laboratoire, nous avons fixé des substrats artificiels avec nos embryons dans chaque zone respective de l'espèce. De plus, ces substrats ont été placés sous deux micro-habitats différents, sous la canopée et sur des surfaces exposées. Au cours de la marée basse, les variations de certains paramètres de fluorescence ont été mesurés : le rendement quantique maximum (Fv/Fm), le rendement quantique effectif (Y), la vitesse relative du transfert d'électrons (rETR) et l'extinction de fluorescence non-photochimique (NPQ). Les résultats indiquent une diminution générale de Fv/Fm, du Y, du rETR et une augmentation du NPQ durant la période d'émersion. Ces variations des Fv/Fm, Y, rETR et NPQ sont plus dramatiques sous les conditions météorologiques les plus sévères, c'est à dire,

sous des conditions ensoleillées et venteuses. Quelles que soient les conditions météorologiques, l'espèce placée la plus basse sur l'estran, *F. distichus* ssp *edentatus*, était la plus affectée. Les paramètres de fluorescence sont généralement significativement différents entre les deux espèces occupant les zones les plus hautes, *A. nodosum-F. vesiculosus* et la plus basse, *F. distichus* ssp. *edentatus*. Ces différences pourraient suggérer un caractère inné des juvéniles à supporter les mêmes conditions que leurs adultes respectifs. La restauration du transfert d'électrons est très rapide pour les trois espèces étudiées sous toutes les conditions météorologiques et aucune différence n'a été détectée entre l'évolution des propriétés de fluorescence des différentes espèces. Sous la canopée, la diminution du rendement quantique maximum est plus graduelle ; les faibles valeurs du rETR de ces juvéniles par rapport à celles des thalles placés en conditions exposées indiqueraient une photoacclimatation des thalles qui se comportent comme des plantes d'ombres.

4.2 Abstract

Pulse amplitude modulated (PAM) fluorescence was used to compare the photosynthetic responses of embryos of three Fucales (*Ascophyllum nodosum* (L.) Le Jolis, *Fucus vesiculosus* L., *Fucus distichus* L. ssp.) in the Saint Lawrence estuary during and immediately after the period of low tide under four defined meteorological conditions. These three species are vertically distributed in overlapping zones, within which we selected three areas in which each species was dominant. In the laboratory we seeded artificial substrata with zygotes from each species and then outplanted them into their respective zones. In addition, we also outplanted disks with embryos into two different microhabitats: under an adult canopy and on exposed surfaces. During low tide the variations of several fluorescence parameters were measured from which the following were calculated: maximum quantum yield (Fv/Fm), effective quantum yield (Y), relative electron transport (rETR), and non-photochemical quenching (NPQ). Generally, there was a decrease in Fv/Fm, Y, rETR and a fluorescence increase of non-photochemical quenching during the course of low tide. These changes were most dramatic under the most severe meteorological conditions, i.e., sunny and windy. Regardless of the meteorological conditions, the lowest species, *F. distichus* ssp *edentatus*, was most affected. In general, fluorometric parameters did not differ between the two highest species, *A. nodosum* and *F. vesiculosus*, but they were significantly different from the lowest species. These differences could suggest a inborn e characteristic of juveniles to support the same conditions than their respective adults. Recovery upon re-immersion was fast for all species under all meteorological conditions, and there were no significant differences in the fluorometric parameters among the three species. Under a canopy of adults, decreases in maximum and effective quantum yields were more gradual than for embryos in exposed locations. The weak values of rETR of these juveniles under canopy in comparison to these placed in exposed conditions could indicate a photoacclimatation of thalli which behave like a shade plants.

4.3 Introduction

Benthic organisms living in the intertidal zone are regularly exposed to aerial conditions during low tide, at which time they experience pronounced changes in temperature, light intensity, and desiccation. This suite of variations increases with increasing tidal height; as the period of emersion becomes longer. Thus, aquatic organisms living higher on the shore will generally experience more stressful conditions than those living lower on the shore. This physical gradient running up the shore has long been invoked to explain the often conspicuous patterns of zonation seen for intertidal organisms (Colman 1933, Doty 1946, Stephenson & Stephenson 1949, Lewis 1964, Underwood 1978, Schonbeck & Norton 1979, 1980, Druehl & Green 1982, Swisbanks, 1982, Chapman 1986, 1995). The fundamental hypothesis has been that the vertical position on the shore reflected specific physiological tolerances related to regime of exposures and their associated levels of stress. Both survival and the ability to compete within a given zone were thought to depend largely on physiological capacities to endure to such abiotic factors (Chapman 1966, 1995). In more recent years, this basic idea has been expanded to include biotic interactions, but these are generally to be more important in determining the proximate factors for setting the lower limits of a species distribution (Connell 1961, Paine 1979, Lubchenco 1980). The upper limits of intertidal species are still generally believed to be directly controlled by physical factors, and ultimately it is these factors that control the biological interactions as well.

Past studies have attempted to relate the physiological abilities of intertidal organisms to the actual environment in which they are normally distributed (Foster 1971, Newell 1979, Foster 1985). Many have focused on marine macroalgae (seaweeds), conspicuous members of many temperate intertidal communities. These photoautotrophs face several challenges when exposed to terrestrial conditions during low tide including excessive light, extreme temperatures, and dehydration. Of these factors, intolerance to dehydration is thought to be the major factor determining upper limits of distribution for intertidal algae (Baker 1909, Chapman 1966, Dring & Brown 1982, Smith & Berry 1986, Johnson et al. 1974, McLachlan 1974, Quadir et al. 1979, Beer & Eshel 1983, Oates 1985, Johnston & Raven 1986, Smith & Berry 1986, Madsen & Maberly 1990, Harker et al. 1999). These investigations have focused primarily on three types of responses to desiccating conditions: (1) the rate of water loss, i.e., dehydration; (2) photosynthetic performance during emergence; (3) the recovery of photosynthetic performances upon re-immersion.

Several studies have compared rates of water loss among intertidal macroalgae concluding generally that macroalgae cannot retard water loss from an exposed surface. The rates of dehydration are thus most closely related to the ratio of surface area to volume ratio (Denny et al. 1985). The idea that macroalgae are zoned based on their rate of water loss has been dismissed as desiccation rates have not been found to be causally related with the vertical position (Dromgoole 1980, Dring & Brown 1982, Beer & Kautsky 1992). The degree of water loss is due primarily to the position on the shore (Johnson et al. 1974, Quadir et al. 1979, Dring & Brown 1982, Oates & Murray 1983, Hawkins & Hartnoll 1985, Madsen & Maberly 1990), and it appears that species growing higher on the shore are simply more able to withstand greater levels of water loss, an idea first proposed by Baker (1909) and supported by subsequent studies (Smith & Berry 1986, Madsen & Maberly 1990). Unlike most terrestrial plants, life in arid conditions depends more on tolerating water loss rather than preventing it.

Photosynthesis during emersion has also been found to vary among different intertidal species and between ecotypes of given species of seaweeds (Madsen & Maberly 1990). Early studies demonstrated an increase in the photosynthetic rate during the early stages of drying due to the greater availability of CO_2 in air relative to water (Stocker & Holdheide1937). This phenomenon has now been observed for a wide range of intertidal algae (Johnson et al. 1974, Brinkhuis et al. 1976, Quadir et al. 1979, Oates 1985). This peak in photosynthesis is usually followed by a gradual decline in photosynthetic rate (Kanwisher 1966, Quadir et al. 1979, Dring & Brown 1982, Beer & Kautsky 1992) as the surface of the alga dries therefore preventing further dissolution and uptake of CO_2 by the alga. Photosynthesis eventually ceases at a critical stage of dehydration (Gessner & Schramm 1971, Brinkhuis et al. 1976, Quadir et al. 1979, Madsen & Maberly 1990) when the low water content of the thalli damages the function of the photosynthetic apparatus (Madsen and Maberly 1990). Some intertidal algae can, however, maintain a positive net carbon balance until they lose 50% of their fully hydrated water content (Hodgson 1981, Dring & Brown 1982, Beer & Eshel 1983). It appears that it is the dehydration that impedes photosynthesis as the rate of photosynthesis does not decline over time if the thallus is not dried out (Dring & Brown 1982).

The zonation of the different species is due partly to their ability to photosynthetise better when exposed to air (Madsen & Maberly 1990) and partly to withstand desiccation The stress imposed by desiccation could be fatal, but the most likely scenario is likely to be chronic, sub-lethal stresses that affect the rate of recovery once re-immerged. This recovery of photosynthetic abilities appears to depend largely on the degree of desiccation experienced by the alga. For example, recovery of *Fucus* was complete up to levels of dehydration of 20-30% (Madsen & Maberly 1990). The exact threshold varies among species (Dring & Brown 1982, Smith & Berry 1986, Brown 1987, Beer & Kautsky 1992), with species living higher on the shore being able to recover from a greater degree of dehydration (Schramm 1968, Dring & Brown 1982, Smith & Berry 1986, Beer & Kautsky 1992), even as much as 70% for some fuoid species (Beer & Kautsky 1992).

From these studies, we now have an emerging understanding of the ecological and physiological response of macroalgae to this unique physical environment. This knowledge is, however, almost entirely based on studies of the adult stages whereas information on juvenile stages, the precursors of adults, remains scarcely investigated (Vadas et al. 1992; but see, for example, McLachlan 1974, Gerard 1990, Brawley & Johnson 1991, Davison et al. 1993, Hanelt et al. 1994, Davison & Pearson 1996, Major & Davison 1998, Aguilera et al. 1999, Pearson et al. 2000). The ability of the younger stages of algae to survive in certain areas contributes to the patterns of intertidal zonation observed in adult stages. Several scenarios are possible. First, the physiological tolerance of juvenile stages is similar to the adult stages so that neither juveniles nor adults can survive outside of certain physical limits. A more likely alternative is that juvenile stages are less tolerant of such stressful conditions, thus more benign conditions are required for recruitment to occur (e.g., moist microhabitats, cool periods). Such conditions would provide "windows of opportunity" for the juvenile stages to grow to a size or stage that could better tolerate initial conditions. Mathieson (1982) measured the lethal desiccation of brown algae germlings after exposure to air in the shade and in the sun and found that germlings could withstand air exposure of 10 min in direct sunlight. Thus, the behaviour of germling stage of algae may play an important role in determining the ultimate distribution of the adult stages.

Our knowledge of the ecology of juveniles is hampered by their small size (e.g., Davison et al. 1993). In addition, our ability to conduct investigations on sublethal stresses in the field has been quite limited. Generally, the evaluation of photosynthesis in benthic marine primary producers is defined as the quantum yields for O_2 production or carbon fixation (Brinkhuis et al. 1976, Hodgson 1981, Beer & Eshel 1983, Madsen & Maberly 1990, Henley et al. 1991), but these kinds of experiments were done in the laboratory because the techniques used were not practical in nature: the young stages are difficult to see, difficult to manipulate, and the absolute level of their physiological response is small, often beyond the limits of detection. Recently, however, the application of pulse amplitude modulated (PAM) fluorometer has allowed rapid, non-intrusive, and extremely sensitive measurement of photosynthetic performance down to the level of isolated chloroplasts (Bradbury & Baker 1984, Schreiber et al. 1986). These chlorophyll fluorescence measurements permit the successful estimation of the functioning of photosynthetic apparatus, especially that of PSII (Genty et al. 1989, Schreiber et al. 1995) as well as its dynamic properties in withstanding damage from environmental factors. During the past ten years, it has been used successfully on adult seaweeds (Hanelt 1992, Henley et al. 1992, Franklin 1994, Häder et al. 1996, Hanelt et al. 1997, Hanelt 1998, Magnusson 1997, Hanelt 1998, Beach et al. 1999, Beer et al. 2000) but has yet to be applied to juvenile stages.

The littoral Fucales (brown algae) offer an excellent opportunity to conduct such studies. They have been intensely studied and due to their simple diplontic life cycle (i.e., no alternation of generations), and substantial work has been done on the ecology of the early post-settlement stages (e.g. Brawley et al. 1999). They are dominant macroalgae on temperate shorelines and form well-defined belts which appear to depend, in part, on adjustements that take place in the photosynthetic apparatus in response to a combination of several factors like excessive light, temperature and desiccation (Chapman 1995). Moreover, fucoid gametes and embryos are easily obtained (Quatrano 1980) in large quantities and the zygotes can be settled onto a variety of surfaces including epoxy disks (Brawley & Johnson 1991) that facilitate the manipulations.

In Québec, in a maritime estuarine habitat where the tide is semi-diurnal, the organisms are exposed to the atmosphere by the low tide twice a day. Three species of Fucales form a typical zonation pattern within the intertidal environment. *Ascophyllum nodosum* (L.) Le Jolis grows highest up in the zone and is exposed to air for many hours every day; *Fucus vesiculosus* L. dominates the mid-intertidal zone and is found on both submerged and emerged rock surfaces. Finally, *F. distichus* ssp. *edentatus* occurs in the low zone and is exposed to shorter periods of emersion.

This study uses PAM fluorometry to investigate the abilities of the earliest stages of these algae to tolerate the emersion during ebbing tide and the recovery once re-immersed. By transplanting juveniles to areas outside the normal distribution of the adult stages, we could determine if environmental factors affecting early stages might be able to determine the eventual distribution on adult stages. Moreover, we investigated the potential of different microhabitats to provide refuges for these early stages from these environmental factors.

4.4 Methods

4.4.1 Study area

The study was conducted in the intertidal zone at Pointe-Mitis near Mont Joli, Québec, Canada (48°41'N, 68°2'W), a semi-sheltered shore (Bourget et al. 1985) consisting of a series of rocky ridges interspersed with consolidated boulder fields. These boulder fields and the lower portions of adjacent ridges are dominated by three species of fucoid algae that form dense canopies covering up to 80% of the substratum. Perennial algae are not able to colonize the more exposed rocky surfaces (e.g., the top portions of the ridges) due to abrasion by ice (Archambault & Bourget 1983). Study sites were separated by approximately 5 meters along a transect perpendicular to the shoreline which was oriented towards the northwest (Chapter 2, Figure 2.1).

4.4.2 Fertilization in the laboratory

At each location, we haphazardly collected receptacles (Figure 1.1) without obvious epiphytes which were then transported back to the laboratory on ice and stored at 10°C in dark conditions. Before use, the receptacles were briefly washed with seawater and a toothbrush to remove any epiphytes (e.g., diatoms) from the receptacle surface. The receptacles were then osmotically shocked to induce gamete release by air drying for 20 min followed by 5 min in cold tap water. After this treatment, sets of 4 receptacles were put into petri dishes filled with filtered (5 μm) seawater.

4.4.3 Artificial substrata

The gametes that were subsequently released then accumulated on and fixed to small artificial substratra placed on the bottom of the petri dish. The number of embryos per disk ranged from 200 to 300. These artificial substrata consisted of small disks (4 mm thick; 20 mm in diameter) made of a white epoxy-filled putty made by Sea Goin' Poxy Putty ™ , Permalite Industries, Newport Beach, CA, USA) through a molding and casting process (Brawley and Johnson 1991, Johnson 1994). Before beginning experiment, embryos were grown for 3 weeks under 60 μmol photon $m^{-2}.s^{-1}$ polychromatic light with photoperiod of 8:16 (Light/Dark) at 10°C. The seawater was replaced twice a week. The disks were transferred into the field for the duration of each period of field measurements, usually no more than 7 h after which they were returned to the laboratory culture conditions.

To study the performance *in situ*, disks with the attached embryos were outplanted into field sites by attached them with small stainless steel screws to small, polycarbonate base plates (3 mm thick, 85 mm in diameter) which were then attached to the substratum using stainless steel screw driven into plastic wall anchors placed in holes drilled into the rock surface (Johnson 1989). Each experimental unit consisted of three disks with embryos attached to a single base plate (Figure 4.1); the mean value for the three disks was then used as the datum for that particular experiment unit.

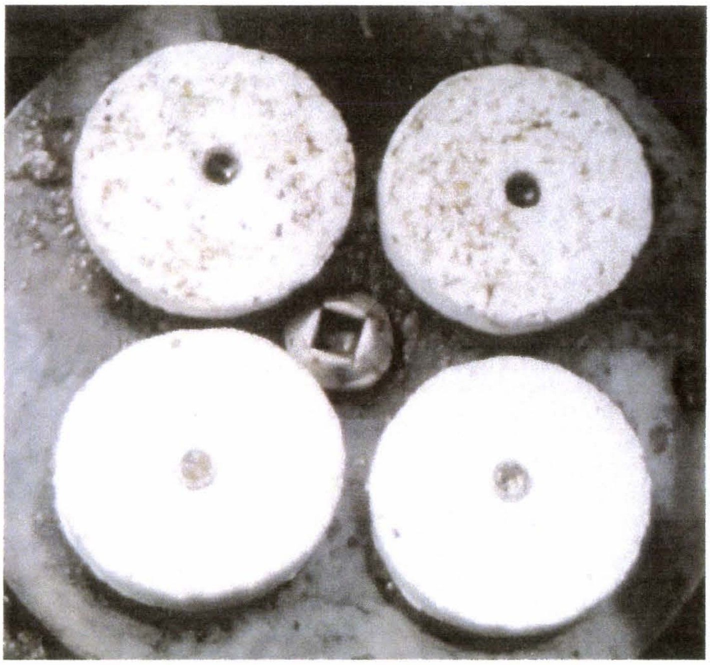


Figure 4.1: Four artificial substrata consisted of small disks (4 mm thick; 20 mm in diameter) made of a white epoxy-filled putty made by Sea Goin' Poxy Putty ™ , Permalite Industries, Newport Beach, CA, USA (Brawley and Johnson 1991).

4.4.4 Fluorometry

The photosynthetic responses of the embryos were examined ***in situ*** during the emersion using pulse-amplitude modulated (PAM) fluorometer (Walz GmbH, PAM 2000 portable fluorometer). Measurements were taken at 30 min intervals using the following protocol: (1) each location was covered with an opaque plastic covering for the first 15 min to dark-adapt the embryos, an obligatory step in certain fluorescence measurements; (2) the disks were uncovered and fluorescence measurements made for the embryos on each disk; (3) the disks were then left exposed to the natural conditions of their respective microhabitat for the reminder of the 30 min period. This protocol obviously reduced the levels of insolation and desiccation experienced by the plants, thus the resulting patterns are conservative estimates of the stresses experienced by these plants.

For each experiment, one saturating 800 ms flash was given to the embryos which were provided by the fibre optic sensor held to a constant distance away from the disk surface. Preliminary measurements were taken to determine the distance needed to achieve levels of 300-400 needed for accurate measurements (Walz 1993). The first measurement provided the following fluorometric parameters: (1) Fo and Fm, the minimal and maximal fluorescence yields, respectively, after dark-adaptation [the difference (Fm-Fo) is termed Fv]. A subsequent saturating flash at the end of the light period (100 ms) provided Fo' and Fm', the minimal and maximal fluorescence yields, respectively with light-adaptation. From these parameters, the ratio Fv/Fm, Y, rETR, qP and NPQ can be calculated.

The ratio Fv/Fm [=(Fm-Fo)/Fm] is derived from measurements made after the first saturating pulse and provides information on the maximum quantum yield of PSII after the plant is dark-adapted and all reaction centers are open. An alternative measure, the effective quantum yield of PSII (Y) conversion is calculated as:

$$Y = (Fm'-Ft)/Fm' = \Delta F/Fm'$$

where Ft is the fluorescence measured at time t: From values of effective quantum yield, values of rETR, the relative electron transport rate of PSII, can also be calculated:

$$rETR = Y \times PAR \times 0.5$$

Where Y is the effective quantum yield of PSII, PAR is the actinic irradiance in μmol photon m^{-2} s^{-1}; 0.5 is a multiplication factor needed to account for the fact that the transport of a single electron requires two photons.

The maximal fluorescence yield (F'm) in the presence of light can be reduced by two different processes: photochemical processes (i.e., photosynthesis) and heat dissipation. Quenching parameters (photochemical and non-photochemical) can provide insights into the relative importance of these two mechanisms in controlling the overall photosynthetic activity. The former is estimated by the photochemical quenching (qP) which is related to the fluorometric parameters as follows:

$$qP = (Fm'-Ft)/(Fm'-Fo)$$

The latter process is estimated by the non-photochemical quenching (NPQ) which is defined:

$$NPQ = (Fm-Fm')/ Fm'.$$

The origin of non-photochemical quenching is in change in the antenna of PSII implying the xanthophylls cycle (Demmig-Adams 1990). The formation of these xanthophylls in the antenna tends to facilitate the loss of energy excitation via radiationless decay. To calculate each NPQ during the experiment, we used the initial value of Fm obtained at the beginning of emersion.

4.4.5 Differences among species

The first experiment consisted of outplanting disks with attached embryos into three locations (i.e., replicates, separated by a distance of 30 cm) in each of the zones typically dominated that our fucoid species, i.e., a total of 9 experimental units (3 species/zones x 3 replicate locations). Each location was an exposed rock surface located approximately 15 cm from the edge of any algal canopy. Based on related research (Chapter 2) and the results of other studies (Lüning 1980, Brawley & Johnson 1991) that suggest that fully exposed conditions are lethal for fucoid embryos, we attempted to moderate the severity of the conditions in these exposed rock surfaces by adding a screen of mosquito netting (a closed cylinder of 1mm mesh, 10 cm in diameter and 5 cm high) around the base plate which reduced the incident solar radiation by 50%.

Each experiment was initiated when the lowest locations in the *F. distichus* ssp. *edentatus* zone were emerged by the ebbing tide. At that time the base plates with attached disks were set out quasi-simultaneously in all locations. They were then immediately covered by an opaque plastic sheet for a 15-min period of dark adaptation. Fluorescence measurements were then taken in the ensuing 15-min period, and then the cycle was repeated every 30 minute periods until the lowest locations were re-wetted by the rising tide; at this moment all experimental units were put in filled seawater petri dishes and the recovery response were examined for two additional periods separated by 15 min. After the experiment, the plates were returned to culture conditions until the next experiment. The disks were regularly inspected to assure that there was no substantial mortality of embryos after experimental or culture periods.

This experiment was conducted on 12 different dates in July and August 1999. As disks with embryos were reused for subsequent days, they were thus several weeks older by the end of the experiment. Because of the daily advance of the tidal cycle (approximately 45 min each day), the timing of the low tide varied among the different days but always fell between 10:30am and 3:30pm. To compare the physiological responses under different physical conditions, the days were selected to fall into four categories: (1) "sunny-calm" – no clouds or wind, and light intensities (PAR) between 900 et 1200 µmol photon $m^{-2}s^{-1}$ (2) "cloudy-calm" – clouds present and light intensities between 200 and 500 µmol photon $m^{-2}s^{-1}$ (3) " sunny-windy " as for sunny-calm but with wind speeds averaging 33 km/h (4) "cloudy-windy" – as for cloudy-calm but with wind speeds averaging 33 km/h.

4.4.6 Effect of microhabitat

In the second experiment, embryos on disks were outplanted on disks (again 3 disks per baseplate) into two contrasting microhabitats: under the canopy of adult plant and onto the exposed rock surface. The locations for the "adult canopy" treatment were selected at places where mature plants 20-40 cm long would create a single layer over the plate. "Exposed rock" locations were simply areas of smooth bare rock surfaces selected within 10-40 cm of canopy positions. On sunny days (PAR = 1200 µmol photon.$m^{-2}.s^{-1}$) the light levels in the different microhabitats (as measured with a LiCor 2032) were 600 μmol photon.$m^{-2}.s^{-1}$in the exposed microhabitats (with screen shades), 150 μmol photon.$m^{-2}.s^{-1}$ under the canopy. The three replicates for any given species were set out within several meters of each other. This design resulted in 18 experimental units (3 zones x 2 microhabitats x 3 replicate locations).

Measurements were again taken at 30 min intervals but for this experiment, disks were outplanted not quasi-simultaneously in all zones but instead set out sequentially as each zone was exposed by the ebbing tide. Thus, measurements taken in the *A. nodosum* zone, the highest of the three, began 30-60 minutes before those taken in the lower zones. The experiment was, however, synchronized for the recovery measurements by placing all the disks into petri dishes with seawater at the moment when the lowest locations (*F. distichus* ssp. *edentatus*) were first submerged by the rising tide. Three subsequent fluorescence measurements were then taken at 15-min intervals to assess recovery after re-immersion. The experiment was again conducted over 12 dates during August and September 2000 with the days again chosen to provide contrasting meteorological conditions (see above). As before, disks and embryos were reused on subsequent days and held in the laboratory between trials.

4.4.7 Analysis

The fluorescence data were tested using a repeated-measures ANOVA model and analysed using SAS statistical package (SAS-Institute 1999-2001). The slopes of change in fluorescence parameters over time were calculated; comparisons between the slopes were tested using student's t-test.

4.5 Results

At the beginning of the experiments, Fo and Fm levels were determined in the dark to characterize original values state of PSII in these embryos. The maximum quantum yield (i.e., the ratio Fv/Fm) of the three species varied from 0.68 to 0.5, somewhat lower than the values observed in adults thalli (0.75; M. Lamote, unpubl. data). However the initial maximum quantum yield of *F. distichus* ssp. *edentatus* was consistently 0.1 lower than the two other species. During the period of low tide, we generally observed an immediate (i.e., within 30 min) and regular decrease of Fv/Fm and the effective quantum yield (Y) of the embryos from the three species, leading to zero values in all cases (see below).

4.5.1 Differences between species

Under the most severe desiccating conditions (sunny-windy) but within the normal zones of each species, the maximum quantum yield (Fv/Fm) of *A. nodosum*, and *F. vesiculosus* had similar time courses and decreased less rapidly than for *F. d.* ssp. *edentatus*, which reached a zero value 60 minutes sooner than the two others, just after 2 h (Figure 4.2 A, Table 4.1).

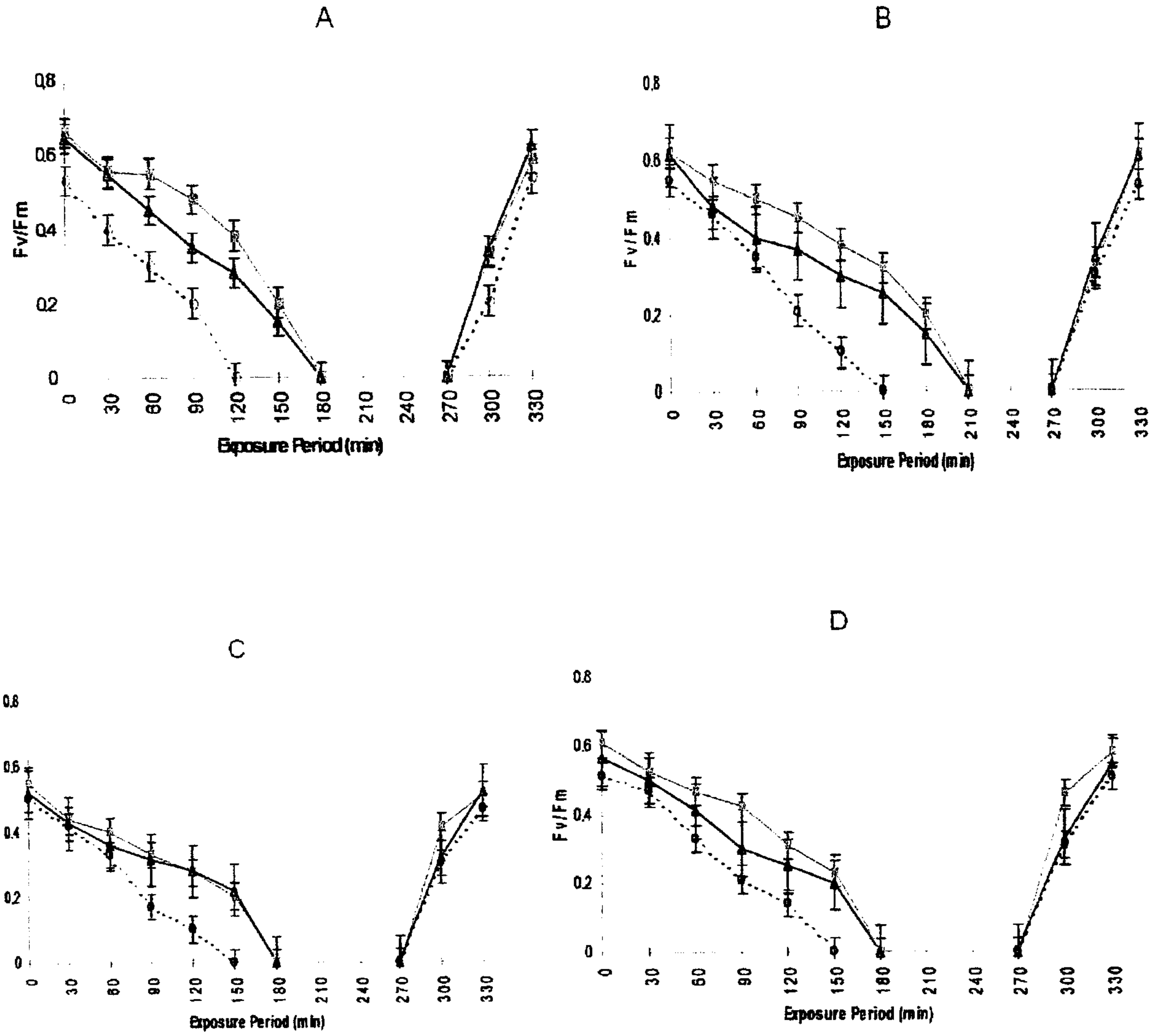


Figure 4.2 : Time course of Maximum Quantum Yield (Fv/Fm) of three species, (-■-) *A. nodosum*, (-Δ-) *F. vesiculosus* and (--o--) *F. distichus* ssp. during low tide and at the beginning of flooding tide (270 min) under (A) sunny-windy conditions, (B) cloudy-calm conditions, (C) cloudy-windy conditions and (D) sunny-calm conditions. Error bars are 95% confidence intervals (n=3).

Table 4.1: Comparison between slopes of fluorometric curves (Effective quantum yield) of species (An = ***Ascophyllum nodosum*** (L.) Le Jolis, Fv = ***Fucus vesiculosus***, and Fd = ***Fucus distichus*** ssp.) placed in each area under four meteorological conditions; SW, "sunny-windy", C, "cloudy-calm", W, "cloudy-windy" and S, "sunny-calm". Different letters are significantly different at $p<0.05$.

Meteorological Conditions	Species	An	Fv	Fd
Sunny-windy		A	A	B
Cloudy-calm		A	A	A
Cloudy-windy		A	A	B
Sunny-calm		A	A	B

Under the begnin cloudy-calm conditions, the decrease of Fv/Fm to a null value for *F. d.* ssp. ***edentatus*** required an additional 0.5 hr, and those for ***A. nodosum (L.)*** Le Jolis and ***F. vesiculosus*** were again 1 hr later (Figure 4.2 B). The statistical difference between ***F. vesiculosus*** and *F. d.* ssp. ***edentatus*** was marginal (Table 4.1; p=0.06). Under cloudy-windy conditions, the decrease of Fv/Fm of *F. d.* ssp. ***edentatus*** was as fast as under cloudy-calm conditions, but the decline in the other two were more like the pattern seen under sunny-windy conditions (Figures 4.2 A-C). The responses under sunny-calm condition were similar to those of cloudy-windy although values for ***A. nodosum***, the high intertidal species, were more distinctly above those of the other two species for the first two hours (Figures 4.2 C-D). Although the data suggest a trend of increasing resistance with increasing height in the intertidal zone, the two higher species did not statistically differ from each other whereas *F. d.* ssp. ***edentatus*** was distinctly different in 3 of the 4 conditions (Table 4.1). The decrease of the Fv/Fm ratio with time was generally due to both an increase of Fo and a decrease in Fm (data not shown).

The variations of effective quantum yield (ϕ_{PSII}) exhibited almost identical patterns to those described for Fv/Fm during emersion (Figures 4.2 and 4.3).

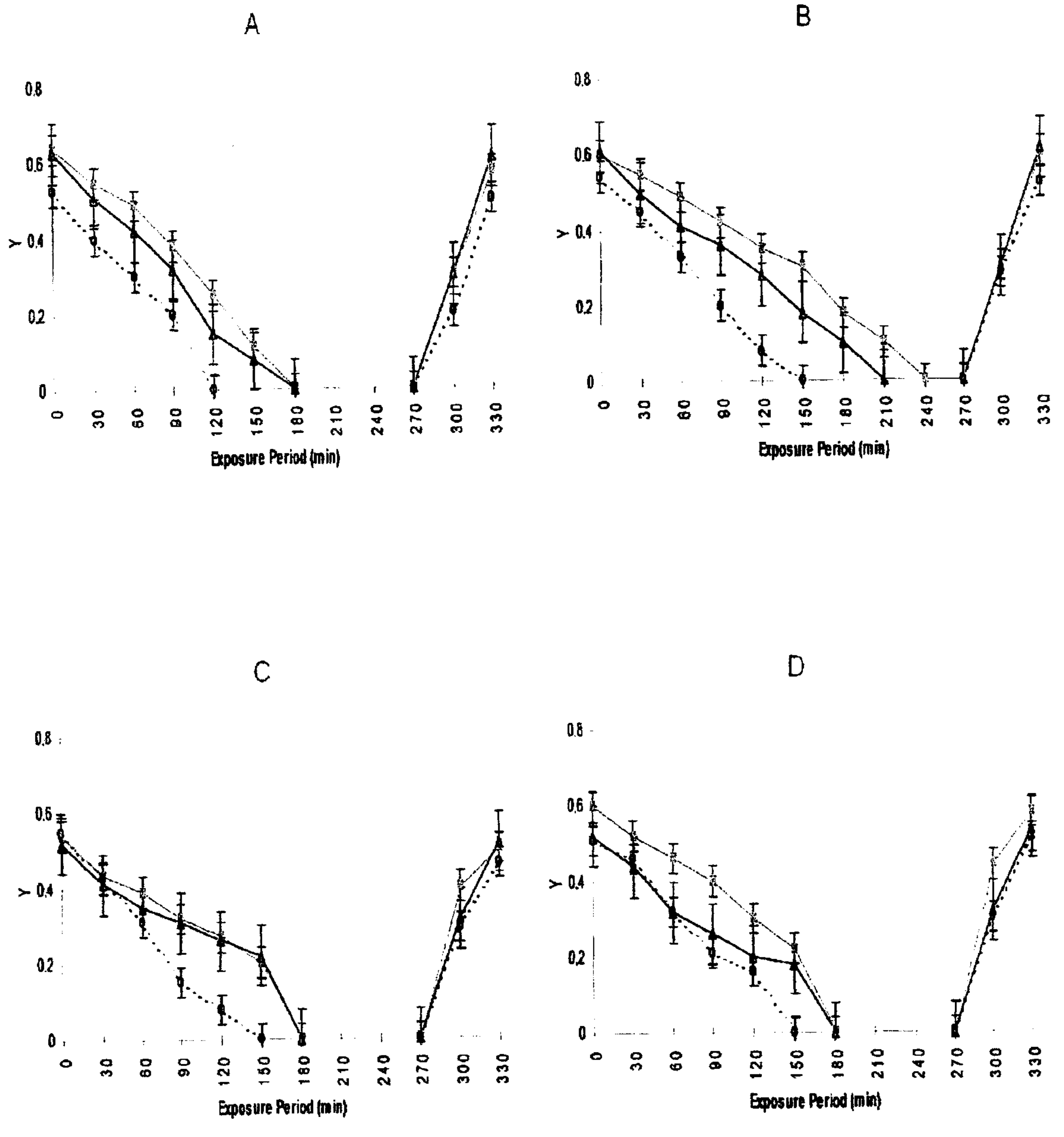


Figure 4.3 : Time course of Effective Quantum Yield (Y) of three species, (-■-) *A. nodosum*, (-Δ) *F. vesiculosus* and (--o--) *F. distichus* ssp. during low tide and at the beginning of flooding tide (270 min) under (A) sunny-windy conditions, (B) cloudy-calm conditions, (C) cloudy-windy conditions and (D) sunny-calm conditions. Error bars are 95% confidence intervals (n=3).

Changes in rETR during the period of low tide largely mirrored those seen for Y (Figures 4.3 and 4.4) but were also affected by PAR.

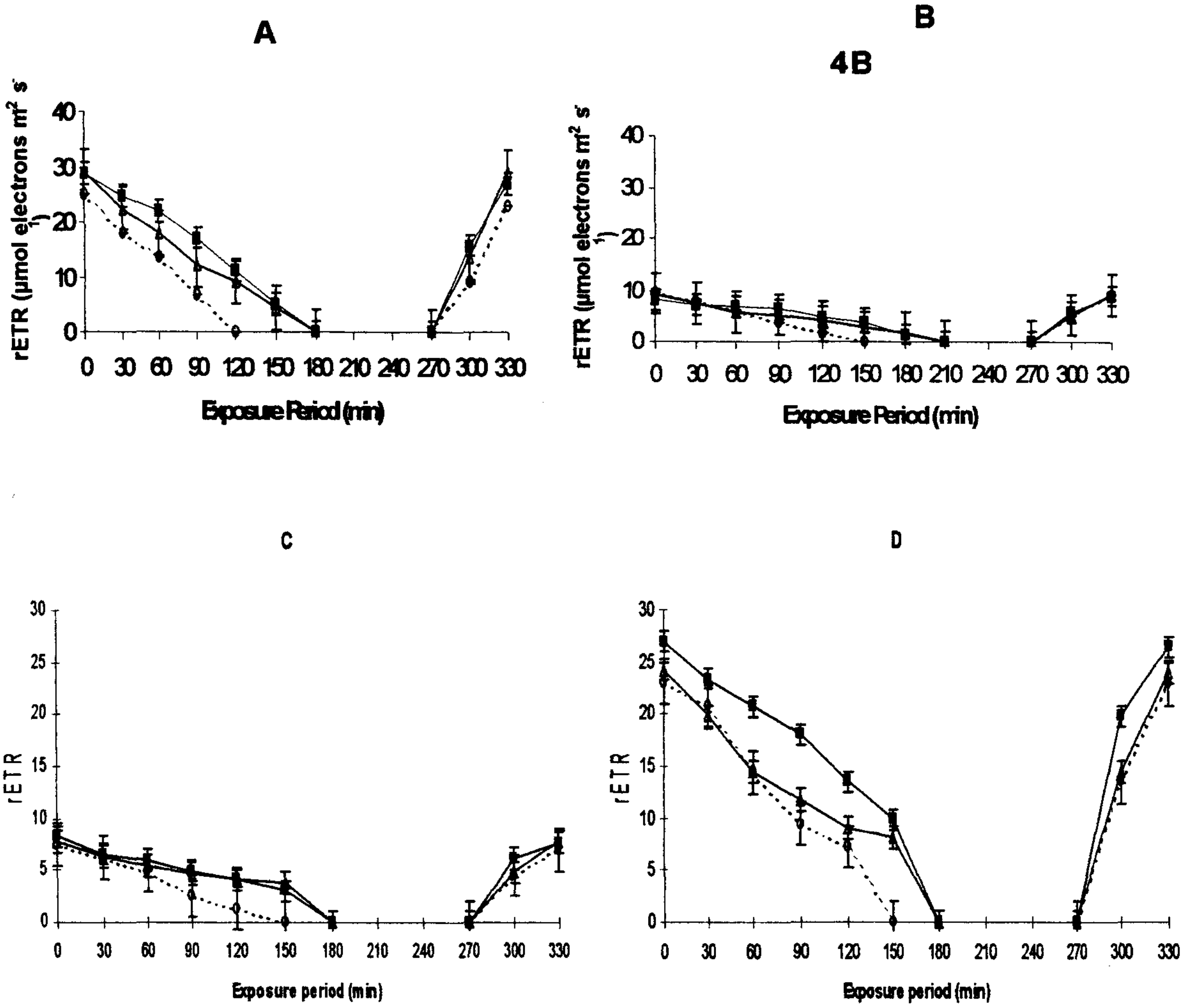


Figure 4.4 : Time course of Relative Electron Transport Rate (rETR, µmol electron m^{-2} s^{-1}) of three species, (-■-) *A. nodosum*, (-Δ-) *F. vesiculosus* and (--o--) *F. distichus* ssp. during low tide and at the beginning of flooding tide (270 min) under (A) sunny-windy conditions, (B) cloudy-calm conditions, (C) cloudy-windy conditions and (D) sunny-calm conditions. Error bars are 95% confidence intervals (n=3).

Under cloudy conditions (Figures 4.4 B, C), the values of rETR were weak in comparison to the values in sunny conditions (Figures 4.4 A, 4.4 D). Among the different species, the rETR for *F. d.* ssp. *edentatus* reached a null value 30 to 60 min before the other species except under conditions of sunny-calm when all species reach a null value at the same sampling time (Figure 4.4 D).

The increases of NPQ reflected the decreases in the effective quantum yield (Y). This increase was most rapid for *F. d.* ssp. *edentatus*, especially under sunny-windy conditions where separation of the three species was most evident (Figure 4.5). In all cases, increases of NPQ were slowest for *A. nodosum*, but not significantly different from *F. vesiculosus*. Under cloudy-calm conditions, there were only slight differences between species (Figure 4.5 B).

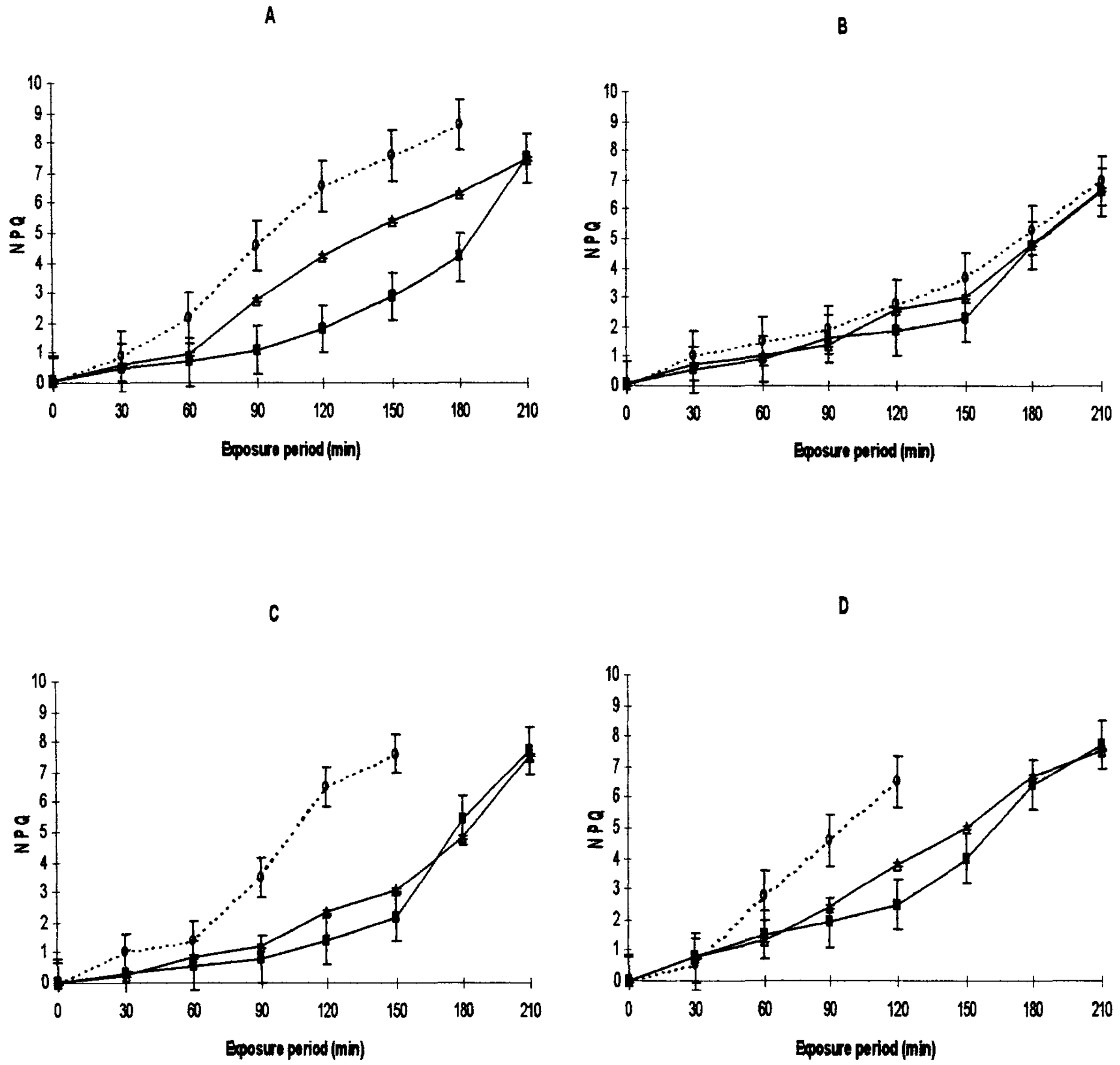


Figure 4.5 : Time course of NPQ of three species, (-■-) *A. nodosum*, (-Δ-) *F. vesiculosus* and (--o--) *F. distichus* ssp. during low tide and at the beginning of flooding tide (270 min) under (A) sunny-windy conditions, (B) cloudy-calm conditions, (C) cloudy-windy conditions and (D) sunny-calm conditions. Error bars are 95% confidence intervals (n=3).

Using the PAM-2000 software, we also obtained during the low tide the light-saturation curves of the three species; these curves were derived from plotting rETR values against PAR values. The light-saturation curves showed that rETR values of *A. nodosum* increased faster than the other two species (Figure 4.6) but that three species all reached the same saturation value.

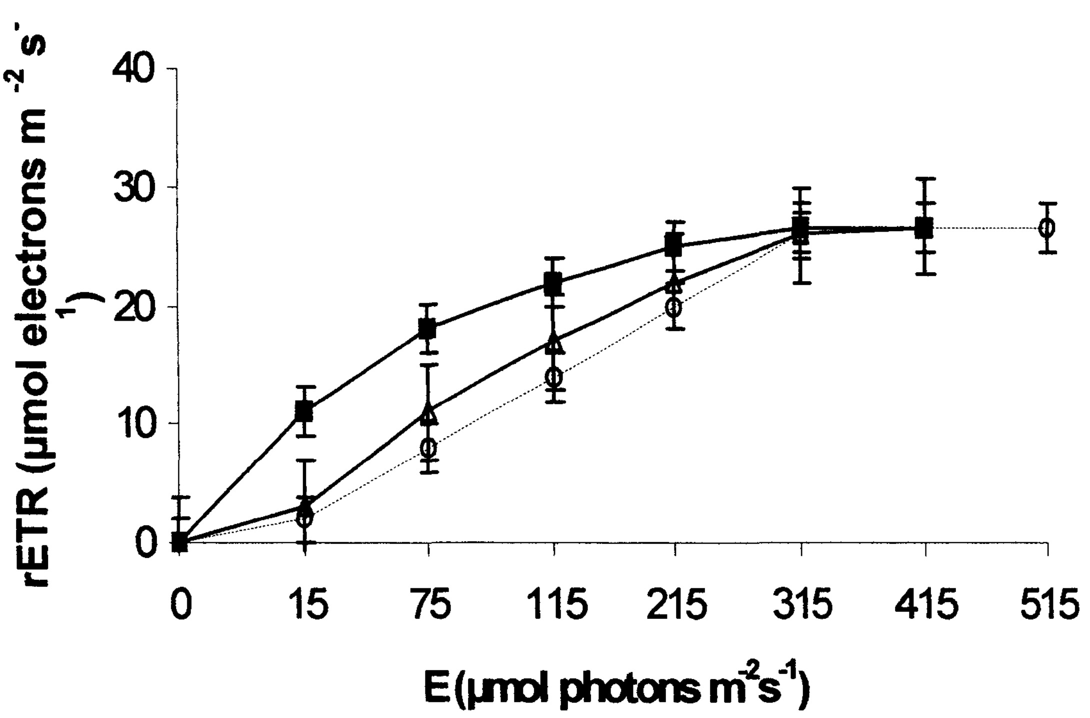


Figure 4.6: Pulse induction saturation curves for net photosynthesis (rETR, µmol electron m^{-2} s^{-1}) versus irradiance (E). (-■-) *A. nodosum* (L.) Le Jolis, (-Δ -) *F. vesiculosus* and (--o--) *F. distichus* ssp. Error bars are 95% confidence intervals (n=3).

4.5.1.2 Recovery

The recovery of Fv/Fm, Y, and rETR were fast for the three kinds of embryos in all meteorological conditions and reached the initial values seen at the beginning of low tide one hour later (Figures 4.2-4.3-4.4). After the first splash of seawater, the values of Fv/Fm, Y and rETR reached a third of the initial value by just 15 min later (data not shown). The recovery of Fv/Fm of *F. d.* ssp. *edentatus* was slightly slower, and statistically significant from the other two species, under sunny conditions (Figures 4.2 A, D). Under cloudy-calm conditions, this difference was marginally significant ($p=0.064$), but there were no significant differences between *F. vesiculosus* and *A. nodosum* ($p= 0.97$).

4.5.2 Canopy vs. exposed microhabitats

The general difference observed between species was also observed for embryos under the canopy although ...There was a general decrease of the maximum quantum yield, and rETR during the emersion for the embryos of the three species under a canopy of adult algae. Under sunny-windy conditions, Fv/Fm was lowest for *F. d.* ssp. *edentatus* and reached a null value 30 minutes before the other two species (Figure 4.7 A). The decrease of both other species was similar. Under cloudy calm conditions, there was a slightly decrease even under the canopy for the three species (Figure 4.7 D). There were no significant differences between the responses of the higher species, *A. nodosum* and *F. vesiculosus* ($p>0.05$).

The higher PAR available on sunny days was responsible of a higher rETR than on cloudy days (Figure 4.8). After Y decreased to zero, the rETR remained null in all species for a period of one hour at the end of low tide on sunny days. These rETR values were about one third of those observed on exposed thalli (Figure 4.8/Figure 4.4). Under all meteorological conditions, rETR values were small and all species responded in the same way, a decrease and to a null value after 3.5-4 hours (Figure 4.8). Under the more benign conditions of cloudy skies with little or no wind, photosynthetic efficiency (ETR) remained constant for the first half of the low tide after which it decreased within a period of 60-90 minutes (Figure 4.8 B).

This permitted the algae to continue photosynthesizing normally a large part during the low tide.

Related to the decrease of the effective yield of PSII, an increase of NPQ was observed during low tide. Under sunny-windy conditions, the NPQ of *F. distichus* reached a high level after 2 hr (Figure 4.9 A) followed by a slower increase. In the two other species, the increase of NPQ was delayed. Under cloudy conditions, the NPQ values were similar in the three species and the increase with time was slower (Figure 4.9 B).

4.5.2.2 Recovery

When the thalli were soaked with sea water, the recovery of rETR was achieved in all species within 1 hr. No significant differences between the three species was observed (Table 4.2; $p>0.05$).

This experiment examined the importance of the location of young embryos at the beginning of low tide under the protection of adults or in exposed conditions. The protection of adults should provide a refuge from the physical environment experienced during low tide by reducing the desiccation and light intensity experienced by the embryos.

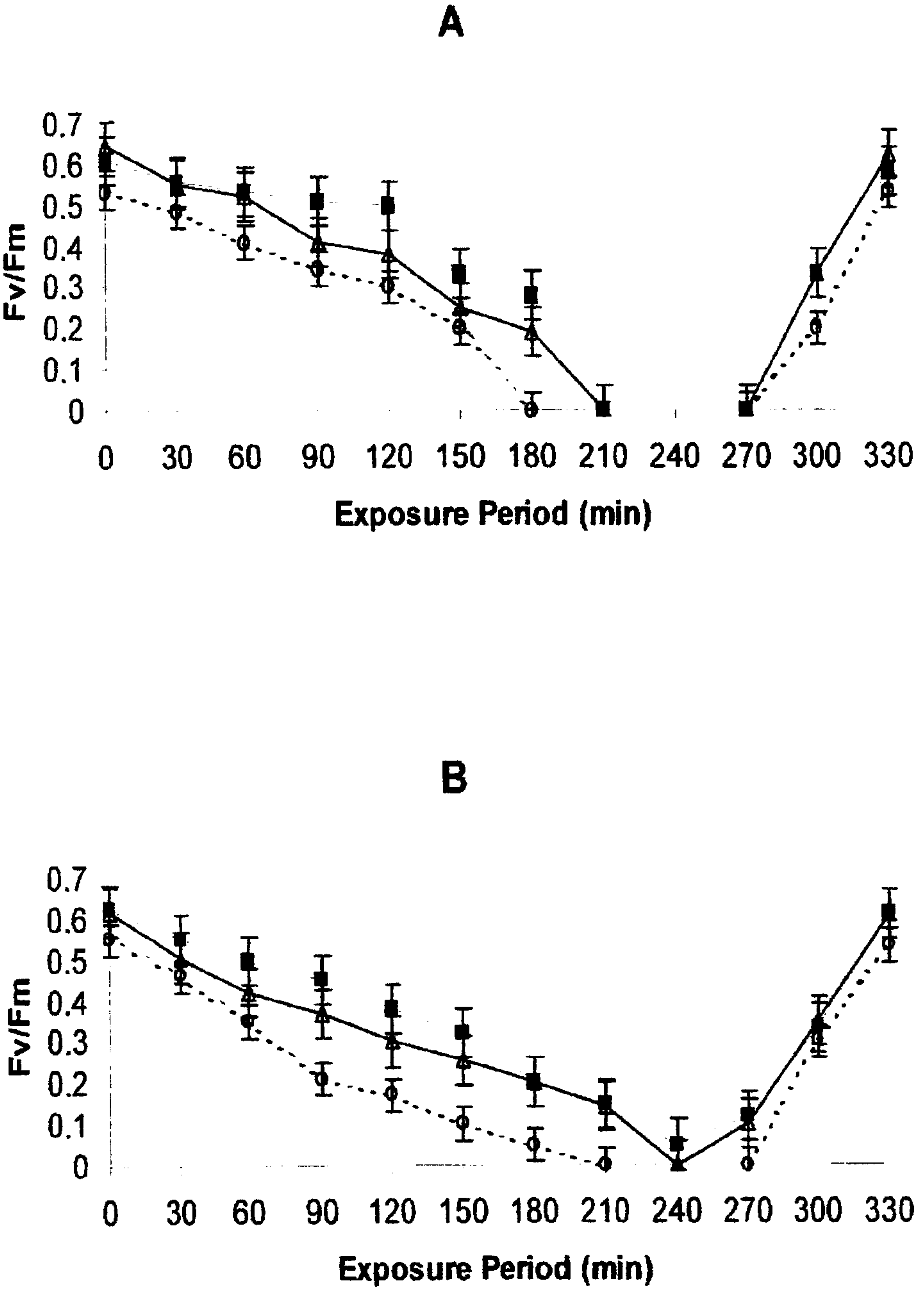


Figure 4.7: Time course of Maximum Quantum Yield (Fv/Fm) under canopy under (A) sunny-windy conditions and (B) cloudy-calm conditions; (-■-) *A. nodosum* (L.) Le Jolis, (-Δ-) *F. vesiculosus*, and (- -○- -) *F. distichus* ssp. Error bars are 95% confidence intervals (n=3).

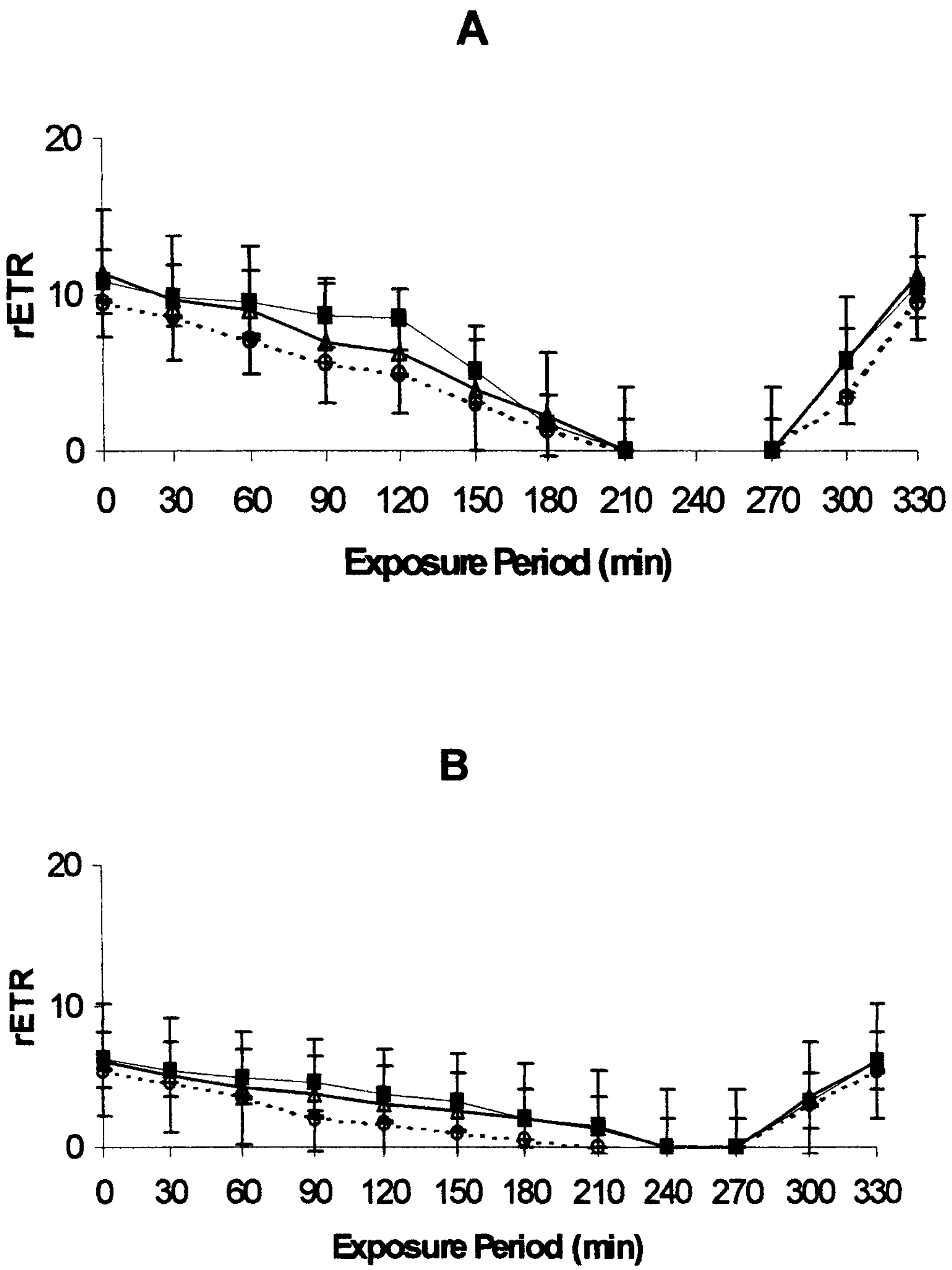


Figure 4.8 : Time course of Relative Electron Transport Rate (rETR, μmol electron m^{-2} s^{-1}) under canopy under (A) sunny-windy conditions and (B) cloudy-calm conditions; (-■-) *A. nodosum* (L.) Le Jolis, (-Δ-) *F. vesiculosus*, and (--o--) *F. distichus* ssp. Error bars are 95% confidence intervals (n=3).

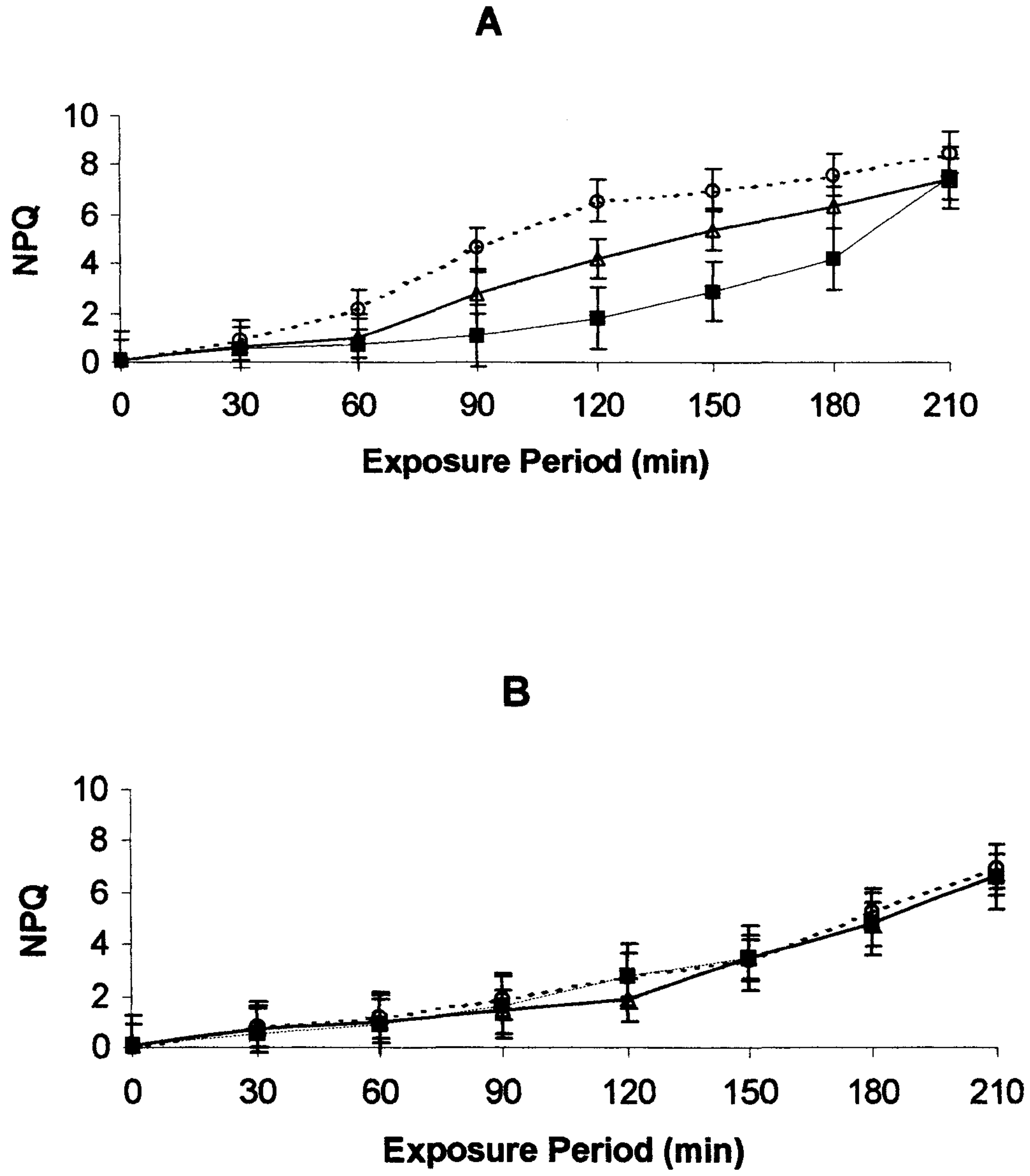


Figure 4.9: Time course of Non-Photochemical Quenching (NPQ) under canopy under (A) sunny-windy conditions and (B) cloudy-calm conditions; (-■-) *A. nodosum* (L.) Le Jolis, (-Δ-) *F. vesiculosus*, and (--o--) *F. distichus* ssp. Error bars are 95% confidence intervals (n=3).

4.6 Discussion

4.6.1 Effect of emersion of PSII

Embryos of all three species lost photosynthetic abilities during emersion under all meteorological conditions. This pattern was true regardless of the exact manner in which performance was assessed, not a surprising result given that all these measures (Fv/Fm, yield, rETR, and NPQ) are derived from subsets of a shared pool of parameters. The photosynthetic behavior of fucoid embryos followed the behavior pattern seen in the adults of other macroalgae, namely, an induction of photoinhibition, declines of effective (Fv/Fm) and maximum (Y) quantum efficiency, and an increase in non-photochemical quenching in macroalgae, including green, red, and brown macroalgae as well as unicellular algae (Leverenz et al. 1990, Franklin 1992, Hanelt 1992, Larkum & Wood 1993, Häder et al. 1996, Häder & Figueroa 1997, Beer et al. 1998, Franklin et al. 2001).

These fluorescence data for the exposed thalli of exposed juveniles did differ from previous observations on adult brown algae (Büchel & Wilhelm 1993, Häder et al. 1996, Davison & Pearson 1996). Usually the values of the maximum PSII quantum yield (Fv/Fm) for brown algae are near 0.7 (Büchel & Wilhelm 1993, Dring et al. 1996), but for the embryos, even after two months of growth, the maximum quantum yields were lower than levels commonly observed in adults. Two hypotheses could explain these low maximum PSII quantum yields: (1) the embryos might be too young and their photosynthetic apparatus not fully developed or (2) embryos behave like shade adapted plants. Shade-plants may be able to survive photoinhibition by maintaining a large population of photoinhibited (inactive) PSII reaction centres resulting in a high Fm but a low Fv, and thus a low Fv/Fm ratio (Oquist et al. 1992). Fv/Fm values were especially low for *F. d.* ssp. *edentatus*, the lowest species, and this observation, combined with the more rapidly saturating rETR curve and its high sensitivity to all meteorological conditions (see below) suggest that the embryos of this species are particularly likely to be adapted to shaded conditions such as those found under an adult canopy.

The decline in Y is thought to be a consequence of the energy-dependent or ΔpH quenching, also creating an increase in NPQ (Franklin 1992). This ΔpH initiates the xanthophylls cycle, thereby reducing photodamage of PSII by enhancing the thermal dissipation of energy associated with increased NPQ (Krause 1988, Demmig-Adams & Adams 1996). Quenching reduces the PSII antennae cross-section to minimise photoinhibition and to adjust the energy transfer to the transducer with excess energy dissipated as heat (Schreiber et al. 1994). Elevation of NPQ (heat dissipation) also help to reduce the deleterious effects of the accumulation of active oxygen species formed during desiccation stress in Fucales (Collen & Davison 1999).

Although the general decline in performance confirms that periods of emersion are indeed stressful (i.e., they induce suboptimal levels of photosynthetic processes), differences did occur among the different species and the different meteorological conditions, demonstrating that this response depends on both the biological and the physical aspects of the system. Among the three species, *F. d.* ssp. *edentatus*, the species distributed lowest in the intertidal zone, was consistently the most rapidly affected by conditions during emersion. Between the other two species, *A. nodosusm*, the species distributed highest on the shore, was often the least affected, but the differences observed between it and *F. vesiculosus* were never statistically different. It is, however, worthwhile to note that the general order of the species in term of their resistance to the stresses induced by the conditions during emersion matched that of their vertical distribution. Species normally distributed higher on the shore were more resistant to these stressful conditions.

Two possible ecological consequences could arise from the decline in photosynthetic performance during periods of emersion: (1) the direct effect of a reduction in the total carbon fixed by the alga, and (2) the indirect effect of any delay in the recovery of full photosynthetic ability after re-immersion in water. With regards to the first, it is clear that these algae rapidly lose photosynthetic ability during low tide, but without a better knowledge of the complete carbon budget for these species, it is difficult to determine how substantial this loss might be. This is especially true for the differences between the species, and at this point, we cannot assess what the impact that an additional hour of active photosynthetic activity during the low tide might be. In general, a considerable portion of the total carbon fixation of marine macroalgae occurs during low tide (Dring & Brown 1982, Bird & McLachlan 1985), and as been estimated to account for 20% of the total carbon budget for *Fucus spiralis* (Madsen & Maberly 1990), a species found in both Europe and North America, but not at our site.

The more indirect effect of stress during emersion on the recovery of photosynthetic performance after re-immersion appears to have little, if any, effect on the overall performance of these algae. Recovery was rapid in all cases, requiring less than 1 h for complete recovery. Moreover, while there were some statistical differences among the species, differences were never large. These results contrast with those from other studies on adult macroalgae recovery after air-drying was more complete in high intertidal algae than in low intertidal algae (Dring & Brown 1982, Brown 1987) and the ability of a species to recover photosynthesis after osmotic dehydration has been found to be correspond to its vertical position on the shore (Smith & Berry 1986). Our examination of recovery did not, however, mimic exactly the normal conditions that these algae experience. By initiating the experiment at the moment the lowest species, *F. d.* ssp. *edentatus* was exposed by the ebbing tide and terminating the exposure period when this same species was first re-submerged by the flooding tide, we effectively reduced the emersion period for othe two species by up to 2 h in the cased of *A. nodosum*. How an extension of the period of emersion might affect the recovery of the two highest species remains to be determined.

Exposure to terrestrial conditions during emersion subjects the algae to higher levels of insolation, desiccation, and temperature extremes, in this case higher temperatures than found in the water. The exact aspect causing stress in these algae was not addressed in this study, but, based on previous studies, it was most likely due to either dehydration or reduced availability of carbon in the form of bicarbonate. Unlike terrestrial plants, algae are unable to regulate their water loss and lose water freely (Schonbeck & Norton 1980, Dring & Brown 1982), and thus emersion almost always results in dehydration unless the relative humidity is very high or the algae are located in a protected microhabitat (e.g., under a canopy of other algae). Whereas desiccation tolerance of intertidal seaweeds varies among species, these differences are often related to their vertical distribution (Dring & Brown 1982, Brown 1987, Beer & Kautsky 1992). Many common intertidal algae including fucoids, can maintain a positive net carbon balance while losing more than 50% of their fully hydrated water content (Hodgson, 1981, Dring & Brown 1982, Beer & Eshel 1983). However, even if there is no tissue dehydratrion, photosynthesis can become limited by a lack of bicarbonate which easily forms at the air-water interface, but then becomes unavailable when the thallus surface dries out.

In all situations, rates of evaporation depend on the local environment. For example, a solitary alga will dry out faster than one located within a bed of algae, primarily due to the fact that a greater proportion of its surface area is exposed to the dry air. This effect of the local environment is likely to be even more important for young stages such as the embryonic stages examined in this study. Not only is the surface area to volume ratio higher for smaller individuals (assuming isometric growth), but local landscape features are relatively larger. In our specific case, the proximity of other embryos could have retained additional extracorporal water as could the small topographical depressions of the disk surface. In this study, we could not assess the exact moment at which the "external" was completely evaporated away and dehydration of the embryos began. Whereas this aspect has no bearing our relative comparisons among species and meteorological conditions is may be an important aspect for absolute determination of the suitability of specific microhabitats (e.g., rock surfaces of different textures). For example, the presence of a surrounding algal turf greatly increases short-term survival of fucoid embryos (Brawley and Johnson 1991) and is the microhabitat most likely to support recruitment on new individuals (Johnson and Brawley 1998). Similarity, high settlement densities increased survival of an intertidal green alga, presumably form reducing desiccation stress (Hruby & Norton 1979). The substratum used in this latter study was, however, glass slides, which do not offer the water absorption and retention of more natural surfaces.

Meteorological conditions also produced substantial variation in the photosynthetic responses of the fucoid embryos. Assuming that desiccation is the principal stress affecting macroalgue during emersion, we expected the most stressful conditions to occur under "sunny-windy" conditions when higher algal and air temperatures (from higher levels of insolation) and higher advective removal of water vapour at the substratum/algal surface (from higher wind speed) would synergtically increase rates of evaporation. The least stressful conditions should thus have occurred during "cloudy-calm" conditions with the other two scenarios falling somewhere in between. The fluorometric responses of the embryo were generally consistent with these expectations with the decline in Fv/Fm and Y being more rapid under sunny-windy conditions relative to cloudy-calm conditions. The intermediate conditions did not, however, elicit intermediate responses; both cloudy-windy and sunny-calm were quite similar to that of sunny-windy, suggesting that either sunny or windy conditions were sufficient to accelerate the decline in photosynthetic performance. The benefit of the more benign conditions of cloudy-calm was most obvious for the two highest species, and there was virtually no difference for *F. d.* ssp. *edentatus*, the lowest species, among the different meteorological conditions. This latter observation suggests that *F. d.* ssp. *edentatus* is sensitive to any type of emersion.

Differences in meteorological conditions were most pronounced for rETR, a measure more closely related to actual rates of photosynthesis. Because of the high availability of PAR during sunny conditions, levels of rETR were initially 3-fold greater under both sunny-windy and sunny-calm conditions relative to either cloudy condition. This advantage far outweighed the slight extension in the photosynthetically active period under cloudy-calm conditions. Thus, from an embryo's perspective, it appears better to have bright, sunny conditions even if it may result in more greater stress later during the low tide. The potential artifacts of shading the embryos must, however, be kept in mind as the full sun could have had lethal effects (Brawley & Johnson 1991) whereas the unshaded light levels during cloudy conditions (200 to 500 μmol photon.m^{-2}s^{-1}) would have been near the saturation level (400 μmol photon.m^{-2}s^{-1}) of the embryos.

The presence of an algal canopy appears to mitigate slightly the effects of emersion, but overall impact is surprisingly minor with just an extension of 30 to 60 min of the period of photosynthetic activity. Given that desiccation rates are expected to be quite low under an algal canopy (Brawley & Johnson 1993), a greater impact would have been expected. This observation in combination with those above suggest that the effects of desiccation may be much less that otherwise believed and that other processes (i.e., changes in temperature, limited supplies of bicarbonate) may also be important and may interact sygeristically with desiccation in affecting photosynthetic performance during emersion.

4.7 Conclusion

Fluoresent analysis of these three species indicates clearly that they cannot photosynthesize normally during extended periods of emersion, but that their ability to resist this stress varies among different species and under different environmental conditions, including conditions that vary markedly over short time scales (e.g., meteorological conditions) and those that are less temporally dynamic (e.g., the presence or absence of an algal canopy). Variations among species and meteological conditions approximated expectations although differences were often slight, even between extreme comparisons. Thus, the performance of these juvenile stages may only play a subtle role in determining the ultimate zonation of the adults, and a more complete knowledge of the carbon budget for these algae will be needed before we can determine if such the cumulation of such effects can have an major impact on the ecology of these species. Given the small differences in performance observed between extreme temporal (i.e., meteorology) and spatial conditions (i.e., canopy), we also need to readdress the perceived importance of desiccation in affecting the physiology of marine macroalgae.

CHAPITRE 5

Photosynthetic and demographic responses of an intertidal alga to a vertical gradient of desiccation and light

5.1 Résumé

Le comportement photosynthétique des thalles juvéniles de l'algue brune *Fucus distichus* ssp. *edentatus* (Fucales, Phaeophyta) a été étudié à différentes hauteurs de marée le long d'un mur rocheux dans la zone intertidale localisée dans la Péninsule de Gaspé, Québec, Canada. Au cours de la marée descendante, nous avons utilisé la fluorescence modulée (PAM) pour examiner le rendement quantique maximum du PSII (Fv/Fm), le rendement quantique effectif (Y), la vitesse de transfert d'électrons relatif (rETR) et l'extinction de fluorescence non-photochimique (NPQ) des juvéniles de fucales à cinq niveaux intertidaux séparés d'une distance de 1,2 mètre. Ces observations ont été réalisées sous quatre conditions météorologiques différentes, définies comme « ensoleillé-venteux », « nuageux-calme », « nuageux-venteux » et « ensoleillé-calme ». Le taux d'évaporation et les niveaux d'irradiance ont été mesurés simultanément. Un gradient de réponses a été généralement observé: les deux plus hauts niveaux montrèrent un déclin du Fv/Fm, du Y, et du rETR avec une augmentation concomitante du NPQ dans le temps. Les niveaux plus bas séparés de 60 à 120 cm des deux niveaux les plus hauts, moins rapidement exposés à la lumière et à la dessiccation ne montrent pas de diminution du rendement quantique, ni du transfert d'électrons mais une légère augmentation de l'extinction de fluorescence non-photochimique au cours de la prise de

mesures de fluorescence. L'association du vent et du soleil crée les conditions climatiques les plus sévères alors que les moins difficiles étaient les conditions nuageuses pour lesquelles seul le niveau le plus haut a montré une décroissance des rendements quantiques. Les corrélations entre les paramètres de fluorescence chlorophyllienne et le taux d'évaporation traduisent l'influence majeure du stress hydrique chez les très jeunes thalles et se manifestait par une densité de population et une croissance plus faible aux deux niveaux les plus hauts. Les stress de dessiccation et de lumière sont plus forts dans les niveaux les plus hauts et causent des retards de croissance et des réductions plus rapides de photosynthèse que dans les niveaux les plus bas.

5.2 Abstract

The photosynthetic behaviour of juvenile stages of the brown alga *Fucus distichus* ssp. *edentatus* (Fucales, Phaeophyta) was examined at different tidal heights along a rock wall at an intertidal site located in the Gaspé Peninsula, Québec, Canada. During the course of the low tide, we used PAM fluorometry to examine the maximum quantum yield (Fv/Fm) the effective quantum yield (Y), the relative electron transport rate (rETR), and the non-photochemical quenching (NPQ) of fucoid juveniles at five intertidal levels spanning a vertical distance of 1.2 m. Observations were made under four different meteorological conditions, defined as "sunny-windy", "cloudy-calm", "cloudy-windy" and "sunny-calm". The rates of evaporation and incident light levels were measured simultaneously. A gradient of responses was generally found: the highest two levels showed declines in Fv/Fm, Y, and rETR with a concomitant increase in NPQ over time. The lowest levels, separated vertically by only 60-120 cm from the highest levels, experienced less desiccation and irradiance and showed no decline of Fv/Fm, Y, and rETR with just a slightly increase in NPQ except under sunny-windy conditions. The combination of wind and sun created the most severe meteorological conditions whereas the less intense meteorological conditions were cloudy-calm conditions during which only the highest level showed a decrease in quantum yields. The correlations between fluorometric parameters and rates of evaporation suggest that desiccation is a major stressor and could result in decreases in population density and growth rates. The stress of desiccation and excess of light are much stronger in the two highest levels and caused delays of growth and more rapid decreases of photosynthesis than the lower levels.

5.3 Introduction

The intertidal zone can be a harsh environment due to the continuous alternation of immersion and emersion conditions resulting from the rise and fall of the tides. During the ebbing tide, emersion often results in the desiccation of organisms due to the combined effects of solar radiation, air temperature, and air movement, and the loss of water can cause substantial decreases in tissue hydration. This stress combined with high levels of light exposure alternates with the more typical marine conditions of wave action and low light thereby creating an inhospitable habitat, especially for photosynthetic organisms. This extreme alternation of physical environment can be expected to play a prominent role in controlling the distribution of intertidal species.

Tides set up a vertical gradient across the shoreline, and many studies have attempted to relate the zonation of marine organisms, including seaweeds, to gradients in stress that must exist across this environment. Colman (1933) first introduced the concept of critical tide levels where distributional limits were related to abrupt changes in the period of emersion. Since then much has been written about the shore zonation (e.g., Stocker & Holdheide 1937, Schonbeck & Norton 1979, 1980), to the extent that many biologists consider intertidal zonation to be a well-understood pattern of nature. However, in spite of these efforts, our knowledge consists largely of elementary descriptions of most shores, which have mostly addressed only to the largest scale patterns of zonation (i.e., the zonation patterns of multiple species across the shore).

A major limitation in past studies stems from the fact that zonation patterns are probably the result of accumulated sub-lethal stresses induced by the physical conditions experienced there. The earliest studies on the causes of zonation (Colman 1933, Doty 1946, Swisbanks 1982) emphasized physical factors in an attempt to present a unified frame of reference for describing intertidal communities. Early description of zonation tended to be imprecise, and zones were often described simply by the presence of a given species, a system that was possibly only applicable for certain shores. More recently, Druehl & Green (1982) have developed a more realistic approach by demonstration that emersion/submersion events do play a role in zonation, but must include considerations of factors such as accumulated time submerged, duration of longest submergence, and the role of wave action.

The underlying theme of these zonation studies is that intertidal zones are related to the physiological abilities of the algae to withstand periods of emersion, and recent efforts have concentrated on relating physical factors to physiological responses. However, difficulties in assessing responses to stress in the field have prevented detailed studies of physiological responses under natural conditions. Instead, such studies have been conducted in the laboratory where there is more control but less realism (e.g., Johnson et al. 1974, Smith & Berry 1986, Madsen & Maberly 1990, Major & Davison 1998).

Ecophysiological experiments that have been conducted in the field have been handicapped by cumbersome equipment or the need for destructive sampling, which has limited the scope and resolution of the research. For studies on the ecology of intertidal algae in which the parameters of interest are usually related to the photosynthetic process, researchers have relied on techniques that measure the evolution of oxygen (Ramus & Rosenberg 1980, Beer & Eshel 1983, Smith et al. 1986, Kirst 1990, Dring 1994, Brenchley et al. 1997) or the uptake of ^{14}C-bicarbonate (McLachlan 1974, Quadir et al. 1979, Oates 1985, Johnston & Raven 1986).

A new technique, pulse amplitude modulated fluorescence (PAM), has been developed over the past decade as a rapid, non-intrusive, and extremely sensitive way to examine photosynthesis under field conditions (Bradbury & Baker 1984, Schreiber et al. 1986) by assessing the function of the photosynthetic apparatus, especially of PSII (Schreiber et al. 1995). The use of this technique permits an examination of the dynamic properties of photosynthesis, as acclimation or photoadaptation and responses to environmental damage (e.g., photoinhibition) when changes in the environment exceed the rate or limits of acclimation.

Over the past ten years, PAM fluorometry has been used successfully in the study of marine algae, but most studies have only examined subtidal species (Leverenz et al. 1990, Hanelt 1992, Magnusson 1997, Häder et al. 1996, Hanelt et al. 1997, Beach et al. 2000). The few studies on intertidal algae (Davison & Pearson 1995, Beer et al. 1998) have shown that photosynthesis performance declines during emersion, presumably due to the high light conditions and desiccation that can produce substantial photoinhibitory stress in algae under natural conditions. Different species appear to respond differently and their physiological responses appear to vary according to their habitat.

A consistent shortcoming of all these studies is their exclusive examination of the photobiology of the adult stages, and no study has yet examined juvenile stages in the field. This lack of information on the photobiology of juveniles is somewhat understandable as the challenge of conducting physiological studies in the field is further compounded by the small size of juvenile stages. Still, given that the distributional limits of adults ultimately depend on the survival of juvenile stages, an understanding of the responses of juvenile is essential.

In this study we examined the physiological responses of the juvenile stages of *Fucus distichus* ssp. *edentadus* in the field over its vertical distribution to understand how the physical conditions encountered during low tide might set the upper distributional limit for this species. Because physical conditions and any associated stresses will depend on climatic conditions, we measured physiological responses under a variety of meteorological conditions.

5.4 Methods

5.4.1 Study area

The study was conducted in the intertidal zone at Pointe-Mitis near Mont Joli, Québec, Canada (48°41'N, 68°2'W), a northeast-facing, semi-sheltered shore consisting of a series of rocky ridges interspersed with consolidated boulder fields (see Bourget et al. 1985 for additional information). Fucoid algae, including *Ascophyllum nodosum* (L.) Le Jolis, *Fucus vesiculosus* L., and *F. distichus* ssp. *edentatus*, are the most common macroalgae in the boulder fields and on the lower portions of adjacent ridges. Perennial algae are not able to colonize the more exposed rocky surfaces of this coastline due to abrasion by ice during winter (Archambault & Bourget 1983). *Fucus distichus* ssp. *edentatus* was the last mature plants producing gametes during the appearance of embryons on our rock wall selected.

5.4.2 Site selection

For this study we selected a rock wall 2 m high located in the low intertidal zone. The wall was oriented towards the northwest and was partially protected from the full effects of wave action by a rocky ridge some 10 m offshore. The wall was generally smooth and flat except for prominent crevices that ran vertically to divide the surface into distinct sections. We selected 3 adjacent sections of the wall, each an area of approximately 2-3 m^2.

The selection of this site was not haphazard and was instead made to facilitate certain aspects of the study. First, we wanted to reduce environmental variation due to the aspect or inclination of the rock surface (e.g., north-facing vs. south-facing surface) – the wall provided a consistent surface where just the effect of tidal level could be examined. Second, we wanted to minimize the effects of herbivory – the lack of crevices and holes eliminated the refuges that herbivorous gastropods use (Addy & Johnson 2001). Third, we wanted an area that had consistent recruitment of juvenile stages – we had observed massive colonization of these areas by fucoid algae in 1998 and 1999. These populations did not, however, persist through the winter as it appears that the annual ice scour removes all recruits from these surfaces, thereby eliminating any historical effects.

On each of the 3 sections, five levels separated vertically by 30 cm were marked with stainless steel screws. Level 1, the highest, began at 30 cm from the top the wall at the level where the upper limit of colonization was observed in previous years. Level 5 (the lowest) was just above the zone of adult *F. distichus* ssp. *edentatus*. At each level, we determined photosynthetic responses during low tides and the density and size of fucoid juveniles during a 2-month period in late summer of 2000. We also measured aspects of the physical environment, specifically PAR and evaporation rates, across this short but sharp gradient.

5.4.3 Fluorometry

Photosynthetic responses were examined *in situ* by pulse-amplitude modulated (PAM) fluorometry, using a PAM 2000 portable fluorometer (Walz GmbH, Effeltrich, Germany). The fluorescence responses were measured directly from individual algae ranging in size from 5 to 20 mm in length. The measuring modulated light intensity was approximately 0.1 μmol photons m^{-2} s^{-1} and sufficiently low as to not produce any significant photosynthesis.

These measurements provided the following fluorometric parameters: (1) Fo and Fm, the minimal and maximal fluorescence yields after a 15 min dark-adaptation, and (2) Fo' and Fm', the minimal and maximal fluorescence yields after light adaptation. Fo is observed when all PSII reaction centers are open as occurs after dark-adaptation whereas Fm is observed when all the PSII reaction centers are closed. The parameter Fo' corresponds to the minimal fluorescence yield in the dark of a light-adapted sample (again with all PSII centers open) whereas Fm' is the maximal fluorescence yield of an illuminated sample after a pulse of saturating light. From these parameters, several estimates of photosynthetic ability can be calculated: (1) Fv/Fm [=Fm-Fo)/Fm], the overall quantum yield of PSII photochemistry which is an index of the maximal photochemical efficiency (Genty et al. 1989); (2) Φ_{PSII} or Yield [Y = ((Fm'-Ft)/Fm' = ΔF/Fm')], the effective quantum yield which reflects the operating PSII efficiency in an illuminated sample where Ft represent the fluorescence at the time t (Schreiber & Bilger 1993); (3) rETR (= Y x PAR x 0.5), defined as the relative electron transport rate between the two photosystems where Y is the effective quantum yield, PAR is the actinic irradiance in μmol photons m^{-2} s^{-1}, 0.5 is a multiplication factor because transport of a single electron requires 2 photons; (4) NPQ [= (Fm-Fm')/Fm'], defined as the coefficient of non photochemical quenching which reflects any process other than photochemistry that lowers the yield of variable fluorescence, e.g., dissipation of heat (Schreiber et al. 1994). These latter two estimates of photosynthetic condition depend on the state of the PSII reaction centers. We always used the initial Fm value recorded at the beginning of the experiment for these calculations.

For any given low tide period (i.e., emersion), fluorescence measurements were taken during 30 min intervals beginning immediately after the plants were exposed to air by the ebbing tide. For the first 15 min of each interval, plants were covered with an opaque plastic cover to dark-adapt them before fluorometric measurements. Although this cover was necessary, it undoubtedly reduced emersion stresses, particularly desiccation. Dark-adapted algae then received a 600 ms pulse of high intensity light (1200 µmol photon m^{-2} s^{-1}) white light to cause transient closure of the PSII photochemical reaction centers. This was followed by a second pulse for determining fluorescence levels under light-acclimation conditions (i.e., Fm' and Fo'). The plants were then left uncovered for the rest of the 30 min interval at which time they were covered again to dark-adapt them for the next series of measurements. At each sampling time, three replicate fluorescence measurements were taken for each section of the rock wall (i.e., 9 total for each level taking approximately 3 min for each level). Because all levels were not exposed at once, we began with levels 1 and 2 and added lower levels as the tide ebbed. Measurements continued until at least five 30-min intervals were sampled at each level. Because of the daily advance of the tidal cycle (approximately 50 min each day), the timing of the low tide varied among the different days but always fell between 10:30am and 15:30pm.

5.4.4 Evaporation rate and PAR

Evaporation rate and PAR were also measured at each level at the same time of photosynthetic measurements. For evaporation rates, we measured water loss from 3 saturated cellulose filters (PFE 25 µm, Campbell Scientific) placed on the bare rock surface at each level (one in each section) and then weighed every 30 min to the nearest 0.001 g. Data were calculated as the percentage of initial wet weight, excluding the weight of the dry filter. As the filters were adjacent to the measured algae, they were also covered by the opaque plastic sheet for 15 min of each measurement period. For PAR, we used the quantum sensor (Li-Cor 2032) integrated into the PAM fluorometer; preliminary observations indicated that PAR did varied little within a given level, and thus only a single measurement was taken for each level.

5.4.5 Meteorological conditions

Measurements were taken for 12 days under differing meteorological conditions. The days were categorized as: (1) "sunny-calm" – no clouds or wind, and light intensities (PAR) between 800 and 1200 µmol photon $m^{-2}s^{-1}$; (2) "cloudy-calm" – clouds present and light intensities between 200 and 300 µmol photon $m^{-2}s^{-1}$; (3) "sunny-windy " as for sunny-calm but with wind speeds averaging 33 $km.h^{-1}$; (4) "cloudy-windy" – as for cloudy-calm but with wind speeds averaging 33 $km.h^{-1}$. Light was estimated based on PAR measurements (see above) whereas wind speeds were obtained from the Mont Joli airport, 15 km from the field site. For each of these conditions, measurements were taken on three different dates in September 2000.

5.4.6 Plant density and size frequency

Every two weeks in September and October (the sampling in August was the point of depart), we sampled juvenile (defined as being between 1 and 20 mm in length) densities and size-frequency by scraping a 7 cm^2 area with a scalpel. Three replicates were collected from each level (one from each section) selected haphazardly from areas where fucoid recruitment occurred (e.g., away from crevices or holes where grazers aggregated). For size-frequency, only individuals with no signs of damage or deterioration were used. The rate of growth was calculated by the size measurements, [(length+1-length)/length] x 100 at time t following the period of plant collects.

5.4.7 Statistical analysis

The fluorescence data were tested using a mixed ANOVA model and analysed using SAS statistical package (SAS-Institute 1999-2001). The slopes of fluorescence parameters were also calculated and comparisons between the slopes of different treatments (e.g., Level) were tested using the "Student Test". The correlations were made using Statistica (Statistica 1999) and the differences between each correlations was tested with t-Test.

5.5 Results

5.5.1 Meteorological conditions

Meteorological conditions and tidal levels interacted to influence the photosynthetic performance of these algae (significant Condition x Level terms; Table 5.1).

Table 5.1: ANOVA with Repeated Measures. Conditions represent Meteorological Conditions, the Level refers to the defined Levels on the rocky wall and Exposure Period (min) is the Period of emersion at low tide.

Effect	df	F value	p
Conditions	3	803.13	<.0001*
Level	4	476.88	<.0001*
Conditions x Level	12	43.79	<.0001*
Exposure Period	5	425.03	<.0001*
Conditions x Exposure Period	12	223.11	<.0001*
Level x Exposure Period	19	158.28	<.0001*
Conditions x Level x Exposure Period	35	27.88	<.0001*

For the three more benign meteorological conditions (cloudy-calm, cloudy-windy and sunny-calm) Fv/Fm remain near the initial value for the entire period, except at the uppermost level (Level 1) where it declined under all conditions, declining to 0.4 in sunny-calm conditions and 0.43 in cloudy-windy conditions after just 60 min of exposure and to 0.44 after 90 min in cloudy-calm conditions, the most benign conditions. Level 2 also declined under cloudy-windy conditions (Figure 5.1 C)), occurring just 30 min later than at Level 1and under sunny-calm conditions (Figure 5.1D), 60 min after the decrease of the first Level.. Under cloudy-windy conditions, the experiment was only made during a low tide of 150 min, the tide rised too quickly to continue the measurements. In general, there were no significant differences between the lowest Levels, i.e., 3, 4 and 5 (Table 5.2).

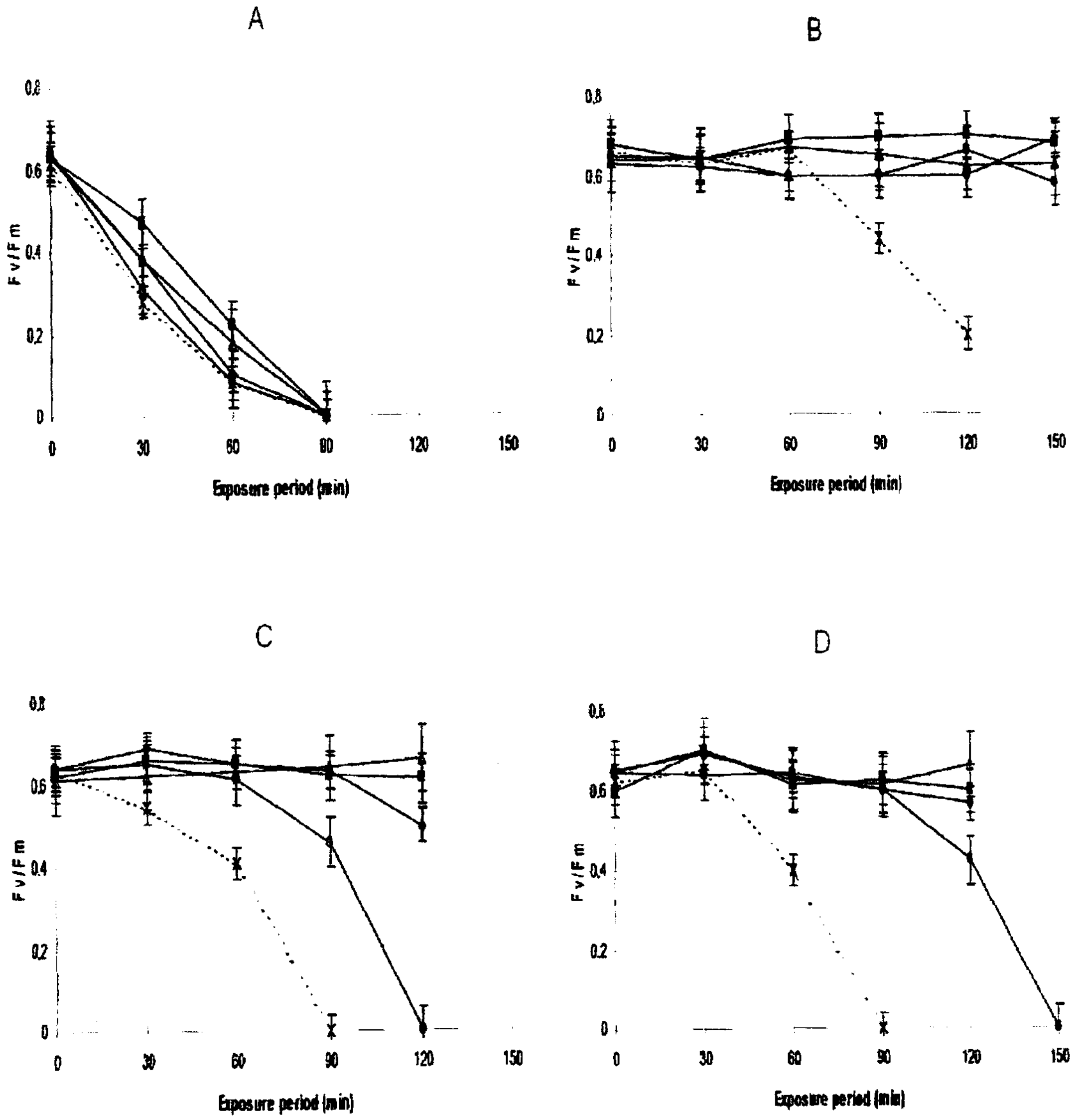


Figure 5.1 : Time course of Maximum Quantum Yield of PSII (Fv/Fm) during low tide under (A) sunny-windy conditions, (B) cloudy-calm conditions, (C) cloudy-windy conditions and (D) sunny-calm-conditions ; (-■-) fifth level, (-Δ-), fourth level, (-♦-) third level, (-o-) second level and (--x--) first level. Error bars indicate the 95% confidence intervals (n=3).

Table 5.2 : Values of Test T at each slopes of ϕ_{PSII} curves at different Levels (L) under the Meteorological Conditions; “Sunny-Windy”, “Cloudy-Calm”, “Cloudy-Windy” and “Sunny-Calm”. Values with different letters are significantly at $p<0.05$.

	Tidal Levels				
Conditions	L1	L2	L3	L4	L5
Sunny-Windy	-0.22^{a}	-1.3^{a}	-4.43^{b}	-6.8^{b}	-8.59^{b}
Cloudy-Calm	-0.25^{a}	-8.72^{b}	-8.3^{b}	-8.87^{b}	-9.43^{b}
Cloudy-Windy	-0.68^{a}	-4.51^{b}	-6.08^{c}	-6.85^{d}	-7.16^{d}
Sunny-calm	-0.48^{a}	-3.82^{b}	-6.41^{c}	-6.67^{c}	-9.01^{c}

However there was a slight decrease of Fv/Fm at Level 3 under cloudy-windy conditions resulting in a weak difference (Table 5.2, p_4=0.048, p_5=0.06). This decrease was intermediate between the Fv/Fm value at Level 2 and those at Level 4 and 5. These decreases in Fv/Fm at Levels 2 and 3 under cloudy-windy conditions but not under sunny-calm conditions suggest that wind was more influential than solar radiation.

The combination of wind and sun produced a strikingly different pattern (Figure 5.1 A). Whereas the initial values of Fv/Fm were similar to the other conditions, by the second measurement, Fv/Fm values had declined at all levels; The decline of Fv/Fm of the Level 1 and 2 was faster than the others Levels after the first 30 min, however by 90 min, , values had fallen to 0 at all levels. There was no difference between the patterns of the decline of Fv/Fm at Levels 3, 4, and 5 (Table 5.2).

The decrease of the maximum quantum yield could be the result of a decrease of Fm or an increase in Fo. Except for the Level 1, decreases in Fv/Fm corresponded with the decreases observed in Fm (data not shown). No large changes were observed in Fo for the three lowest levels (data not shown). The decrease of Fv/Fm at Level 1 was principally due to changes in

Fo under all conditions. A slight increase of Fo was seen for Level 2 under windy and cloudy conditions.

The changes in effective quantum yield, ϕ_{PSII} followed those of maximum quantum yield (Figures 5.2).

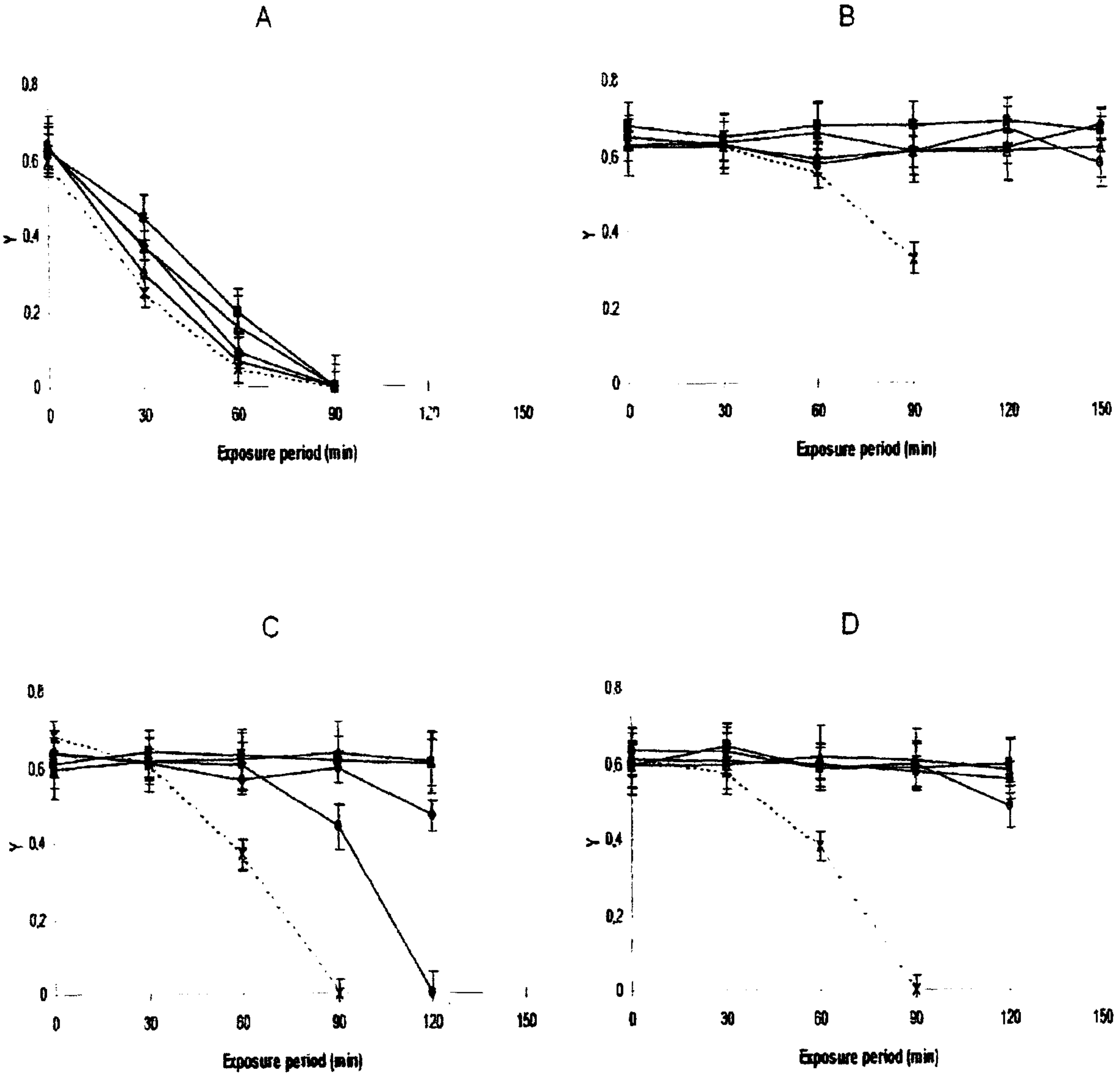


Figure 5.2 : Time course of Effective Quantum Yield of PSII (Y) during low tide under (A) sunny-windy conditions, (B) cloudy-calm conditions, (C) cloudy-windy conditions and (D) sunny-calm conditions ; (-■-) fifth level, (-Δ-) fourth level, (-◆-) third level, (-o-) second level and (--x--) first level. Error bars indicate the 95% confidence intervals (n=3).

Under sunny-windy conditions, the decrease of ϕ_{PSII} at all levels was rapid and reached a null value after 90 min (Figure 5.2 A). In all conditions, the ϕ_{PSII} at Level 1 paralleled the values of Fv/Fm. Similarly, rETR followed the response of the effective quantum yield at all levels (Figure 5.3).

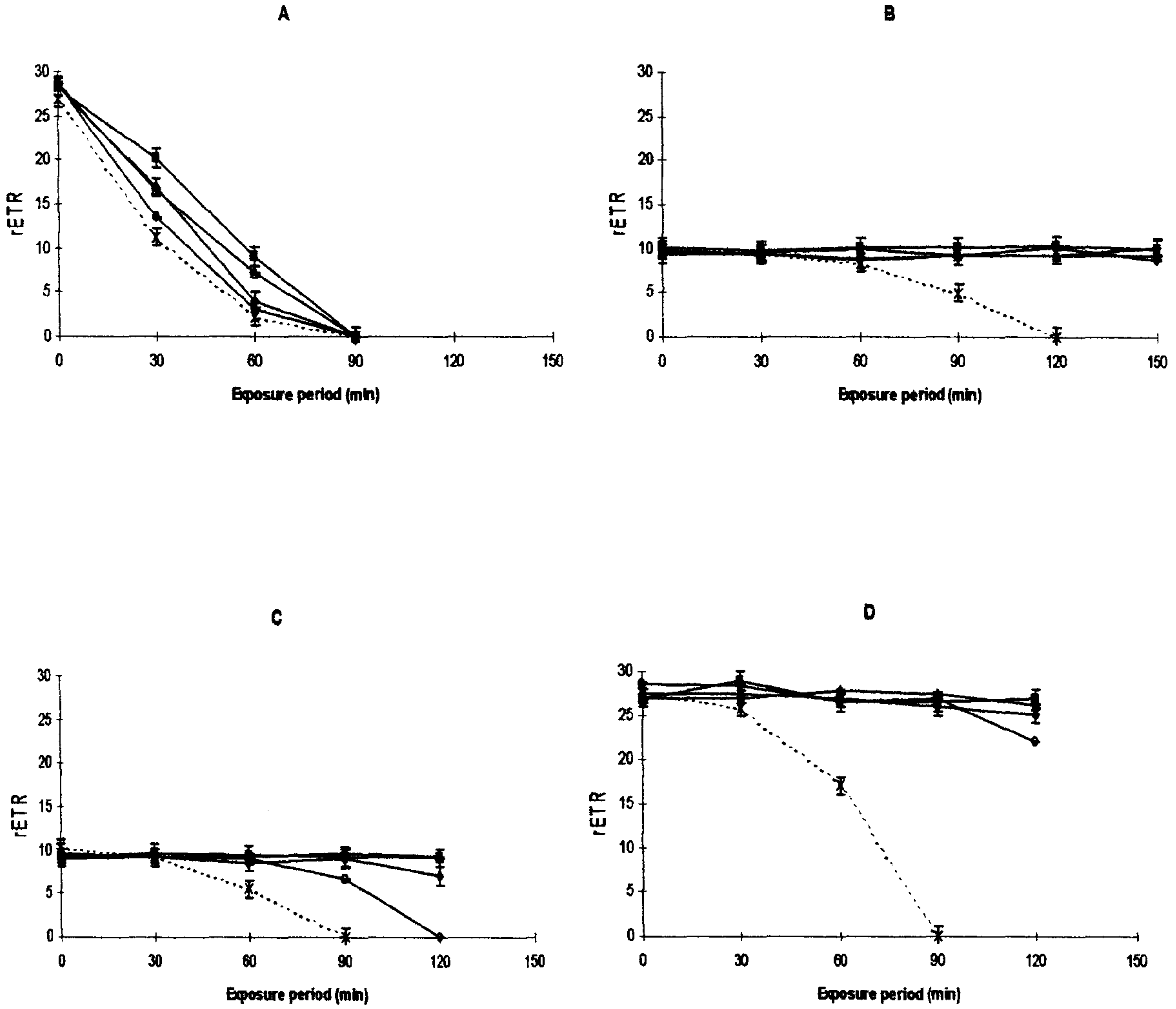


Figure 5.3 : Time course of Relative Electron Transport Rate (rETR, μmol electrons m^{-2} s^{-1}) during low tide under (A) sunny-windy conditions, (B) cloudy-calm conditions, (C) cloudy-windy conditions and (D) sunny-calm conditions ; (-■-) fifth level, (-Δ-) fourth level, (-♦-) third level, (-o-) second level and (--x--) first level. Error bars indicate the 95% confidence intervals (n=3).

Under sunny-windy conditions rETR declined for all levels. Under all other meteorological conditions, rETR remained stable except at Level 1 in all conditions and Level 2 for the cloudy-windy conditions. The initial value of rETR in sunny-calm conditions and sunny-windy were higher than the initial values in cloudy-calm and cloudy-windy conditions due to the higher levels of PAR available under sunny conditions. It was, however, only under sunny-calm conditions that the algae were able to maintain this high level of rETR. In sunny-windy conditions, rETR fell below the levels seen under those for cloudy condition within 60 min.

Dissipation of excess energy by other pathways (NPQ, non-photochemical quenching) was generally high and variable (Figure 5.4), but, as expected, the NPQ increased as ϕ_{PSII} decreased (Figures 5.2 and 5.4).

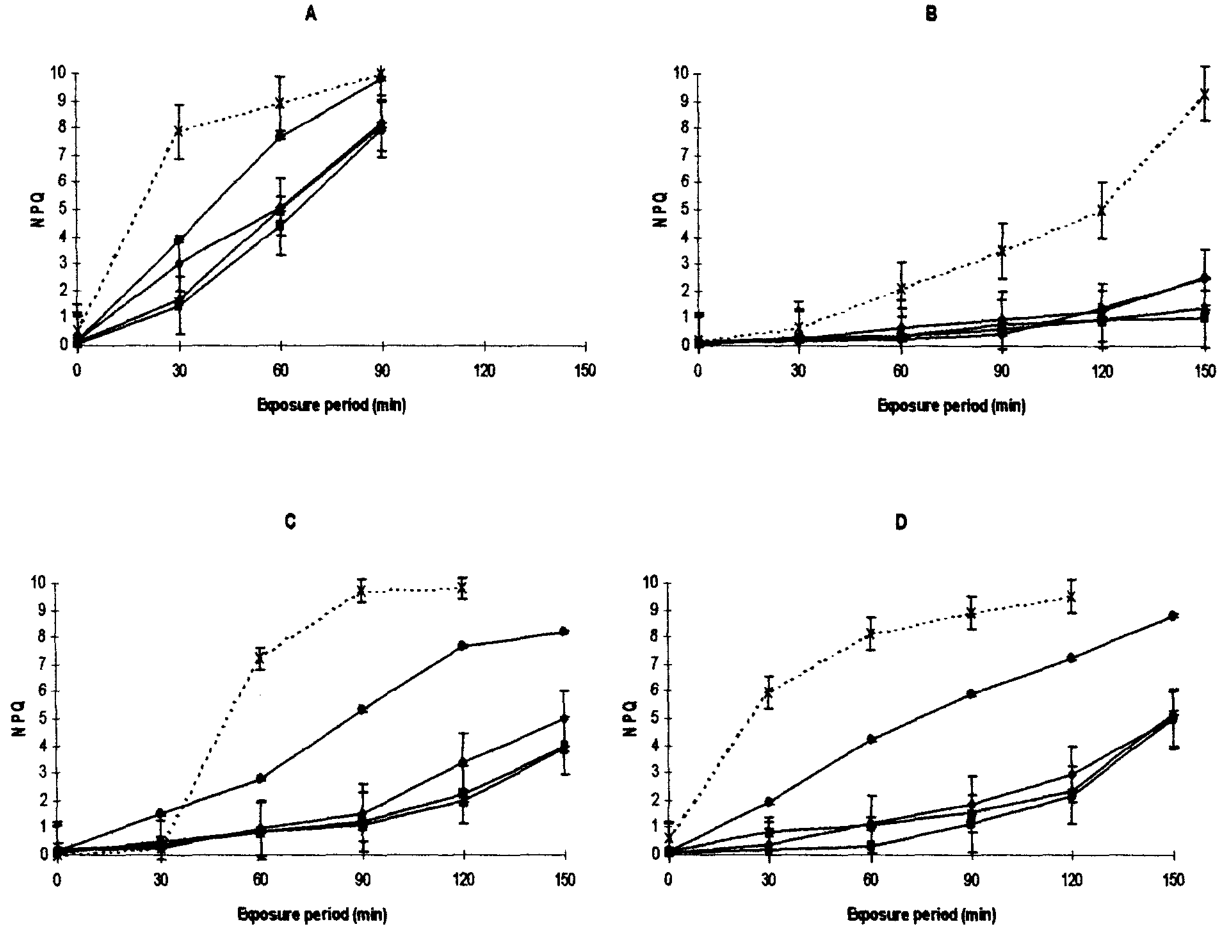


Figure 5.4 : Time course of non-photochemical quenching (NPQ) during low tide under (A) sunny-windy conditions, (B) cloudy-calm conditions, (C) cloudy-windy conditions and (D) sunny-calm conditions ; (-■-) fifth level, (-Δ-) fourth level, (-◆-) third level, (-o-) second level and (--x--) first level. Error bars indicate the 95% confidence intervals (n=3).

Variation corresponded largely to the meteorological conditions. For the most benign conditions (cloudy-calm), there was an increase in NPQ (Figure 5.4 B) only for Level 1 and only a slight increase for the other levels. For more stressful conditions (cloudy-windy and sunny-calm), the increase was progressive and appeared first as an increase at Levels 1 and 2 followed by a gradually increase of the other levels (Figure 5.4 C and D). For the most severe conditions (sunny-windy), increases were seen at all levels, with those of Levels 1, 2, and 3 preceding those of Levels 4 and 5 (Figure 5.4 A).

5.5.2 Evaporation rate and PAR

The evaporation rate was constant throughout a given measurement period but varied according to the level and the meteorological conditions. Generally, the decrease in percent dehydration was much faster for the uppermost Level 1 and 2 and slowest for the lowest Levels 4 and 5 (Figure 5.5). Under the most severe condition (the highest level with sunny-windy conditions) the evaporation rate was 45% h^{-1} (i.e., the filter was almost completely dry after the 2 h observation period) in contrast to a rate of 37% h^{-1} at Level 2 and less than 27% h^{-1} for the lowest levels (Figure 5.5).

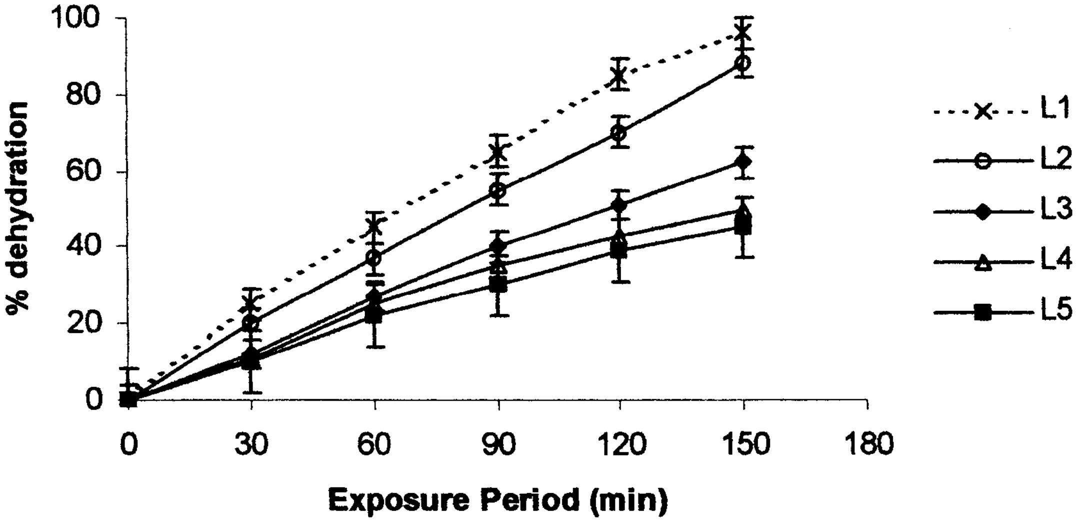


Figure 5.5 : Average % dehydration of filters (n=9 during low tide among levels). The values represent average of 3 days under sunny-windy conditions; † 95% confidence intervals.

Evaporation rates and changes in fluorometric parameters exhibited the same general patterns (Figures 5.1-5.5). Using ϕ_{PSII} as a representative fluorometric parameter, there were significant correlations (r values ranging from 0.96 to 0.65) between ϕ_{PSII} and % dehydration of filters during the course of the period of emersion (excluding the first measure of each day at which time the filters were, by definition, at 100% hydration) for all levels under sunny conditions but just for the highest two or three levels under other conditions (Table 5.3).

Table 5.3: Correlations between the % dehydration of filters and the Effective Quantum Yield (ϕ_{PSII}) among levels, the r values at each Level under meteorological conditions were compared using the test t.; values with different letters are significantly different at $p<0.05$.

	Tidal Levels				
Conditions	L1	L2	L3	L4	L5
Sunny-Windy	-0.97^{a}	-0.88^{b}	-0.63^{c}	-0.46^{d}	-0.33^{e}
Cloudy-Calm	-0.94^{a}	-0.85^{b}	-0.71^{c}	-0.78^{c}	-0.77^{c}
Cloudy-Windy	-0.96^{a}	-0.8^{b}	-0.64^{c}	-0.51^{d}	-0.48^{d}
Sunny-calm	-0.95^{a}	-0.91^{b}	-0.78^{c}	-0.75^{c}	-0.74^{c}

PAR data were pooled into two categories: sunny and cloudy. PAR was higher for sunny conditions (an average of 40% greater over all the levels), but for both conditions, we observed a gradual reduction in PAR with decreasing tidal height that was slightly more pronounced for sunny conditions. PAR was maximal at Level 1 and decreased to 30% of this value at the lowest levels (Figure 5.6). PAR and rates of evaporation were highly correlated (r=0.99 to 0.998).

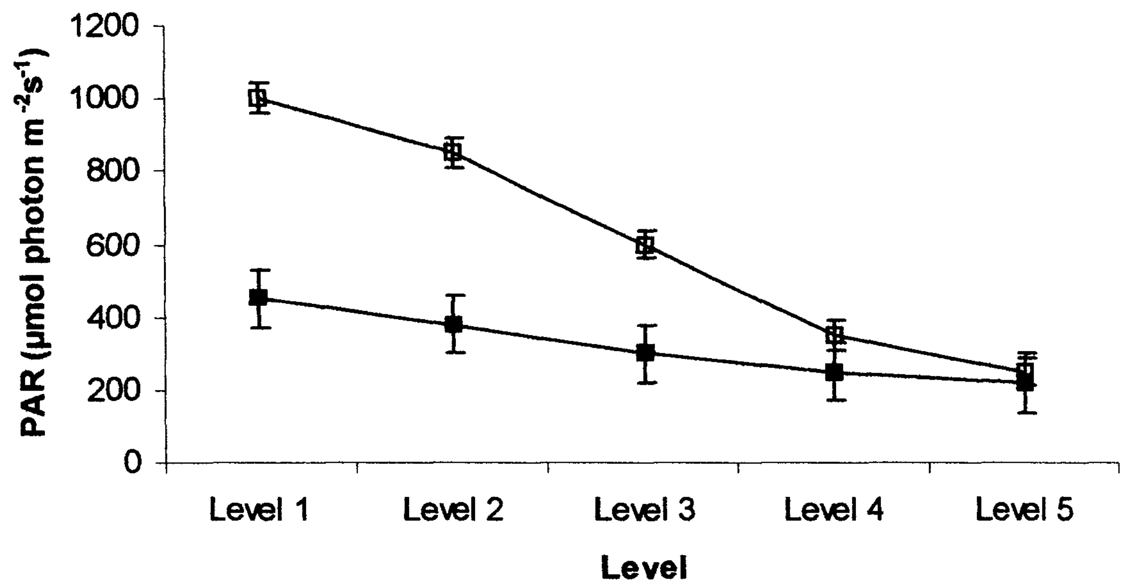


Figure 5.6 : PAR values at different levels under (□) sunny conditions and (■) cloudy conditions (n=9). Error bars represent 95% confidence intervals.

Table 5.4: Correlations between the PAR (µmol photons m^{-2} s^{-1}) and the Effective Quantum Yield (ϕ_{PSII}) among levels;the r'values at each Level under meteorological conditions were compared using the test t.; values with different letters are significantly different at $p<0.05$.

Comparisons were made with the Student test; values with different letters are significantly different at $p<0.05$.

	Tidal Levels				
Conditions	L1	L2	L3	L4	L5
Sunny-Windy	-0.97^{a}	-0.88^{b}	-0.54^{c}	-0.33^{d}	-0.1^{e}
Cloudy-Calm	-0.96^{a}	-0.8^{b}	-0.39^{c}	-0.34^{c}	-0.32^{c}
Windy-Cloudy	-0.84^{a}	-0.77^{b}	-0.55^{c}	-0.24^{d}	-0.19^{d}
Sunny-calm	-0.96^{a}	-0.7^{b}	-0.46^{c}	-0.44^{c}	-0.4^{c}

5.5.3 Density and growth

Densities decreased during the period of experiment, presumably due to self-thinning processes, but population densities were always greater at the bottom levels, e.g., 5 times more thalli at Level 5 compared to Level 1 in September (Figure 5.7).

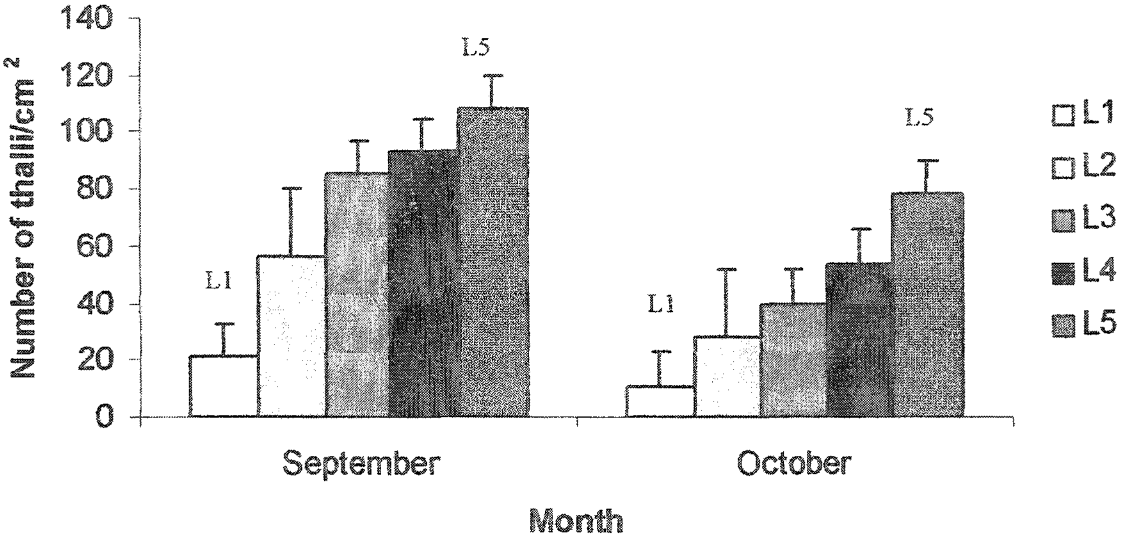


Figure 5.7 : Density of young thalli at two months for the 5 levels. Error bars indicate 95% confidence intervals (n=9).

Moreover, growth rates at the lowest levels were very similar to one another but were much higher than at the upper levels, especially in September when they grew over twice as fast (Figure 5.8). Growth rates in general decreased in October as light levels and temperatures decreased. However, growth still remained highest for the lowest levels.

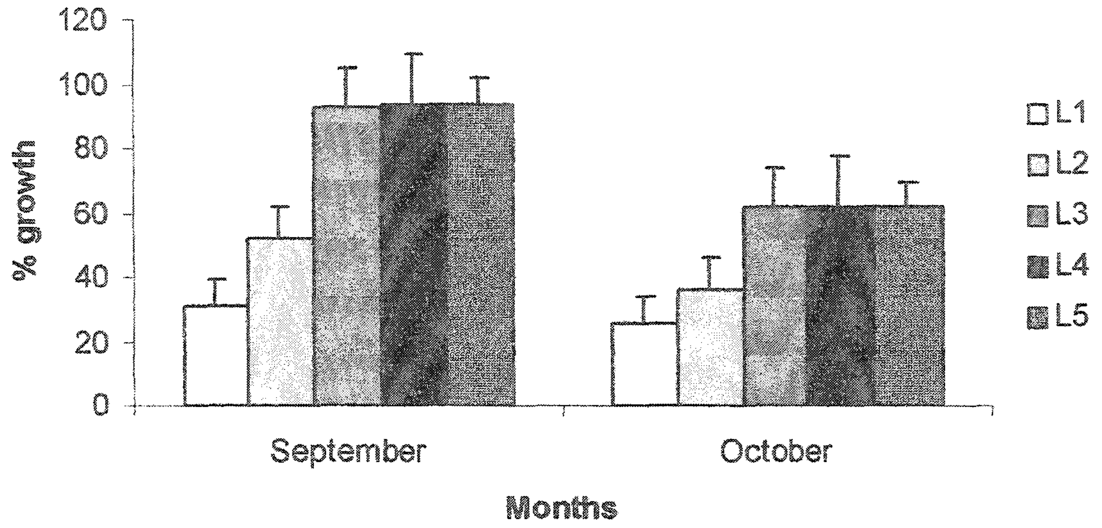


Figure 5.8 : % Growth (size) of young thalli at the 5 tidal levels (n=9). Error bars indicate the 95% confidence intervals.

5.6 Discussion

5.6.1 Environmental factors at low tide

Zaneveld (1937) proposed that there was a direct relation between the rates of desiccation and the intertidal height at which an alga can live, i.e., algae that lived at higher levels should inherently lose water less rapidly.

In theory, evaporation rates should have been constant for all levels. Our measurements of evaporation rates demonstrated that they were not constant across this vertical gradient and were lower at the lowest levels. This decrease did not, however, occur in a linear manner: the top two and the bottom two levels had similar rates of evaporation, respectively. We hypothesize that the increased rates at higher levels were due to variation in the wind speeds which should have been higher near the crest of the ridge on which the wall was located. Regardless, these higher evaporation rates combined with the longer periods of emersion at higher levels ensured that the algae at higher levels experienced greater desiccation stress.

Desiccation can also vary in predictable manner depending on meteorological conditions. Among our four somewhat arbitrarily-defined conditions, clear differences were seen that are consistent with our intuition. The highest rates of dehydration occurred on sunny-windy days when the solar radiation augmented evaporation, and the wind maintained a steep humidity gradient over the rock surface (i.e., increased convective loss of water) by removing the water vapour. This proposition assumes that either a substantial fraction of the photosynthesis occurs during low tide or that stresses experienced during low tide can affect subsequent photosynthesis (i.e., retard the recovery of photosynthesis once re-immersed). While the validity of this assumption may depend on the exact species, modelling growth of intertidal fucoid algae has demonstrated that the period of low tide is not simply “lost time” for photosynthesis, and that photosynthesis during low tide can contribute substantially to the overall energy budget (Madsen & Maberly 1990).

In spite of a lack of any physiological or structural means to retard water loss (Schonbeck & Norton 1979), some mechanism must exist that allows survival and growth at these high levels on the shore where water loss becomes significant. Desiccation is often related to the surface-area-to-volume ratio rather than inherent factors such as mucilage content and cell wall thickness (Dromgoole 1980). Several algal species have been found to maintain positive net photosynthesis at relatively high degrees of water loss (Quadir et al. 1979, Beer & Eshel 1983). Jones & Norton (1979) suggested that shrinkage of tissue during water loss can serve as a mechanism to reduce the surface area over which evaporation occurs. Earlier studies showed that the efficiency of the photosynthetic apparatus of germling or juveniles (McMurtry 1988) is lower than that of the adults (Prezelin 1981), and that adults could better withstand air exposure than the juveniles (Mathieson 1982).

Beyond the problems of dehydration, being out of water deprives the seaweed of their source of nutrients (Thomas & Turpin 1980) including inorganic carbon (Chapman 1966). Fucoid algae use the bicarbonate in seawater as a source of carbon (Bidwell & McLachlan 1985, Raven and Johnston 1991), and the small amount of bicarbonate available in the surface film of water on the plant can be replenished from the atmosphere as long as the thallus remains wet. Photosynthetic rates during emergence can even exceed those during submergence for some intertidal algae (e.g., Johnston & Raven 1986), but in several cases, net photosynthesis while emerged was found to be lower than when submerged. Regardless, once dry, the alga must rely on accumulated inorganic carbon (Bidwell & McLachlan 1985, Reiskind et al. 1989). In some fucoid algae, this store of inorganic carbon was depleted after 2 hours, and photosynthesis ceased at that time (Kawamitsu & Boyer 1999). In some cases, rates of photosynthesis were found to be greatest at slightly desiccated levels (Chapman 1966, Hodgson 1981, Harker et al. 1999), but this slight increase could not be seen in our fluorometric parameters because they reflect only the behaviour of the PSII, not the workings of the Calvin cycle.

5.6.2 Fluorescence parameters

Variations in the physical environment appears to affect directly the physiology of these algae as all the fluorometric parameters varied in a similar manner to that of evaporation rates and PAR, i.e., changes in fluorometric parameters, related to photosynthesis (Y and qP) occurred during times and in locations of high rates of evaporation and higher level of solar radiation.

The decrease was more pronounced for the Level 1 than for the others levels, and once started, the decrease was rapid. Normally the decrease of Fv/Fm can be due to the increase of Fo (interpreted as photodegradation of PSII reaction centers, Hanelt et al. 1993) and/or the decrease of Fm (Krause & Somersalo 1989, Franklin 1992). In our experiments, changes in Fo occurred at Level 1 under all conditions and at Level 2 only under windy conditions. There were no changes in Fo for the lowest Levels, and the decrease of the Fv/Fm was due to changes of Fm. Decreases in Φ_{PSII} and rETR appeared to be due primarily to decreases in Fm, which is generally interpreted as reflecting mechanisms of photoprotection (Hanelt 1992, Magnusson et al. 1994, Beer et al. 1998). Under such conditions, the energy used for photosynthesis declined as seen by the slight decreases in Φ_{PSII} and qP (data not shown) while the excess energy was dissipated as heat (as seen by increases in NPQ; Figure 5.4) which can be considered to be photoinhibition (Hanelt et al. 1993).

The variation of the fluorescence parameters for these juvenile thalli agrees with previous observations on brown algae (Büchel & Wilhelm 1993, Häder et al. 1996). For example, photosynthetic responses of various fucoid algae exposed to the air changed gradually as the algae desiccated, until photosynthesis finally ceased at a critically low water content (Gessner & Schramm 1971, Madsen & Maberly 1990). Field experiments conducted on *Fucus* adults demonstrated a decline in the Φ_{PSII}, suggesting that photoinhibition experienced during natural emersion is a sub-lethal stress, temporarily impairing photosynthesis (Davison & Pearson 1995). Measurements of recovery are needed to determine if photodamage occurred, but we were not able to make these measurements without endangering the equipment. In a similar study on adult stages (unpublished data, M. Lamote), fluorescence measurements during recovery demonstrated that recovery was fast but was lower than initial values, suggesting that some photodamage can occur.

Although the rETR in low light conditions (i.e., cloudy-calm and cloudy-windy, Figures 5.3 B, C) never reached the saturating values obtained in sunny conditions, it was maintained throughout the period of emersion thereby resulting in a similar integrated rETR to those in sunny-windy conditions. It was only under the conditions of sunny-calm that the algae were able to have a high level of rETR that could be maintained during the entire period of low tide (Figure 5.3 D). It is not, however, clear if these juveniles were ever at saturating levels of PAR, but a greater exposure to sunlight would have certainly increased desiccation rates, thereby negating any advantage of higher light levels (e.g., Level 1).

Juvenile stages were able to dissipate excess energy into heat as revealed by NPQ measurements (Figure 5.4). This dissipation of energy was most likely accomplished by the xanthophyll cycle (Sapozhnikov 1957) as Harker et al. (1999) has shown a correlation between NPQ values and violaxanthin de-epoxydation in brown algae. The increase of energy dissipating through NPQ began earlier and reached higher values for Levels 1 and 2, corresponding generally to the magnitude of the stress (i.e., % dehydration; Figures 5.4 and 5.5).

Under the most severe conditions, the combined actions of high light and the drying effect of the wind resulted in the greatest impact on photosynthetic performance. Although evaporation rate correlated well with the decrease of ϕ_{PSII} (Table 5.3), it was also highly correlated with PAR measurements (Table 5.4), and thus it is not possible to attribute the physiological responses we observed entirely to desiccation. However, the more pronounced response to windy conditions (i.e., the decreases observed for levels 2 and 3 only under windy conditions) suggests that desiccation is the primary factor involved in provoking this response. Given that the rock wall on which the study was conducted faced the northwest, these algae rarely received direct sunlight, and thus were probably never in saturating conditions. However, the dehydration could affect these relations so that even in cloudy conditions, the light was probably saturating for thalli at Level 1 (shown by decreases of ϕ_{PSII}) because of dehydration suffered by young thalli. Physiological limitations at the highest level can probably be attributed to damage to the primary processes of photosynthesis resulting from severe dehydration (Hodgson 1981, Smith et al. 1982, Smith et al. 1986).

This effect of dehydration was less likely at the lower levels of evaporation there. Compared to a rate of dehydration of 45% h^{-1}, the maximum percentage of dehydration undergone by the higher levels, the lower levels experienced considerably less, approximately half (27% h^{-1}). Thus, at lower tidal levels the young thalli were able to photosynthetize for a longer period, indeed, longer than the periods observed in studies on adult fucoid algae (Mathieson 1982, Dring & Brown 1982, Davison et al. 1993, Davison & Pearson 1996). This difference is likely due to reduced levels of desiccation from low levels of solar radiation and wind movement, but also perhaps to the retention of water by the high density of algal fronds (Figure 5.7).

5.6.3 Demographics

The higher up on the shore a species grows, the longer it is exposed to terrestrial conditions. In our case, there was a gradual vertical gradient of stress, manifest as changing levels of light and evaporation. Both factors can affect photosynthetic rates and ultimately the growth and survival of individuals. We found juveniles at much higher densities at the lower levels (Figure 5.7), and while this may be due to more benign conditions there, the higher densities could also modified the milieu as well because the dense turf formed by the juveniles could maintain the local humidity. Similarly, the smaller size of juveniles at the higher levels (Figure 5.7) could have made them more susceptible to desiccation as smaller organisms generally have higher surface area to volume ratios. Regardless, the patterns of density, size and growth all suggest that the end result of the differences in meteorological conditions experienced by the juvenile stages is a massive impact on the recruitment of this species. Growth appears to be light-independent in fucoid algae (Major & Davison 1998). Thus the highest juveniles were most stressed by sunshine and air movement, their capacity of photosynthesis and growth being restricted by the longer exposure to air which leads to greater levels of desiccation.

5.7 Conclusion

In this study, we have shown that the physiological responses of an intertidal alga can vary dramatically over space and time in response to both predictable and unpredictable aspects of its environment. Factors affecting the desiccation of these algae (i.e., solar radiation and wind) appear to determine to a large degree their photosynthetic abilities during the ebbing tide, but further work is required to tease apart the dual roles of the light environment in both enhancing desiccation and provoking photoinhibitory responses. The effects of the physical environment can be manifested in the growth and perhaps the survival of individual plants, and clearly plays a key role in determining the upper limits of this species distribution in the intertidal environment. As these physical factors depend ultimately upon the climate, changes in weather patterns (e.g., number and frequency of sunny days) may have a large impact on the interannual and long-term abilities of this species to maintain its populations in this environment.

CHAPITRE 6 - Conclusion Générale et Perspectives

La présente étude démontre l'utilisation facile des jeunes stades de Fucus dans les études écologiques et physiologiques. Bien que les cultures de Fucales ont fréquemment été utilisées pour les études cytologiques et de développement, peu d'études ont examiné l'écophysiologie avec le respect des stades juvéniles. C'est une étude qui examine les jeunes stades de trois espèces se développant dans des zones horizontales adjacentes.

La première partie étudie l'évolution du recrutement des embryons de Fucales ; Les Fucales sont dominantes dans les zones intertidales. Cette dominance et l'importance de ces organismes dans le milieu doivent être assurées par le recrutement et l'installation des jeunes stades. Les rapides changements dans les conditions environnementales induisent la libération des gamètes et la fécondation. Cette fécondation est très sensible aux conditions du milieu car elle se réalise à l'extérieur de la plante adulte. Les jeunes embryons sont alors exposés à tous les stress que subissent les adultes et de part leur petitesse, ils y sont beaucoup plus sensibles. Afin de mieux connaître l'écologie des Fucales sur mon terrain d'étude, j'ai examiné l'installation et le recrutement d'un assemblage de multiples espèces de Fucales dominantes du milieu (*Ascophyllum nodosum* (L.) Le Jolis, *Fucus vesiculosus* L. and *Fucus distichus* ssp.) sur des substrats artificiels dans différents micro-habitats et sur des périodes d'échelles de temps différentes sur des côtes rocheuses semi-exposées à la Péninsule de Gaspé, Québec (Canada) durant les trois années de mon doctorat. Ces mesures ont été prises à différentes fréquences de temps dans trois micro-habitats ;

- surfaces rocheuses exposées,
- sous la canopée des adultes de Fucales et
- dans les cuvettes durant la période de reproduction des trois espèces.

La première des observations est que les propagules tendent à s'installer partout sans discrimination. Leur nombre compté sur des substrats artificiels est très important. Les résultats montrent de plus sans surprise que l'installation des embryons variait dans le temps et suivant les différents lieux de mesures. L'installation des embryons se trouvait plus faible dans l'année 2000 et surtout dans le mois de septembre. Ces données suivent le même profil que les données de phytoplancton de l'estuaire du St-Laurent qui m'ont été gracieusement fournies par l'institut Maurice-Lamontagne. Les résultats de l'institut concluent à une action dominante des facteurs environnementaux et plus particulièrement par la température et la vitesse du vent. Nos résultats indiquent que cette installation ne dépendait pas de la quantité de plantes matures mais plutôt des facteurs de l'environnement et plus particulièrement de la vitesse du vent. Le taux d'installation des embryons varie avec la vitesse du vent. C'est à dire que plus la vitesse du vent était forte et plus le taux d'installation était faible. Le vent cause des mouvements de mer qui vont déplacer les jeunes embryons ; cette action du vent démontre soit que la fixation du zygote n'est pas efficace ou soit que les propagules fixés ne sont pas fécondés. En effet, les oosphères et les zygotes lorsqu'ils sont libérés se « collent » au substrat ; la fixation se solidifie avec le développement de la paroi qui n'a lieu que 8 heures après la fécondation. Cette première fixation ne semble pas efficace lors des mouvements de marées et vont déplacer les jeunes embryons.

La majorité des propagules dans la zone intertidale sont régulièrement détruites par les conditions physiques adverses. En effet, malgré une forte installation, le taux de mortalité post-installation est extrêmement important car il n'y avait pas de différences dans le nombre de recrues mesurés dans l'échelle de temps entre 1 et 30 jours. Ces résultats de mortalité sont à prendre avec modération car ces pourcentages de mortalité peuvent comprendre des effets de déplacement. Malgré une bonne fixation des embryons sur les substrats, il ne faut pas négliger l'action des vagues sur les substrats. Suivant les micro-habitats, le taux d'installation était plus fort sous la canopée qu'en surfaces exposées ou dans les cuvettes. Les conditions exposées ne sont pas favorables à un bonne installation, des surfaces lisses peu propices à la fixation d'une sphère. De plus, l'action des vagues est plus frappante sur ces surfaces que sur des surfaces chaotiques que créent les crampons des algues ou les algues elles mêmes. L'installation sous la canopée est importante, et les embryons doivent profiter de la protection des adultes qui assure une meilleure fixation par les crampons. Le pourcentage de mortalité est plus faible sous la canopée que sur les surfaces exposées et dans les cuvettes.

Les jeunes stades sous la canopée sont de plus, moins exposés aux herbivores que sur les surfaces rocheuses ou même dans les cuvettes. Le taux de survie est probablement très bas mais la taille des états microscopiques de la population reste inconnue. L'installation et le recrutement sont influencés par les facteurs physiques constituant les stress environnementaux de la zone intertidale.

Après l'installation, l'étude s'intéressait à la mise en place de l'appareil photosynthétique chez les jeunes embryons lors de leurs premiers jours de croissance. Les premières mesures concernaient le contenu pigmentaire. Les œufs contiennent les mêmes pigments que ceux trouvés dans les adultes. On y trouve une forte concentration en fucoxanthine, une xanthophylle, les deux types de Chlorophylles *a* et c et du β-carotène. Lors de la croissance, ce contenu pigmentaire augmentait rapidement suivant les différents stades. La fucoxanthine et la chlorophylle *a* ont des concentrations qui vont doubler alors que la concentration en β-carotène augmente peu. Des spectres de fluorescence ont mis en évidence que les pigments sont libres dans l'œuf et que ces pigments vont en s'attachant former les appareils photosynthètiques. Les embryons de *Fucus* devenaient photosynthétiquement opérationnels après les 30 premières heures de croissance. L'appareil photosynthétique étant complètement fonctionnel, un embryon sur le terrain va devoir supporter les cycles des marées.

En effet, les embryons maintenant installés dans leur milieu naturel vont subir les conséquences de l'immersion et l'émersion et pour survivre doivent s'acclimater à ces conditions très variables d'exposition à l'air. En effet, ces conditions vont varier rapidement avec le temps, l'espace et suivant les conditions météorologiques. Cette variation de toutes ces conditions environnementales crée une zonation des espèces dans la zone intertidale. Les algues en conditions émergées doivent affronter l'excès de lumière et le dessèchement causé par le vent et la température.

L'estimation de l'activité photosynthétique chez les macroalgues peut être maintenant réalisées *in situ* en utilisant une nouvelle technologie de mesure de la fluorescence chlorophyllienne en lumière modulée. Cette technique met en oeuvre un fluorimètre portable

(PAM 2000) qui permet des mesures rapides et non-destructrices. Celles-ci rendent compte de l'état de l'appareil photosynthétique des végétaux à un instant donné et mettent en évidence l'incidence des paramètres de l'environnement et des stress. Nous avons utilisé cette technique pour comparer les réponses d'embryons aux facteurs environnementaux de trois Fucales ;

- *Ascophyllum nodosum* (L.) Le Jolis,
- *Fucus vesiculosus* L.,
- *Fucus distichus* ssp.

La fluorescence a été mesurée sur ces algues dans l'estuaire du St Laurent durant la marée basse et au commencement de la marée montante sous quatre conditions météorologiques déterminées suivant les vitesses de vent et l'irradiance. En détail, les conditions ont été déterminées comme « ensoleillées-venteuses », « nuageuses-pas de vent », « venteuses-irradiance moyenne » et « ensoleillées-pas de vent ». Nos trois espèces dominent chacun un niveau de l'estran.

Deux hypothèses pourraient expliquer la zonation par les effets de la dessiccation sur les plantes intertidales. La première hypothèse est que les espèces de haut niveau qui contiennent une faible quantité en eau sont capables de maintenir une photosynthèse plus active que les espèces de bas niveaux. Mes résultats sont en accord avec cette hypothèse. *A. nodosum* qui croit dans les hauts niveaux possède sous toutes les conditions météorologiques une activité photosynthétique plus active que *Fucus distichus* placé plus bas sur l'estran.

La deuxième hypothèse est que le taux de récupération de la photosynthèse après une période d'émersion est plus complet dans les espèces de haut niveau. L'émersion n'est pas préjudiciable à la photosynthèse car la plupart des algues, même les stades les plus jeunes sont capables de tolérer la dessiccation. La perte d'eau commence dès qu'elles sont émergées. Dans le sens étroit du terme, dessiccation est équivalent à la déshydratation mais bien sûr ceci englobe les changements en nutriments car déshydratation et changements en nutriments arrivent en même temps. Il est vraisemblable que l'étendue de la dessiccation que peut tolérer une algue varie suivant les espèces. On peut penser que l'appareil photosynthétique des algues de haut niveau n'est pas plus résistant à la perte d'eau que les algues des bas niveaux, mais l'algue du bas niveau doit perdre plus d'eau. Les jeunes stades de *Fucus* sont un peu plus larges que ceux de *Ascophyllum* ; cette superficie est vraisemblablement responsable d'une plus grande perte d'eau.

Les taux de récupération des jeunes stades sont similaires dans nos résultats. Sous des conditions d'ombre, sous la canopée, les taux de photosynthèse de *Ascophyllum* peuvent dominer ceux des espèces plus basses. Ceci peut être important pour un organisme dont la productivité peut être limitée par de longues périodes d'émersion.

Nos courbes rETR en fonction de l'intensité lumineuse peuvent être assimilées aux courbes P vs E. Les jeunes stades ont une photosynthèse plus faible en réponse aux niveaux de lumière peu élevés trouvés sous la canopée ou sous les conditions nuageuses. Sous des conditions ensoleillées, les jeunes stades utilisent efficacement cette lumière disponible. Même si le taux de photosynthèse dépend de l'irradiance disponible, il apparaît que les jeunes stades des algues se comportent plus ou moins comme des plantes d'ombres. Des expériences préliminaires de transplantation présentées plus tard dans une autre étude pourront démontrées cette affirmation.

Cependant les réponses photosynthétiques des jeunes stades sont différentes de celles des adultes. Ces réponses sont plus faibles que celles des adultes qui contiennent plus de matériel pigmentaire que les plus jeunes.

Les résultats du chapitre 6 indiquent que la taille des jeunes stades face aux stress lumineux et de dessiccation est moins longue et que la croissance est affectée ; ceci indique que les concentrations de pigments, le taux de chlorophylle a sur les pigments accessoires sont réduits. Cette évolution pourrait être un sujet à de plus longues investigations.

Après une exposition à la même intensité lumineuse, les réponses varient entre les algues des différentes zones, avec des plantes vivant dans le bas littoral montrant plus de photoinhibition. Habituellement un excès d'énergie lumineuse atteint l'appareil photosynthétique et différents mécanismes sont impliquées pour protéger la plante de cet excès d'énergie. La formation et l'activation de ces mécanismes résultent dans la photoinhibition, comme la décroissance de l'efficacité photosynthétique. La photoinhibition est le résultat de 2 processus, le premier est une capacité photoprotectrice, se traduisant par la dissipation de l'énergie par la température. Lorsque cette capacité de photoprotection est dépassée, l'appareil photosynthétique peut être dégradé.

L'exposition à l'atmosphère durant la marée descendante, cause un stress auquel les différentes variétés de fucales sont plus ou moins sensibles. Les trois espèces dans notre terrain d'étude se répartissent en zones successives selon un gradient. A partir de fécondations réalisées en laboratoire, nous avons fixé des substrats artificiels avec nos embryons dans chaque zone préalablement définie comme la zone de *A. nodosum*, celle de *F. vesiculosus* et celle de *F. distichus*. Les espèces des embryons correspondaient avec la zone des adultes. De plus, dans le premier chapitre, ayant démontré l'importance de l'installation des embryons sous la canopée, des substrats ont été placés sous deux micro-habitats différents, sous la canopée et sur des surfaces exposées. Au cours de la marée basse, les variations de certains paramètres de fluorescence ont été mesurés : le rendement quantique maximum (Fv/Fm), le rendement quantique effectif (Y), la vitesse relative du transfert d'électrons (rETR) et l'extinction de fluorescence non-photochimique (NPQ). Les résultats ont indiqué une décroissance de trois premiers paramètres et d'une augmentation du NPQ. La réduction de la photosynthèse débute dès que les algues sont émergées. L'excès d'énergie est évacué sous forme de chaleur. La reprise de l'activité photosynthétique prouve que l'exposition des algues à l'air n'est pas destructrice. Les variations des paramètres de fluorescence étaient drastiques sous les conditions météorologiques les plus sévères, c'est à dire, sous des conditions ensoleillées et venteuses. Les espèces qui souffrent le plus sont les espèces qui occupent la partie basse de l'estran quelle que soit les conditions météorologiques. Les paramètres de fluorescence sont généralement significativement différents entre les deux espèces occupant les zones les plus hautes, *A. nodosum-F. vesiculosus* et la plus basse, *F. distichus*. La restauration du transfert d'électrons était très rapide pour les trois espèces étudiées sous toutes les conditions météorologiques et aucune différence n'a été détectée entre l'évolution des propriétés de fluorescence des différentes espèces. Certains résultats pour les paramètres de fluorescence (NPQ) supportent l'hypothèse d'une capacité des embryons de l'espèce placée plus haut sur l'estran à mieux supporter le dessèchement subit pendant la période exposée à l'air que celle des espèces localisée plus bas. Cette aptitude à supporter la dessiccation plus longtemps serait un facteur qui pourrait expliquer la zonation de ces trois espèces. Dring et

Brown (1982) estimèrent que trois hypothèses pourraient expliquer les effets de dessiccation sur les plantes et sur la zonation. Les espèces placées haut sur l'estran sont capables de maintenir une photosynthèse plus active que les espèces plus bas sur l'estran ; ceci est en accord avec nos données ; ***Ascophyllum***, l'espèce la plus haute sur l'estran possède une activité photosynthétique un peu plus active que les deux autres espèces étudiées ; toutefois, cette activité est souvent confondue avec l'activité de l'espèce la plus proche.

La deuxième hypothèse concerne la reprise de la photosynthèse après une période d'émersion est plus rapide dans les espèces placées haut sur la zone intertidale. Cette reprise est plus complète dans les espèces hautes sur l'estran. D'après quelques résultats préliminaires que nous avons réalisés, les adultes répondent d'une façon plus brusque que celle de nos jeunes juvéniles. Les réponses des adultes sont bien espacées dans le temps. Les juvéniles seraient déjà adaptés aux conditions d'émersion mais n'ont pas encore atteint la capacité des adultes à une activité photosynthétique plus active. Leur reprise de l'activité photosynthétique est similaire pour nos trois espèces ; cette reprise n'est ni plus rapide ni plus lente pour aucun des espèces et elle ne peut en aucun cas expliquer la zonation dans nos résultats.

Même si *A. nodosum* peut tolérer plus longtemps l'excès de lumière et les stress de desiccation, une question se pose : pourquoi ne domine-t-il pas l'estran ? Localisé plus bas que sa position originale sur l'estran, les jeunes stades de *A. nodosum* sont moins sévèrement stressés que placés dans leur lieu originel. Cependant, les mesures de recrutement indiquent que *A. nodosum* avait un fort taux de recrutement avant les autres espèces (Chapitre 2). Cependant, la période de recrutement pour cette espèce est courte (trois semaines, pers. obs) alors que *F. vesiculosus* et *F. d.* ssp. *edentatus* maintenaient un fort taux de recrutement pendant une période de temps plus longue (2 mois, Chapitre 2). Même si *A. nodosum* était capable de supporter une plus longue desiccation, l'espèce ne serait pas capable de compétitionner avec les deux autres espèces de *Fucus*.

Sous la canopée, la décroissance du rendement quantique maximum était plus graduelle mais ses faibles valeurs par rapport à celles des thalles placés en conditions exposées et celles du rETR indiquaient une photoacclimatation des thalles qui se comportaient comme des plantes d'ombres. Bien que la canopée entretienne une certaine humidité, les mesures de fluorescence des embryons localisés sous la canopée indiquent une chute du transfert d'électrons. L'humidité ne serait pas suffisante pour conserver l'efficacité du PSII chez les jeunes embryons. La canopée possède alors l'avantage de favoriser l'installation des œufs, de garder une certaine humidité qui ralentit la dessiccation et donc ralentit la chute de l'activité photosynthétique mais cette activité n'est pas totale car cette canopée est aussi un voile à l'énergie lumineuse. La photosynthèse dépend de l'énergie lumineuse disponible. Les paramètres de fluorescence indiquent que les algues subissent à la marée descendante un excès d'énergie lumineuse qui cause la photoinhibition ; cette photoinhibition est accentuée par la dessiccation durant la marée basse. La reprise rapide de l'activité photosynthétique indique que la photoinhibition n'est pas dommageable et on peut parler de la mise en marche du premier processus de la photoinhibition qu'est la photoprotection. Cette photoprotection implique la transformation de xanthophylles qu'il serait intéressant de doser en laboratoire. Quelques données préliminaires de contenu pigmentaire nous ont montré que la transformation de xanthophylles est mise en place très tôt chez les embryons.

Les algues montrent sur un schéma de régulation et de photoprotection commun, de grandes variations individuelles. Les plantes croissant dans des conditions lumineuses variées possèdent des capacités de réaction à « l'agression lumineuse » très différentes. L'habitude des « agressions » permet par une voie que l'on ignore de forger le caractère (Jupin) de ces algues et leur permet d'acquérir, rapidement des adaptations efficaces. Des études reliant les adaptations à court terme et à long terme restent donc à effectuer, mais les connaissances déjà acquises permettent de montrer l'intégration des diverses fonctions dans le thylakïde et leur régulation.

Après une étude des jeunes algues sur des distances qui sont importantes, le comportement photosynthétique des thalles juvéniles de l'algue brune *Fucus distichus* ssp. (Fucales, Phaeophyta) a été étudié sur des petites distances. Mon terrain d'expérience a fourni l'opportunité d'observer des juvéniles de *Fucus* à différentes hauteurs de marée le long d'un mur rocheux dans la zone intertidale. Au cours de la marée basse, nous avons utilisé la fluorescence modulée pour examiner Fv/Fm, Y, la vitesse de transfert d'électrons relatif (rETR) et l'extinction de fluorescence chlorophyllienne non-photochimique (NPQ) des juvéniles de fucales à cinq niveaux intertidaux séparés d'une distance de 1,2 mètre. Ces observations étaient toujours faites sous nos quatre conditions météorologiques définies. Le taux d'évaporation et les niveaux d'irradiance ont été mesurés simultanément. Un gradient de réponses a été généralement observé: les deux plus hauts niveaux montrèrent un déclin du Fv/Fm, du Y, et du rETR avec une augmentation concomitante du NPQ dans le temps. Les niveaux plus bas séparés de 60 à 120 cm des deux niveaux les plus hauts, étaient moins rapidement exposés à la lumière et à la dessiccation ; ils ne montraient pas de décroissance du rendement quantique, ni du transfert d'électrons mais une légère augmentation de l'extinction de fluorescence non-photochimique au cours de la prise de mesures de fluorescence. L'association du vent et du soleil créait les conditions climatiques les plus sévères alors que les moins difficiles étaient les conditions nuageuses pour lesquelles seul le niveau le plus haut a montré une décroissance des rendements quantiques. Les corrélations entre les paramètres de fluorescence chlorophyllienne et le taux d'évaporation traduisaient l'influence majeure du stress hydrique chez les très jeunes thalles et se manifestait par une densité de population et une croissance plus faible aux deux niveaux les plus hauts.

Perspective

Mon travail n'est qu'une ébauche de l'étude des stress environnementaux sur le développement des embryons de *Fucus*. Il reste énormément de recherches complémentaires à effectuer.

Nos résultats indiquent que les facteurs environnementaux sont responsables de l'installation des œufs de Fucus. Une bonne coordination de nos données de recrutement avec les données de température de l'institut Maurice Lamontagne devrait pouvoir démontrer que la température a aussi une action sur l'installation de ces jeunes embryons. Pour le deuxième chapitre, La germination étant dépendante de la température, l'étude du contenu pigmentaire dans les premiers stades de croissance, dans des conditions de températures variables seraient à tenter.

Pour le troisième chapitre, j'ai effectué des expériences de transplantation qui consistaient à poser les substrats des différentes espèces d'embryons indépendamment de la zone définie. Je mesurais la fluorescence ; les résultats ne sont pas présentés dans ce document mais un papier devrait voir le jour car la transplantation dans ce chapitre pourrait caractériser

l'adaptation à une zone restreinte. Les études de Davison (1993, 1998, 2001) émettent l'hypothèse que les réponses de l'appareil photosynthétique aux stress lumineux ou thermiques chez différentes espèces de Fucales seraient dues à une adaptation génétique.
Cette capacité à supporter le dessèchement plus longtemps serait due à une adaptation génétique qui serait facile à déterminer depuis la mise sur le marché des marqueurs génétiques d'algues brunes (Olsen et al. 2002).

En cherchant une explication à cette aptitude à tolérer la dessiccation, j'ai découvert l'apparition de nouvelles protéines lors du développement des embryons des plantes supérieures pendant les stress de dessiccation et de lumière. Ces nouvelles protéines sont appelées ELIPs (Early-Light Induced Proteins). Elles sont synthétisées chez les végétaux supérieurs et chez quelques microalgues durant des stress lumineux et de dessiccation. Par analyse de protéines sur des embryons de fucus stressés par la lumière et la dessiccation, j'ai été en mesure de mettre en évidence cette protéine. Ce sont des expériences préliminaires qui doivent être poursuivies par des analyses génétiques. De plus, l'adaptation discutée auparavant pourrait exister au sein des ELIPs qui sont chez les plantes supérieures des protéines conservées. La découverte des ELIPs pourrait favoriser une recherche plus poussée dans l'apparition de nouvelles protéines face aux stress, comme les LEA qui ont été mis en évidence chez les embryons des plantes supérieures.

Les études de Collen et Davison (1999, 2001) montrent l'impact des molécules d'oxygène singulets sur les embryons ; ces molécules réactives d'oxygène seraient responsables de la zonation des fucales. Pour une étude sur le terrain plus poussée, établir des corrélations entre les paramètres de fluorescence chlorophyllienne et l'activité de dégagement d'oxygène aurait été une étude plus fine afin d'étudier l'effet du stress.

Il s'avérerait judicieux qu'un projet identique au mien se fasse dans le cadre d'une nouvelle coopération entre le Canada et la France. Les mêmes expériences effectuées simultanément de part et d'autres de l'Océan pratiquement sous la même latitude éclaireraient peut être différemment le résultat de mes recherches.

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Résumés

Résumé I

La plupart des organismes benthiques subissent dans la zone intertidale des périodes d'émersion et d'immersion journalières. C'est de la colonisation par les embryons et de leur survie que dépende la continuité de la population. Les Fucales, algues brunes, sont parmi les espèces dominantes des côtes rocheuses atlantiques et plusieurs de leurs espèces sont distribuées verticalement selon un gradient de stress physique. Ce travail montre que la colonisation par les embryons est spatialement et temporellement variable dépendant de certains facteurs climatiques et physiques. La mise en place de l'appareil photosynthétique chez ces embryons est complète après 6 jours de développement. La deuxième partie de cette thèse présente les évolutions des réponses photosynthétiques de trois espèces d'embryons lorsqu'ils supportent le dessèchement et l'excès de lumière occasionnés par différentes conditions météorologiques durant la marée basse et suivant leur emplacement. La dessiccation semble jouer un rôle majeur dans la distribution de ces algues.

Résumé II

La zone intertidale est un environnement extrême en raison de l'alternance de périodes d'émersion et d'immersion. Les organismes benthiques comme les algues brunes doivent être capables de coloniser rapidement cette zone et de survivre dans ces conditions difficiles. Ce travail consiste à étudier, d'une part, la colonisation du milieu par ces algues et, d'autre part, l'effet des paramètres climatiques sur le développement de leurs jeunes organismes dans l'estuaire du St Laurent (Canada). (1) Des dénombrements d'embryons sur des substrats artificiels permettent d'observer une grande variabilité spatiale et temporelle du recrutement, indépendant du nombre d'adultes matures mais conditionné par des facteurs climatiques. Le taux de mortalité s'avère plus important sur les surfaces rocheuses exposées et dans les cuvettes que sous la canopée. (2) Par des mesures de fluorescence chlorophyllienne en laboratoire, nous avons suivi la mise en place de l'appareil photosynthétique de ces embryons qui s'avère fonctionnel après six jours de développement. (3) Des mesures de fluorescence chlorophyllienne réalisées sur le terrain montrent que l'activité photosynthétique de jeunes embryons de trois espèces différentes, confrontés à quatre conditions météorologiques définies, déclinent inégalement durant les marées basses. Ces différences de sensibilité au stress déterminent l'emplacement de ces trois espèces sur l'estran, celle qui occupe le niveau bathymétrique le plus élevé étant capable de maintenir une activité photosynthétique plus longtemps au cours d'une émersion que celles qui vivent à des niveaux inférieurs. De plus, pour chaque espèce, les embryons qui se développent sous la canopée sont moins affectés durant la marée basse mais, en raison de la faible irradiance, leur photosynthèse est plus faible. (4) Durant la marée basse, un gradient dans la rapidité de dégradation de l'activité photosynthétique de jeunes thalles situés à cinq niveaux différents sur une paroi verticale a été mis en évidence par des mesures de fluorescence chlorophyllienne, les thalles des niveaux supérieurs étant les plus affectés. Cette altération est étroitement corrélée à un gradient de dessiccation qui apparaît comme un des facteurs déterminant l'emplacement des algues sur l'estran.

Abstract

The intertidal zone is an extreme environment due to the alternation of emergence and immersion. The benthic organisms, specially the young stages have to be able to colonize quickly this zone and to survive to these extreme conditions. Among benthic species of estuary of St Laurent (Québec, Canada), brown algae are dominant and live their optimum ecological niche. The rigorous winter cause the recovery of the intertidal zone by ice. All organisms live slowly or dye under this ice cover. In adopting an ecophysiological approach, this work aims at the study on the one hand the colonisation of the zone and on the other hand the effect of climatic parameters on the development of juveniles. (1) To estimate the colonisation, counting of embryos on artificial substrata allow to observe a wide spatially and temporally range of recruitment that don't depend to the number of adults; but would be strongly influenced by climatic factors. The mortality rate is variable and proves more important on rocky shores exposed than in basin and under canopy. (2) By measuring fluorescence in laboratory, we followed the set up of photosynthetic apparatus into embryos and these embryos have efficient photosynthetic apparatus after 6 days of growth. (3) Measurements of fluorescence directly in the field have to be realised on embryos of three species located in their niche under four meteorological conditions ; Their photosynthetic answer are similar traducing by a decline during low tide. However the ability of photosynthesis in these juveniles call back those of adults. The embryos located under canopy have a electron transport working longer but weaker than on exposed surfaces. (4) On a wall, five levels with juveniles have been determined; the measurements of fluorescence relative to measurements of desiccation and light showed that the yield of juveniles of higher levels dropped more rapidly than those of lower levels. The correlations proved the action of light get worse with the action of wind. The percentage of mortality increased in the higher levels and the growth rate decreased. The desiccation seems to be greater responsible of the zonation of algae.

Avant-propos

La présente thèse a été rédigée en utilisant la formule mixte. Elle est présentée sous forme de quatre articles rédigés en anglais. Le deuxième article (Chapitre 3), sur l'évolution du contenu pigmentaire au cours du développement embryonnaire de l'algue brune, *Fucus* a été accepté pour publication dans la revue ***Photosynthesis Research*** en collaboration avec le Dr. Benoît Schoefs, le Pr. Yves Lemoine et l'aide précieuse du Dr. Eva Darko. Pour cet article, je témoigne que je suis responsable de l'organisation et l'accomplissement des expériences au laboratoire, l'analyse des données et la rédaction du manuscrit. Et je déclare que les prochains articles qui je l'espère seront soumis avant la soutenance seront tous de mon propre travail. J'atteste également que, bien que mes directeurs, ainsi que les autres co-auteurs m'ont appuyé intellectuellement avec des échanges d'idées stimulants durant toute ma recherche, cette thèse est le résultat de mon propre effort.

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« Ayez le culte de l'esprit critique »
L. Pasteur (1888)

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