Les amoebophryidae (Syndiniales) parasitoïdes de dinoflagellés : cycle de vie, dynamique et spécificité in situ

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TABLE DES MATIÈRES

TABLE DES FIGURES

LISTE DES TABLEAUX

ABBREVIATIONS

ADN	Acide DesoxyriboNucéique
ARNr	Acide RiboNucléique Ribosomique
ARNr 18S (=SSU)	Acide RiboNucléique Ribosomique 18S (=petite sous-unité)
ARNr 28S (=LSU)	Acide RiboNucléique Ribosomique 28S (=grande sous-unité)
ASP	Amnesic Shellfish Poisoning
bp	base pair
Mbp	Mega base pair
ciPCR	culture independent Polymerase Chain Reaction
DSP	Diarrhetic Shellfish Poisoning
FISH	Fluorescent *in situ* Hybridization
FISH-TSA	Fluorescent *in situ* Hybridization coupled with Tyramide Signal Amplification
HAB	Harmful Algal Bloom
HSP70	Heat Shock Protein 70
MALV	Marine ALVeolate
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PSP	Paralytic Shellfish Poisoning
REPHY	REseau de surveillance du PHYtoplanctonique et des phycotoxines
STX	SaxiToXine

Introduction Générale

INTRODUCTION GÉNÉRALE

Tous les organismes, qu'ils soient terrestres ou marins, sont intégrés dans des réseaux d'interactions, positifs ou négatifs, avec leurs congénères. Ces multiples interactions peuvent avoir lieu soit à l'intérieur d'une espèce (intra-spécifique), soit entre espèces (inter-spécifique). Autant les interactions agonistes, par exemple la symbiose (mutualisme) où les deux organismes sont bénéficiaires, que les interactions antagonistes comme le parasitisme où un des organismes exploite l'autre à ses dépends, sont considérées comme des relations durables (Figure 1). A l'opposé, les interactions de type prédateur-proie constituent un transfert d'énergie éphémère où la pression de sélection se déroule à un moment donné [Combes, 2001].

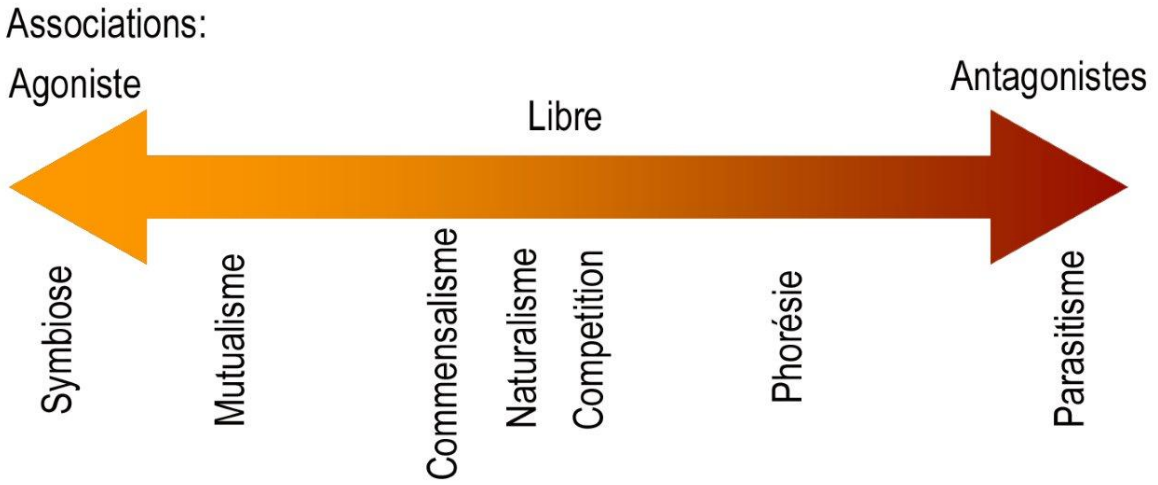


FIG. 1 – Différents types d'associations durables entre les organismes : de l'agonisme à l'antagonisme.

Au sein des interactions durables, le parasitisme est extrêmement fascinant car il impose une pression de sélection constante sur l'organisme cible. Ce type d'interaction peut entraîner la mort de l'un des deux partenaires, comme c'est le cas pour les parasitoïdes, qui tuent obligatoirement leur hôte pour accomplir leur cycle de vie. Tous les organismes subissent, en tant qu'hôte ou en tant que parasite, cette pression de sélection. Le parasitisme est donc un mécanisme essentiel dans les réseaux trophiques modifiant, voire contrôlant, les dynamiques des populations hôtes. Mais de telles interactions ne sont pas stables dans le temps, l'hôte cherchant en permanence une échappatoire au parasite et ce dernier essayant d'améliorer son potentiel d'infection et de contrer les mécanismes de défense de l'hôte. Cette « course aux armements » est une bataille sur le long terme, qui fait appel à l'ingéniosité des deux partenaires [Van Valen, 1973].

Ainsi, dans le cas d'un système hôte/parasite, le génome du parasite peut induire une modification du phénotype de l'hôte et *vice versa* [Combes, 2001]. Par exemple, d'après Waage, ce phénomène pourrait être à l'origine des rayures sur le pelage des zèbres [Waage, 1981]. En effet, différents travaux ont montré que les mouches Tsé-tsé, qui inoculent le parasite *Trypanosoma gambiense*, sont attirées par les grandes surfaces unies synonymes de grands animaux. Ainsi, il n'est pas inconcevable de supposer que la présence de rayures chez les zèbres ait pu être sélectionnée au cours de l'évolution comme moyen de dissimulation face aux mouches Tsé-tsé. Inversement, un parasite, tel que *Plasmodium falciparum*, l'agent de la malaria, doit continuellement modifier ses protéines membranaires pour échapper au système immunitaire de l'hôte [Kidgell *et al.*, 2006]. Tous les organismes, microscopiques ou non, subissent ce type d'interactions, que l'on soupçonne être l'un des moteurs de l'évolution [Van Valen, 1973].

Le parasitisme est universel dans la nature. Cependant, la majorité des études se sont focalisées sur le milieu terrestre, vers des organismes d'intérêts médicaux ou agricoles. A l'opposé, le milieu marin reste encore de ce point de vue relativement inexploré. Néanmoins, depuis quelques années, la découverte des virus marins a permis de mettre en évidence l'impact du parasitisme. Bien qu'aujourd'hui plus aucun doute ne réside sur l'importance des pathogènes dans les dynamiques des populations marines, l'impact des parasites eucaryotes, quant à lui, reste largement sous-estimé.

Dans le cadre de cette thèse, nous nous sommes intéressés à des parasites infectant un des plus importants compartiments dans le réseau trophique marin, le phytoplancton.

Les relations entre différents organismes (d'après [Breuil, 1997] :

La symbiose (du latin *Syn*, « ensemble » et *biose* « vie ») : les deux organismes sont physiologiquement indépendants, ils assument en revanche, l'un vis à vis de l'autre, un rôle assimilable à une fonction organique. Leurs survies respectives sont interdépendantes.

Le mutualisme : interaction facultative entre deux ou plusieurs espèces car les deux partenaires peuvent vivre l'un sans l'autre.

Le commensalisme (du latin *co-*, « avec » et *mensa*, « table » , *e.g.* « compagnon de table ») : interaction dans laquelle l'hôte fournit une partie de sa propre nourriture au *commensal*. Il n'obtient en revanche aucune contrepartie évidente de ce dernier (le bénéfice de cette relation n'est pas réciproque). Le commensalisme est une exploitation non-parasitaire d'une espèce vivante par une autre espèce.

La phorésie (du grec *phoros* « porter ») : l'hôte n'a d'autres fonctions que d'assurer le transport du *phoronte*(transport temporaire dans la plupart des cas). Il s'agit d'une association libre (les sources de nourriture de l'un et l'autre des partenaires étant indépendantes) et non destructrice (le transport en question n'occasionne pas de dommage physiologique particulier).

Le parasitisme (du grec *para* « à coté » et *sitos* « qui mange », « qui mange à la table de l'autre ») : non seulement le bénéfice de la relation est unilatéral, mais le parasite est destructeur pour son propre hôte (différent de pathogène, du grec *pathos* « maladie génique » et gène qui signifie « produit »).

Parasitoide : signifie que le parasite tue son hôte.

Le parasitisme en général

Le parasitisme est un schéma d'interaction universel dans la nature. En effet, tous les organismes sont impliqués dans de telles interactions, en tant qu'hôte ou parasite. Ces interactions peuvent être d'une complexité variable, avec souvent plusieurs parasites pour un même hôte, la nécessité de plusieurs hôtes successifs pour le parasite, ou encore l'existence de parasites de parasites (hyperparasitisme).

Un parasite pour un ou plusieurs hôtes

La question de la spécificité est très variable suivant les organismes étudiés. Par exemple, R.H. Emeson et V. Mladenov ont montré que le copépode *Ophiopsyllus reductus*, parasite d'ophiures (échinoderme marin), ne peut infecter qu'une seule des sept espèces présentes dans le milieu [Emson and Mladenov, 1987]. Dans d'autre cas, il n'existe pas de spécificité apparente, le trématode *Labratrema minimus* pouvant infecter différentes espèces de poissons de la mer Méditerranée.

Différents hôtes pour survivre

Même dans le cas d'une spécificité importante, un parasite peut avoir besoin d'un hôte secondaire pour compléter son cycle de développement. Par exemple, les parasites du genre *Plasmodium* se reproduisent sexuellement dans le moustique, son hôte secondaire, bien que son hôte principal soit l'Homme.

Des parasites de parasites

Certains parasites peuvent aussi infecter d'autres parasites. C'est le cas d'un champignon, *Ampelomyces quisqualis*, qui infecte un autre champignon, le mildiou, responsable d'infection sur un large choix de plantes hôtes. D'autres exemples se retrouvent dans le modèle marin où *Amoebophrya leptodisci*, un protiste unicellulaire marin, infecte un autre protiste parasite du genre *Amoebophrya*, *A. ceratii*, qui infecte à son tour un des plus important groupe phytoplanctonique, les dinoflagellés [Cachon, 1964].

Plusieurs parasites infectant le même hôte

Un hôte peut être infecté par différents parasites, ce qui est très fréquent chez les animaux. Chez les micro-eucaryotes unicellulaires, la co-infection est rare, mais plusieurs espèces parasites peuvent tout de même infecter la même espèce hôte, comme c'est le cas pour *Amoebophrya ceratii* et *Parvilucifera infectans* capables d'infecter tous deux des dinoflagellés comme *Alexandrium minutum*. Cela peut être encore plus complexe dans le cas des attaques virales, où plusieurs virus peuvent infecter en même temps le même hôte.

Dans la nature tous les schémas sont donc envisageables. Néanmoins, malgré son impact reconnu d'un point de vue évolutif ou démographique sur les populations hôtes, le parasitisme n'est pas souvent intégré dans les modèles écologiques, encore moins lorsqu'il concerne un compartiment essentiel du milieu marin, le phytoplancton.

Rôle du parasitisme au sein du plancton marin

Le phytoplancton est à la base de la chaîne trophique au sein du plancton marin. Il permet de transformer des composés inorganiques en composés organiques, et de les transmettre aux échelons trophiques supérieurs. La dynamique du plancton est fortement dépendante de facteurs tout aussi bien abiotiques (tels que température, lumière...) que biotiques (prédation, parasitisme...).
Ainsi, un vaste éventail de parasites eucaryotes ou procaryotes peuvent infecter le phytoplancton. Ces pathogènes peuvent être des virus, des bactéries, des champignons, ou des parasites eucaryotes. Dans la suite de cette introduction, nous avons pris le parti de nous focaliser sur l'impact de deux groupes de parasites sur le phytoplancton, les virus qui sont le sujet de la majorité des études sur le parasitisme en milieu marin et les parasites eucaryotes en général.

Exemple de parasites ubiquistes : les virus

Les virus des milieux aquatiques sont très abondants (de l'ordre de 10^4 à 10^9 virus.mL^{-1}) en milieu marin [Bergh *et al.*, 1989, Bratbak *et al.*, 1994]. Au sein du plancton marin, ils sont capables d'infecter aussi bien les bactéries, les cyanobactéries, que le phytoplancton de grande taille, tel que les dinoflagellés. Les virus pourraient donc influencer la diversité du phytoplancton [Weinbauer and Rassoulzadegan, 2004] voire de toute la chaîne alimentaire marine (Figure 2) [Fuhrman, 1999]. Thingstad et Lignell ont élaboré le concept de « killing the winner » [Thingstad and Lignell, 1997]. Ce concept part du principe que le contrôle de la diversité de la population hôte repose sur la destruction sélective de l'espèce prédominante. Ainsi, le rôle des virus serait d'empêcher un accroissement exponentiel d'une espèce hôte et de permettre l'accroissement d'une autre espèce moins compétitive. Ce concept aboutit à un équilibre dynamique, permettant d'expliquer la coexistence de nombreuses espèces, ainsi que la terminaison brutale des efflorescences [Weinbauer and Rassoulzadegan, 2004].

Le processus de lyse virale permet de transformer de la matière organique vivante en matière organique libre dans le milieu. Les bactéries pourront alors consommer cet apport de matière organique. L'activité virale intervient donc aussi au niveau des flux de matière, en contribuant au recyclage de la matière organique. De plus, en alimentant le réservoir de carbone organique, les virus contribuent à la régénération d'éléments tels que le carbone, l'azote, le phosphore, le soufre et le fer, qui, sortis de la chaîne alimentaire, redeviennent sous forme dissoute disponibles pour de nombreux Procaryotes. Les virus peuvent alors former une boucle ou semi boucle qui est appelée « Shunt viral ». La lyse virale aura alors pour conséquence d'augmenter la respiration procaryotique en diminuant par la même occasion la respiration et la production des protistes et du gros zooplancton [Weinbauer and Rassoulzadegan, 2004].
Enfin, il existe un mécanisme où les phages peuvent agir directement sur la diversité génétique. En effet, ils peuvent avoir un rôle de vecteur de l'information génétique. Le génome viral va alors

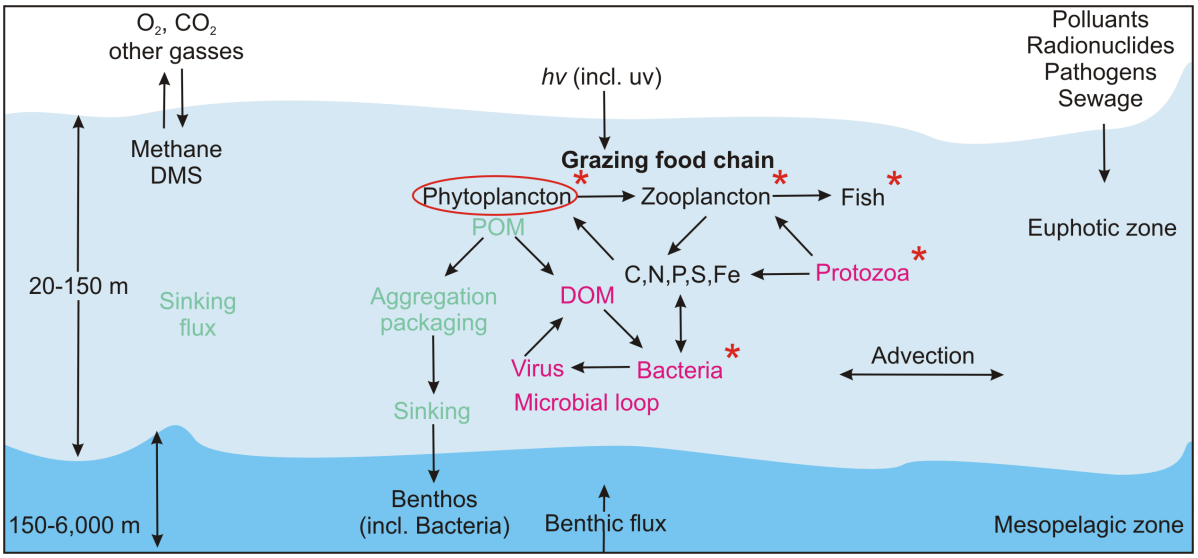


FIG. 2 – Réseau trophique dans le milieu marin [Nybakken, 2001]. Le phytoplancton est encerclé en rouge. *Représente les organismes infectés par des parasites procaryotes ou eucaryotes.

s'intégrer dans le génome de la cellule hôte, ce mécanisme est appelé lysogénie. Il est considéré comme une stratégie de survie lorsque la densité des hôtes est trop faible [Fuhrman, 1999]. Ce mécanisme est réversible, le virus peut ainsi lorsque la densité de l'hôte est suffisante, revenir à un cycle de vie lytique. Par ce mécanisme les phages peuvent intervenir dans l'échange d'ADN par transduction lorsque l'ADN de la cellule hôte est accidentellement encapsidé dans le phage durant l'assemblage. Lorsque ce phage infecte un autre hôte, au lieu d'insérer uniquement son génome, il insère en même temps une partie du génome de l'hôte précédent. Jiang et Paul ont estimé que dans l'estuaire de Tampa en Floride, il y aurait $1{,}3\times10^4$ événements de transductions par an [Jiang and Paul, 1996]. En extrapolant, ils ont déduit que les phages marins pouvaient transférer 10^{28} paires de bases par an dans l'océan mondial [Muyzer and Ramsing, 1995, Fuhrman, 1999]. La présence de ce type de mécanisme est aussi suspectée chez les micro-algues tel que *Phaeocystis pouchetii* (Haptophyte) et pourrait être un des moteurs de la diversité génétique [Monier *et al.*, 2008].

Les parasites eucaryotes

Les parasites eucaryotes sont composés de champignons, Perkinzoa, Amoeboa, dinoflagellés, euglenoïdes, kinetoplastides, et d'autres flagellés. Ils sont principalement connus pour infecter aussi bien les diatomées que des dinoflagellés mais ont aussi la capacité d'infecter d'autres taxons tels que les cyanobactéries, les chrysophytes, les cryptophytes, les chlorophytes, les prymnesiophytes, ainsi que des ciliés et des copépodes... La plupart des études ont été de nature descriptive, ne donnant ainsi que peu de renseignement sur la biogéographie des parasites ou sur leur rôle dans le contrôle de la chaîne alimentaire [Park *et al.*, 2004]. L'impact

des parasites eucaryotes sur la chaîne alimentaire se révèle proche du rôle des virus. Ils interagissent aussi bien avec tous les compartiments du plancton qu'avec des niveaux trophiques supérieurs. En effet, des parasites comme certains champignons peuvent induire la mortalité de diatomées, supprimer ou retarder leur efflorescence. Par exemple, Canter et Lund en 1951 ont montré que le parasite chytrid (champignon) pouvait diminuer l'abondance de la diatomée d'eau douce *Asterionella formosa* et permettre la croissance d'autres diatomées, *Fragilaria crotonensis* et *Tabelarria fenestrata* [Canter and Lund, 1951]. *Amoebophrya* sp. (Syndiniales, dinoflagellés) est également capable d'infecter massivement certaines espèces de dinoflagellés en milieu marin [Coats *et al.*, 1996]. Les parasites eucaryotes peuvent aussi infecter les échelons trophiques supérieurs et réguler l'abondance et la diversité de leurs hôtes. Par exemple des parasites comme *Duboscquella* sp., *Syndinium* sp. (Syndiniales, dinoflagellés) ou encore *Oxyrrhis* sp. (Dinoflagellés) peuvent infecter le zooplancton : ciliés, copépodes... [Park *et al.*, 2004]. D'autre part, ces parasites ont la capacité d'alimenter eux-mêmes la chaîne alimentaire. En effet, ces parasites eucaryotes ont une taille suffisante pour être consommés par des brouteurs. Pour exemple, il a été démontré que la forme libre d'*Amoebophrya* sp. pouvait être consommée par les ciliés [Johansson and Coats, 2002].

Bien que le parasitisme eucaryote soit sous estimé, il est important dans le réseau trophique marin :

- Tous les organismes sont infectés par des parasites eucaryotes, procaryotes, ou viraux.
- Ils permettent de réguler les populations.
- Ils jouent un rôle de pression de sélection sur les populations hôtes.

Objectif et organisation du travail

A travers tous les parasites infectant le phytoplancton marin, nous avons choisit un modèle de parasites eucaryotes, les Amoebophryidae (synonyme des Alvéolés du Groupe II). Les séquences de ces organismes sont retrouvées dans tous les écosystèmes étudiés, laissant supposer un rôle écologique important [Moon-van der Staay *et al.*, 2001,Lopez-Garcia *et al.*, 2001]. Cette thèse a débuté par la caractérisation de la diversité génétique de ces parasites dans le but de comprendre leur impact écologique plus particulièrement lors d'efflorescences, toxiques ou non, de ses hôtes. Enfin une étude plus physiologique a été réalisée pour caractériser leur spécificité d'infection et comprendre leur persistance annuelle dans la colonne d'eau, ce qui reste encore une énigme.

Cette thèse s'est donc déroulée suivant les quatre axes suivants :

- **Etude de la diversité génétique des Alvéolés du Groupe II grâce aux données environnementales (librairies génétiques)**

- **Impact *in situ* et *in silico* des Alvéolés du Groupe II sur les populations hôtes**
- **Etude de la spécificité d'infection**
- **Etude du cycle de vie de ce parasite en culture**

Afin de comprendre la relation hôte parasite, il est important de connaître séparément chacun des acteurs. Dans un premier chapitre de ce manuscrit, un état des lieux a donc été réalisé sur les hôtes, les dinoflagellés, et leurs parasites, les Alvéolés du Groupe II.
Ce travail de thèse a débuté par une collaboration à un travail de synthèse de la diversité génétique des Syndiniales grâce à l'apport de données environnementales, ainsi qu'une évaluation de la biogéographie de ces parasites. Ce travail a donné lieu à une publication dans *Environmental Microbiology* en 2008.

Guillou L., Viprey M., Chambouvet A., Welsh R.M., Kirkham A.R., Massana R., Scanlan D.J., Worden A.Z. (2008). Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). *Environmental Microbiology* 10(12) :3349-3365.

Pour étudier plus précisément les interactions entre les Alvéolés du groupe II et les dinoflagellés, un écosystème particulier, l'estuaire de la Penzé dans la baie de Morlaix, a été choisi. Cet écosystème est caractérisé par la présence récurrente d'un dinoflagellé toxique, *Alexandrium minutum* dont les efflorescences ont été un problème sanitaire majeur jusqu'en 1998. Des analyses *in situ* de la dynamique hôte-parasite, ainsi que l'étude de la spécificité de ces interactions ont été entreprises durant trois années consécutives. Ce travail a donné lieu à une publication dans *Science* en 2008.

Chambouvet A., Morin P., Marie D., Guillou L. (2008). Control of toxic dinoflagellate blooms by serial parasitic killers. *Science* 322 :1254-1257.

Afin de confirmer les capacités de contrôle du parasite sur les populations de dinoflagellés, un modèle comparant pression de broutage par les prédateurs et action plus ciblée du parasitisme a été élaboré, en collaboration avec David Montagnes ainsi qu'Andy Fenton (School of Biological Sciences, Université de Liverpool, Angleterre). Cette étude a donné lieu à une publication dans *Aquatic Microbial Ecology* en 2008.

Montagnes D.J.S., Chambouvet A., Guillou L., Fenton A. (2008). Responsibility of microzooplankton and parasite pressure for the demise of toxic dinoflagellate blooms. *Aquatic Microbial Ecology* 53 :211-225.

Ces travaux ont aboutit à l'hypothèse que l'existence d'efflorescences de microalgues (di-

noflagellés) était due à l'absence de contrôle par les parasites. Cette hypothèse a été testée dans un autre écosystème, l'étang de Thau, où des efflorescences toxiques d'*Alexandrium catenella* se produisaient toujours de façon récurrente depuis son introduction en 1998, entraînant d'importants problèmes sanitaires. Ce travail, effectué en collaboration avec Mohamed Laabir (UMR 5119, CNRS, UM2 Ecosystèmes Lagunaires, Montpellier) et Mario Sengco (Smithsonian Environmental Research Center, Maryland, USA), fait l'objet d'une publication en préparation.

Chambouvet A., Laabir M., Sengco M., Guillou L. *Alexandrium catenella* (Dinophyceae) in the Thau lagoon (Mediterranean Sea, France) was the only dinoflagellate species to not be infected by the parasitoid *Amoebophrya* spp. during toxic blooms. In prep.

Enfin, en 2007, nous avons réussi à isoler une culture de parasite appartenant aux Alvéolés du groupe II infectant le dinoflagellé *Scrippsiella trochoidea* en collaboration avec Wayne Coats (Smithsonian Environmental Research Center, Maryland, USA). De façon empirique, nous avons constaté la présence de nombreux kystes de résistances dans le fond des tubes de culture. Nous avons alors cherché à comprendre si cet enkystement était induit par la présence du parasite, pouvant alors être considéré comme une réaction de défense de l'hôte. Enfin, nous avons recherché la présence du parasite pendant toute la durée de l'infection afin de comprendre les capacités de survie du parasite en l'absence de son hôte. Nous avons synthétisé ce travail dans une publication en préparation.

Chambouvet A., Cueff V., Marie D., Coats D.W., Guillou L. Parasitism and sex : when Nature overrules theory. In prep.

Chapitre I

Etats des lieux des deux modèles : Les parasites Amoebophryidae (Alvéolés, Groupe II) et leurs hôtes (les dinoflagellés)

CHAPITRE 1

ETATS DES LIEUX DES DEUX MODÈLES : LES PARASITES AMOEBOPHRYIDAE (ALVÉOLÉS, GROUPE II) ET LEURS HÔTES (LES DINOFLAGELLÉS)

Sommaire

Le groupe des Alvéolés est constitué de protistes de formes et de fonctions très diverses. Ils sont caractérisés par la présence de vésicules sous-membranaires, les alvéoles, de pores distincts sur la membrane externe ainsi que d'organites excréteurs (voir pour revue [Leander and Keeling, 2003]. Le groupe des Alvéolés est composé de trois groupes majoritaires : les ciliés, les dinoflagellés et les apicomplexes. Basé sur la phylogénie de différentes protéines (HSP 70, β-tubuline...), certains groupes de flagellés comme les colpodellides ainsi que les *Perkinsus* y ont récemment été rattachés [Fast *et al.*, 2002]. Enfin, deux nouvelles lignées appelées le groupe I et le groupe II, découvertes par séquençage systématique de la petite sous-unité du ribosome à partir de différents écosystèmes marins [Lopez-Garcia *et al.*, 2001, Moon-van der Staay *et al.*, 2001], ont pu être rattachées à la base des dinoflagellés, et ont été reconnues comme appartenant au groupe des Syndiniales [Guillou *et al.*, 2008].
La monophylie des Alvéolés est basée sur l'étude de différents gènes codant diverses protéines : HSP70, α-tubuline, β-tubuline ainsi que sur des gènes ribosomaux de la petite et de la grande sous unité du ribosome. Les Alveolata, les Straménopiles, les Haptophyta, ainsi que les Cryptophyta, forment le groupe des Chromalveolata (Figure 1.1).

Les Chromalveolata auraient un ancêtre commun photosynthétique, issue d'une endosymbiose secondaire avec une algue rouge [Delwiche, 1999]. De multiples pertes indépendantes de la photosynthèse se seraient donc produites au cours de l'évolution. Cette théorie est étayée par la découverte récente d'un plaste vestigial chez certains apicomplexes, un groupe pourtant exclusivement composé de parasites [Harper and Keeling, 2003] (Figure 1.2).

Les Alvéolés possèdent des modes trophiques très variés, en effet certains sont phagotrophes comme la plupart des ciliés, d'autres sont photosynthétiques comme de nombreux dinoflagellés. Les interactions entre organismes sont très variées, et le parasitisme représente un mode trophique rencontré chez tous les groupes composant les Alvéolés (Figure 1.3).

1.1 Les dinoflagellés

1.1.1 Histoire évolutive

Les dinoflagellés sont un ancien groupe de microalgues. Des fossiles ont été retrouvés datant de l'ère du Silurien supérieur, c'est-à-dire il y a 400 million d'années [Walker, 1984]. L'ancienne dichotomie entre la phycologie et la zoologie les a fait appartenir pendant très longtemps à deux groupes distincts, le groupe des algues par des botanistes et le groupe des protozoaires par des zoologistes [Taylor, 1987]. On parle aujourd'hui plus communément de dinoflagellés (ou dinoflagellata).

1.1.2 Morphologie

Les dinoflagellés constituent un embranchement comprenant environ 4 000 espèces réparties dans 550 genres [Taylor, 1990]. On les retrouve partout, que ce soit en milieux océaniques ou

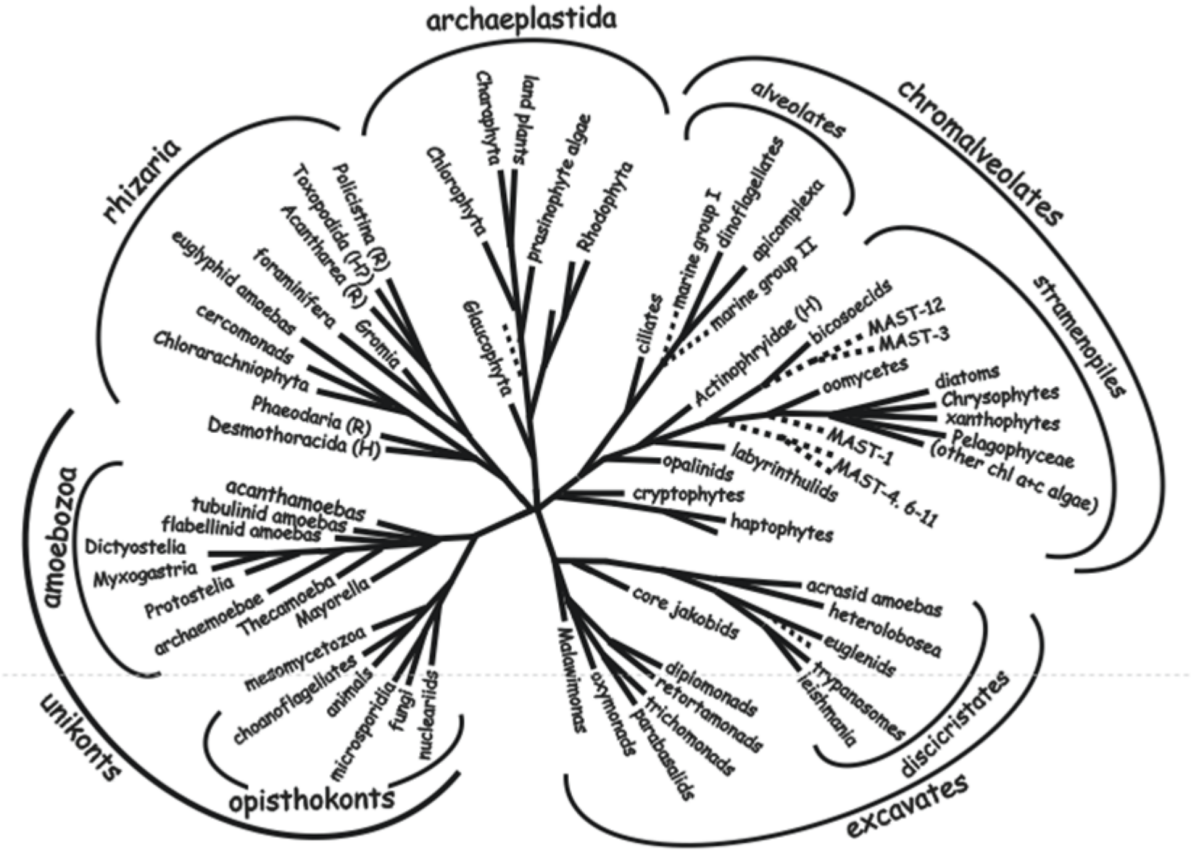


FIG. 1.1 – Schéma de l'arbre phylogénétique consensus des Eucaryotes basé sur l'analyse des gènes codant la HSP70, l'α-tubuline, la β-tubuline ainsi que sur des gènes ribosomaux codant la petite et la grande sous unité du ribosome, mis à jour par Sandra Baldauf. Les séquences environnementales découvertes par ciPCR sont illustrées par des pointillés [Baldauf, 2003].

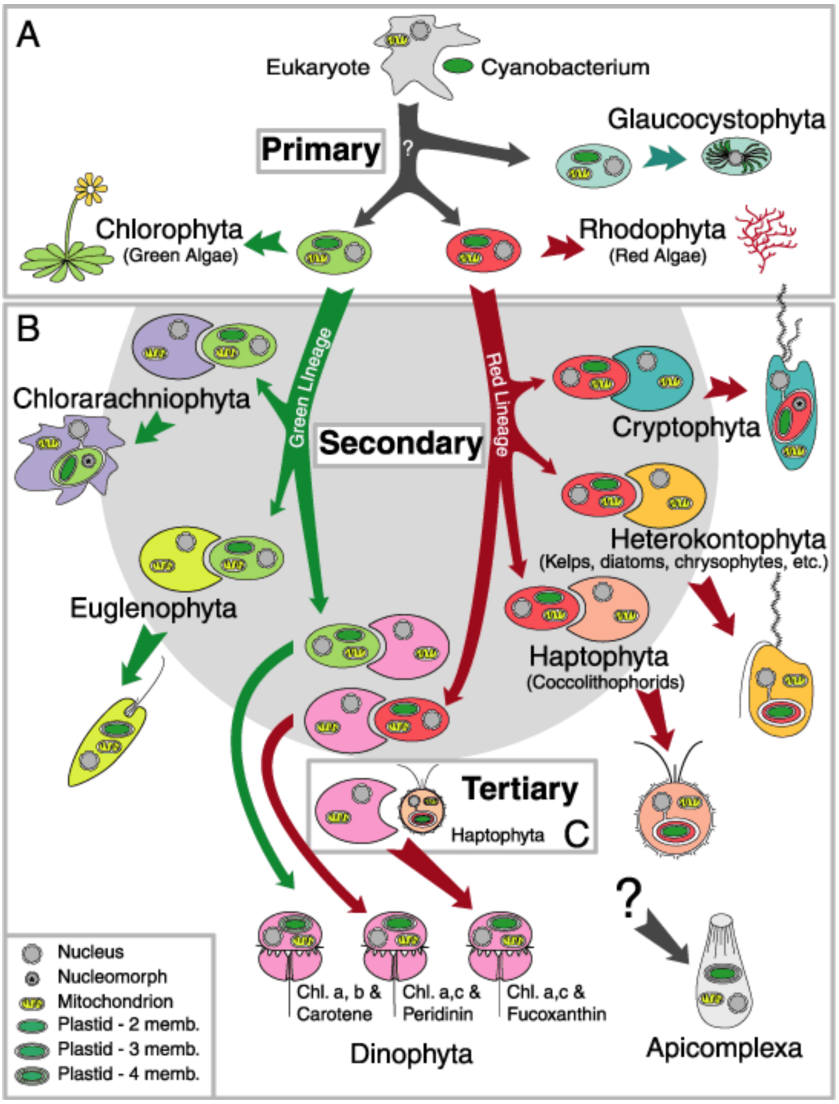


FIG. 1.2 – Schéma récapitulatif représentant une des hypothèses des évènements de l'endosymbiose expliquant la formation des plastes [Delwiche, 1999].

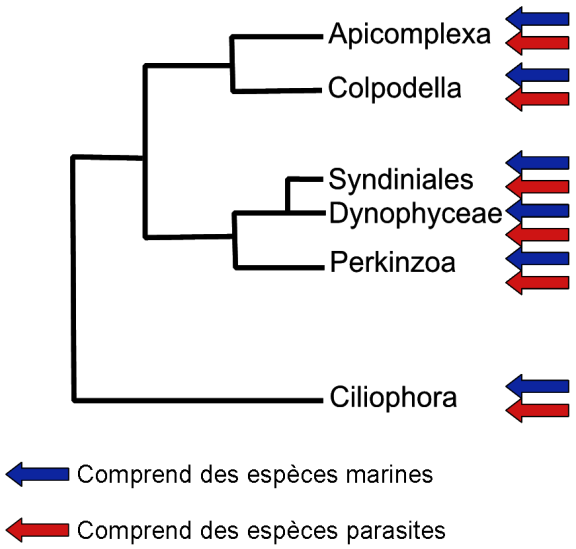


FIG. 1.3 – Schéma de l'arbre phylogénétique des Alvéolés (modifié d'après Leander) [Leander and Keeling, 2003].

côtiers, dans des eaux saumâtres ou des eaux douces [De Reviers, 2003]. Ce groupe est composé d'organismes unicellulaires protégés par une thèque constituée de plaques rigides recouvertes, pour certains, de cellulose. Ils sont caractérisés d'une part par la présence de deux flagelles, un équatorial et un vertical, et d'autre part, par un noyau appelé dinocaryon, dont les chromosomes restent condensés en permanence. Les dinoflagellés ont adopté un large choix de modes trophiques et d'habitats. Ils peuvent être photosynthétiques, hétérotrophes ou mixotrophes. D'autre part, leurs interactions avec d'autres organismes sont multiples : certains sont libres alors que d'autres vivent en symbiose ou sont parasites (Figure 1.4).

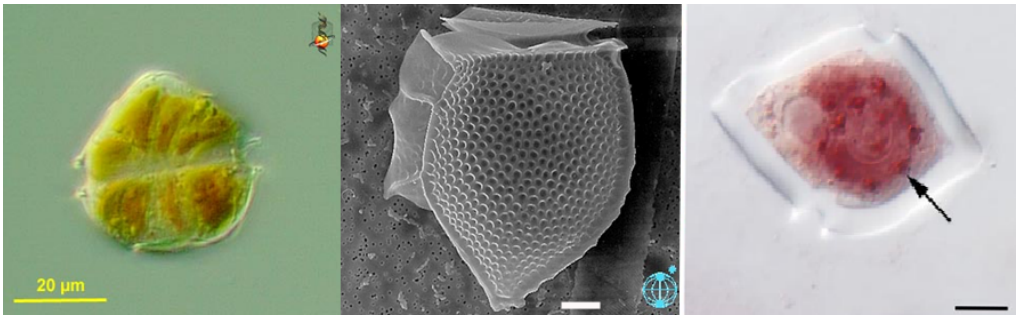


FIG. 1.4 – Trois types de dinoflagellés (de gauche à droite) : *Alexandrium tamarense* (plankton*net), *Dinophysis norvegica*, barre d'échelle 20 μm (plankton*net) et le parasite *Amoebophrya* sp. (montré par un flèche) infectant *H. triquetra*, barre d'échelle 10 μm [Park *et al.*, 2004]

1.1.3 Physiologie

1.1.3.1 Ecologie

Avec leurs physiologies très diverses, il est très difficile de généraliser le comportement des dinoflagellés. Cependant, certaines de leurs caractéristiques permettent de mieux comprendre leur écologie. Premièrement, ces organismes possèdent des flagelles leur permettant d'optimiser leur localisation et d'éviter de sédimenter lorsque l'environnent est très stable. Ces caractéristiques semblent être les éléments clés dans leur compétition avec les diatomées, leurs principaux rivaux dans le plancton (Figure 1.5).
En règle générale, les dinoflagellés sont plus adaptés à des eaux chaudes à faible turbulence. En effet, il a été montré que les turbulences diminuent la capacité de nage suite à la rétractation des flagelles permettant une sédimentation, voire une agrégation des cellules (c'est le cas par exemple du dinoflagellé *Ceratium tripos*) [Havskum *et al.*, 2005]. D'autre part, il est apparu que la croissance est aussi négativement corrélée à un fort hydrodynamisme [Sullivan and Swift, 2003].

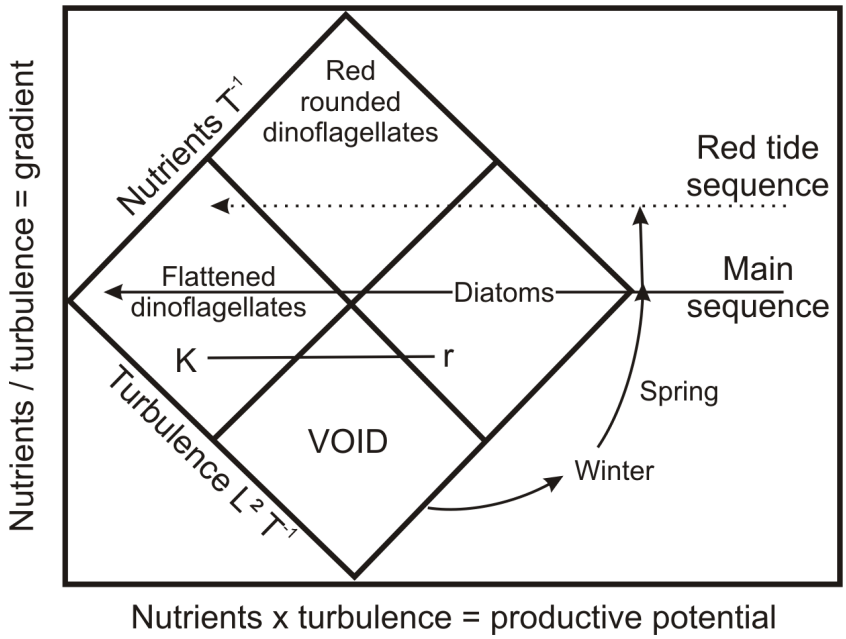


FIG. 1.5 – Illustration des sélections environnementales des différentes espèces phytoplanctoniques suivant les successions saisonnières en zone tempérée (d'après Margalef [Margalef, 1979], redessiné par Smayda [Smayda and Reynolds, 2001].

1.1.3.2 Efflorescences toxiques

Lorsque les conditions environnementales sont favorables, il peut y avoir production massive d'organismes phytoplanctoniques pouvant atteindre des densités de 10^5 jusqu'à 10^6 cellules.L^{-1}. Dans certains cas, une telle abondance de cellules pigmentées peut colorer intensément l'eau de mer, ces phénomènes sont communément appelés eaux colorées ou marées rouges [Sournia, 1995, Gilbert *et al.*, 2005]. Dans certains cas, cette prolifération peut conduire par la même occasion à la production de toxines (HAB, Harmful Algal Bloom) [Smayda, 1997]. En effet, sur 4 000 espèces phytoplanctoniques marines, environ 5 % sont capables de produire des toxines et sont impliquées dans des évènements du type HAB [Zingone and Enevoldsen, 2000]. La plupart de ces espèces appartiennent aux dinoflagellés [Smayda, 1997].
Certaines microalgues peuvent être toxiques même à de très faibles concentrations (cas de *Dinophysis* spp. sur nos côtes). Ces toxines peuvent agir directement (cas des ichtyotoxines tuant les poissons et les coquillages, produites par exemple par le dinoflagellé *Karenia mikimotoi*), soit être concentrées par les organismes filtreurs tels que les bivalves, et/ou se transmettre à travers la chaîne alimentaire. Par exemple, *Gambierdiscus* sp. est un dinoflagellé se développant à la surface des coraux, qui est consommé par des poissons brouteurs qui vont concentrer dans leur chair la toxine qu'il produit, la ciguatoxine. La consommation de ces poissons contenant ces toxines thermorésistantes peut se révéler mortelle pour l'Homme, car il n'y a pas d'antidote (Tableau 1.1). L'interdiction des ventes ainsi que la fermeture des zones contaminées restent les seules mesures préventives actuelles.

Bien que les HABs soient un phénomène naturel, il est apparu que depuis 20 ans de tels évènements augmentent en fréquence, en intensité et se diversifient au niveau des aires géographiques (Figure 1.6).

L'arrivée massive de la plupart des espèces, souvent invasives, pourrait être liée à l'association de plusieurs facteurs, tels que le réchauffement climatique, l'eutrophisation de nos côtes (produit par l'agriculture intensive ou le développement de zones touristiques), ou l'accélération des transports maritimes [Anderson, 1997]. Ainsi, depuis le premier évènement recensé sur les côtes françaises, un réseau de surveillance a été mis en place permettant le recensement des événements toxiques : Le REPHY (Réseau de surveillance du phytoplancton et des phycotoxines).

Les dinoflagellés forment un groupe très important participant à la production primaire. Ils préfèrent les environnements stables (avec peu de turbulence). Cependant, certains d'entre eux ont la capacité de produire des toxines pouvant être mortelles pour l'homme, par exemple sur nos côtes le genre *Alexandrium*.

Syndrome	Paralytic shellfish poisoning (PSP)	Diarrhetic shellfish poisoning (DSP)	Amnesic Shellfish poisoning (ASP)	Neurotoxic shellfish poisoning (NSP)	Ciguatera
Exemple d'organismes	*Alexandrium* sp. *Gymnodinium catenatum*	*Dinophysis acuminata*, *Prorocentrum lima*	*Pseudonitzschia* sp.	*Gymnodinium breve*	*Gambierdiscus toxicus*
Symptômes	Nausée, vomissement, diarrhée, douleur abdominale				
Symptômes extrêmes	Paralysie musculaire, arrêt de la respiration	Exposition chronique qui peut provoquer des tumeurs digestives	Hallucination, confusion, perte de mémoire à court-terme	Altération de la perception, difficulté respiratoire, trouble de la parole	Symptôme neurologique Problème respiratoire
	MORT	MORT	MORT	MORT	MORT
Transmission	Via la consommation d'organismes filtreurs				Via la consommation de poissons brouteurs
Exemple de Localisation	Europe				Tropique

TABLE 1.1 – Symptômes cliniques des différents types de toxines avec leurs organismes sécréteurs associés

1.1.4 Cycle de vie

1.1.4.1 La reproduction végétative

Les dinoflagellés sont des espèces dites « haplontiques » signifiant que le stade haploïde représente la plus grande partie de leur vie [Figueroa *et al.*, 2006] (Figure 1.7). Les dinoflagellés se reproduisent classiquement par voie végétative (c'est le cas lors d'efflorescences). Une cellule se divisera par fission binaire pour donner deux cellules filles identiques à la cellule mère. En cas de stress, les dinoflagellés peuvent former des kystes temporaires (ou kyste ecdysal) [Schmitter, 1979, Uchida, 1991]. Ces kystes à membrane fine peuvent survivre pendant un temps limité, par exemple dans le cas de *Scrippsiella trochoidea*, la période de dormance des kystes temporaires est de 6 à 13 jours [Wang *et al.*, 2007]. Lorsque les conditions redeviennent favorables, ces derniers pourront très rapidement re-germer et retourner vers une multiplication végétative active.

1.1.4.2 La reproduction sexuée

Les dinoflagellés ont aussi la capacité de faire de la reproduction sexuée. Ce phénomène a été observé chez une trentaine d'espèces de dinoflagellés [Von Stosch, 1973, Pfiester and Anderson, 1987]. Pfiester, après observation de la fusion de deux cellules mobiles de *Peridinium stygium*

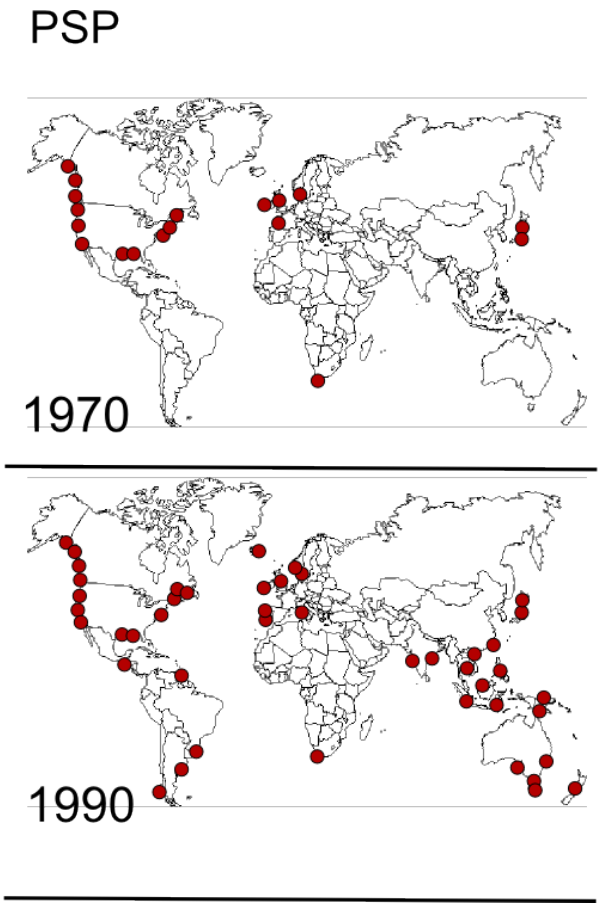


FIG. 1.6 – Distribution globale connue des évènements PSP en 1970 et 1990 [Hallegraeff, 1993]

Ehrenberg, a été un des premiers à décrire la présence d'une phase sexuée [Pfiester, 1977]. Le cycle de vie des dinoflagellés peut être d'une surprenante complexité. C'est le cas de *Pfiesteria piscicida* Steidinger et Burkholder, qui possède au moins 24 étapes dans son cycle de vie, comportant entre autre, une phase amibe, une forme enkystée ainsi qu'une phase libre [Litaker *et al.*, 2002]. La fusion de deux gamètes peut avoir trois issues différentes : un retour rapide après réduction chromatique vers une multiplication végétative, un enkystement à court terme ($<$ 96 heures) ou un enkystement à long terme ($>$ 2 mois).
Une réduction chromatique rapide peut se produire directement après formation du zygote. Ce comportement a également été observé chez *Scrippsiella trochoidea* Loeblich [Uchida, 1991]. Cette voie est avantageuse lorsque les conditions sont encore relativement favorables, car le processus d'enkystement est coûteux en temps et en énergie.

1.1.4.3 Les kystes permanents

La morphologie des kystes de résistance varie suivant l'espèce. En général, les kystes de résistance sont de formes ovale ou sphérique. Leur membrane est formée de plusieurs enveloppes,

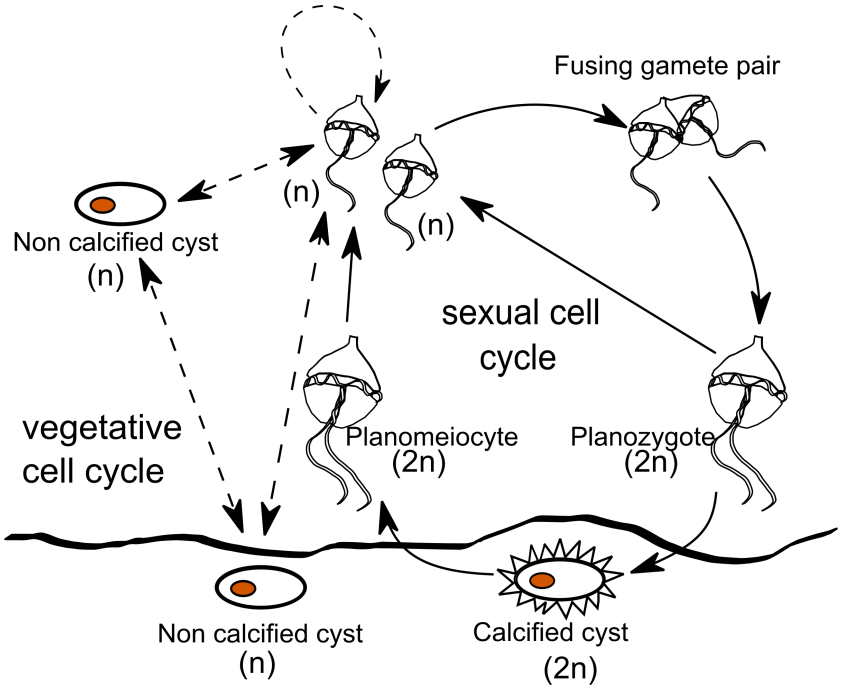


FIG. 1.7 – Schéma du cycle de vie chez *Scrippsiella trochoidea*. En trait plein la reproduction sexuée et en pointillé la reproduction végétative (d'après Figueroa [Figueroa *et al.*, 2006] modifié par Chambouvet 2008).

composées de polymères, chimiquement proches de la sporopollénine, une molécule également présente chez les spores et les grains de pollen des plantes supérieures. Cependant, certaines espèces possèdent une couche supplémentaire en carbonate de calcium fortement ornementée. Chez certaines espèces de *Scrippsiella* sp., par exemple, cette couche est constituée d'une ornementation typique composée d'épines caractéristiques, utilisées en taxonomie pour distinguer les espèces entre elles (Figure 1.8).

Ainsi l'existence de kystes de longue durée permet le passage de l'hiver et explique la présence uniquement saisonnière de certaines espèces [Figueroa *et al.*, 2006]. Les kystes permanents possèdent des périodes de dormance variables, par exemple entre 15 et 60 jours pour *Scrippsiella trochoidea* [Wang *et al.*, 2007, Kim and Han, 2000]. D'autre part, les kystes de résistance assurent le couplage entre la vie benthique et pélagique, et soutiennent le développement et la récurrence des efflorescences [Marcus and Boero, 1998]. L'accumulation dans le sédiment appelée « banque de kystes » [Matsuoka *et al.*, 2003, Garcés *et al.*, 2004] représente la source d'ensemencement importante pour l'initiation des efflorescences [Anderson *et al.*, 2005]. Bien que les raisons de la germination des kystes restent indéterminées, des hypothèses liées aux conditions environnementales ont été émises pour expliquer ce phénomène, c'est-à-dire lorsque les conditions optimales pour le dinoflagellé sont atteintes, que ce soit en lumière [Kremp, 2001],

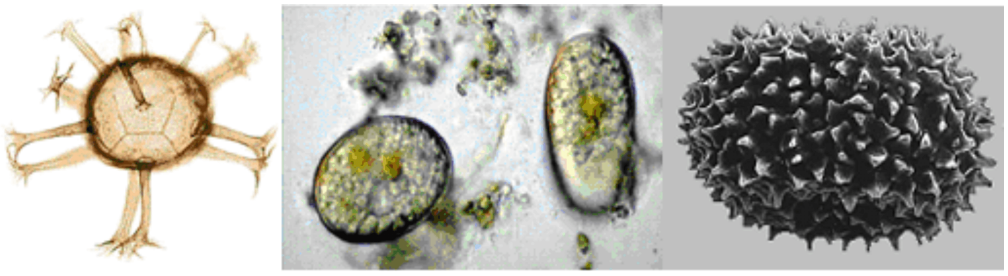


FIG. 1.8 – Différentes morphologies de kystes de résistance. De gauche à droite, *Oligosphaeridium abaculum* (http://www.fugrorobertson.com/technicalservices/biostratigraphy), *Alexandrium sp.* (photo D. Wall, http://www.whoi.edu/cms/images/CYST_cysts_250_47406.jpg), *Scrippsiella trochoidea* (http://www.smhi.se/oceanografi/oce_info_data/plankton_checklist/dinoflagellates/xscrippsiella_trochoidea.htm).

température [Rengefors and Anderson, 1998], sel nutritif ou en salinité [Kim *et al.*, 2002,Binder and Anderson, 1987].

Enfin, la reproduction sexuée permet de générer de la diversité génétique, par rapport à la voie végétative. Ce type de cycle cellulaire permettrait aux dinoflagellés une évolution plus rapide face à des changements biotiques (prédation, parasitisme...) ou abiotiques (changement en sel nutritif...).

1.2 Alvéolés parasites : Le cas des Syndiniales

1.2.1 Les Syndiniales

Chatton a été le premier à rattacher ces organismes aux dinoflagellés [Chatton, 1920]. En effet, malgré une grande variété morphologique, certains caractères sont communs aussi bien aux dinoflagellés libres que parasites, tels que la structure nucléaire (Dinocaryon) ainsi que la mitose (Dinomitosis). Il a également décrit de nouvelles espèces et les a suspectées d'être des organismes polyphylétiques. Il les regroupa donc à l'intérieur d'un seul groupe, les Blastodinida. En 1964, Cachon donna des détails plus précis aussi bien sur la cytologie de ces organismes que sur leur cycle de vie, et conclut, comme Chatton avant lui, à une origine polyphylétique. Il distingua deux groupes, les Blastodinia et les Duboscquodinida [Cachon, 1964]. Le premier groupe est composé d'ectoparasites et le second de parasites intracellulaires. En 1976, en utilisant des données biochimiques, Loeblich proposa que le parasite *Syndinium* (parasite de copépodes) soit séparé des Duboscquodinida formant un seul groupe, les Syndinidae. Il plaça donc ces trois groupes à un niveau taxonomique supérieur, à l'intérieur de l'ordre des Syndiniales. Il instaura donc en 1976 une nouvelle taxonomie de ce nouvel ordre [Loeblich III, 1976](Figure 1.9). Enfin, Loeblich les sépara du reste des dinoflagellés (qu'il appela « vrai dinoflagellés »), et plaça les Syndiniales à l'intérieur des Syndiniophyceae.

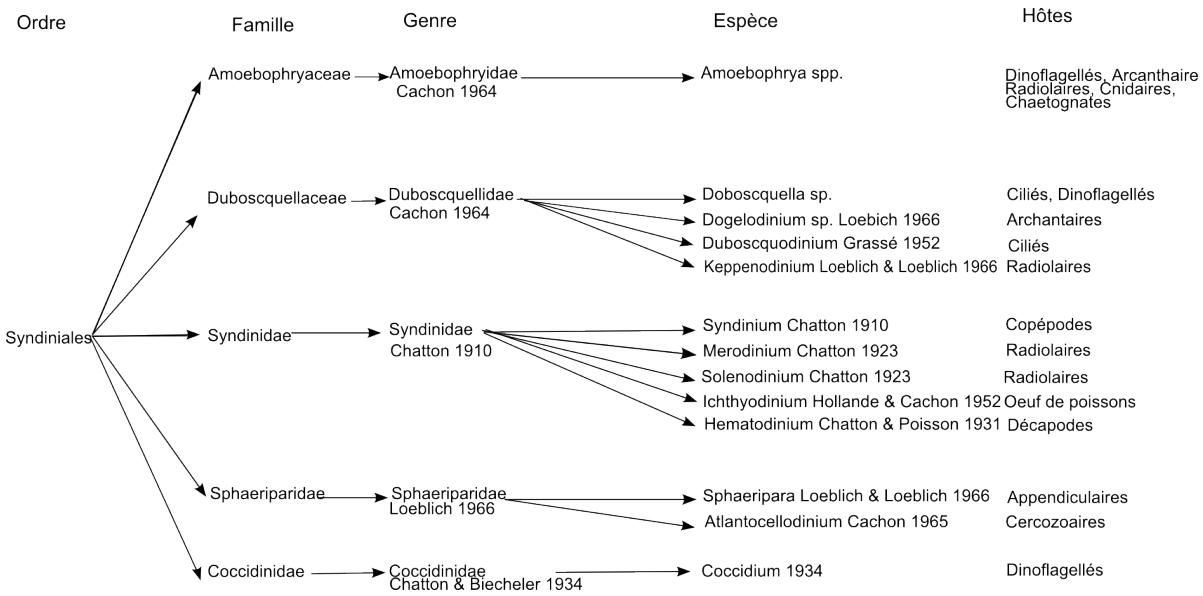


FIG. 1.9 – Taxonomie des Syndiniales et de leurs hôtes, modifiée d'après Loeblich [Loeblich III, 1976]

Les dinoflagellés et les Syndiniales possèdent donc des caractéristiques communes telles que la dinomitosis. En effet, lors de leur ségrégation, les chromosomes restent ancrés à l'enveloppe nucléaire durant la division mitotique. Cependant, pour les Dinophyceae, la ségrégation se déroule à travers la membrane nucléaire grâce aux microtubules, alors que pour les syndiniales, le centriole participe à la formation de l'appareil mitotique [Hollande, 1974, Ris and Kubai, 1974]. Les Syndiniales possèdent aussi d'autres particularités propres, telles que le nombre de chromosomes. En effet, il est compris entre 4 et 10 alors que pour les dinoflagellés, tels que *Gonyaulax* et *Ceratium*, il s'élève à plus de 100 [Loeblich III, 1976]. Ainsi, il semble raisonnable de penser que ces deux caractéristiques (division mitotique ainsi que le faible nombre de chromosomes) aient dérivés d'un modèle initial de dinoflagellé [Xiao-Ping and Jing-Yan, 1986]. A l'inverse, de récentes analyses phylogénétiques à partir de séquences codant pour la grande sous unité du ribosome (ARNr 28S), ont placé *Ichthyodinium chaberladi* (Syndinidae) [Gestal *et al.*, 2006] proche de *Perkinsus* [Massana *et al.*, 2008], à l'intérieur des dinoflagellés. Des séquences supplémentaires sont donc nécessaires pour obtenir une phylogénie plus robuste.

Définitions [De Reviers, 2002, De Reviers, 2003] :

Dinomitosis Mitose particulière chez les dinoflagellés. L'enveloppe nucléaire reste intacte (mitose fermée) et, à la prophase, des faisceaux de microtubules passent à travers le noyau via des tunnels constitués de cette enveloppe. Il existe des contacts entre la membrane externe de l'enveloppe nucléaire et le fuseau, en face des endroits où les chromosomes sont en contact avec la membrane interne de l'enveloppe. C'est ainsi que le faisceau peut malgré tout jouer son rôle d'aide à la séparation des chromosomes.

Taxon polyphylétique Se dit d'un groupe d'êtres vivants n'ayant pas d'ancêtre commun direct.

1.2.2 *Amoebophrya* spp.

1.2.2.1 Historique d'après Cachon [Cachon, 1964]

Les péridiniens, et plus particulièrement le genre *Amoebophrya*, sont connus depuis très longtemps. La nature parasitaire de ces organismes est restée longtemps inconnue. Cet organisme a été tour à tour considéré comme une partie intégrante des hôtes que ce soit du *Sticholonche* (Radiolaire) [Fol, 1883], des Acanthomeoba (noyau) [Hertwig, 1877] ou bien des Péridiniens (chromosomes spiralés) [Butschli and Askenasy, 1885]. En 1864, Koeppen nomme une première espèce, *Hyalosaccus ceratii*, considérée comme un Acinétiens (parasite de vers). Cependant en 1920, Chatton et Biecheler, en se fondant sur la morphologie des spores (dinospores) ainsi que sur les caractères de la mitose (dinomitosis), ont pu déterminer l'appartenance de ces organismes au groupe de dinoflagellés parasites, péridiniens, réunissant par la même occasion *Blastodinida* [Chatton, 1920]. Reprenant en 1964 les travaux de Chatton, Cachon et Cachon ont affirmé, grâce à l'analyse de la cytologie et du caryotype, que les *Amoebophrya* spp. sont bien d'authentiques péridiniens [Cachon, 1964]. Ils ont pu ainsi déterminer que la famille des Amoebophryidae se place à proximité de celle des Duboscquellidae et ne comporte qu'un seul genre *Amoebophrya*. Cachon a ensuite décrit 7 espèces différentes principalement différenciées par le type d'hôte infecté (Tableau 1.2).

Cachon a donc pu, grâce à une analyse cytologique, mettre en évidence le cycle de vie du parasite (Voir section 1.2.2.2). D'autre part, il reporta qu'*Amoebophrya ceratii* était présent sporadiquement dans un nombre important d'espèces hôtes méditerranéennes , mais que le plus haut niveau d'infection avait été trouvé en fin d'efflorescence de dinoflagellés. En 1968, Taylor prouva que ce parasite était également capable d'infecter des dinoflagellés toxiques [Taylor, 1968]. En 1985, Nishitani et collaborateurs relièrent la présence du parasite *A. ceratii* au rapide déclin des populations hôtes, montrant ainsi l'importance de ce facteur lors de la prévention de

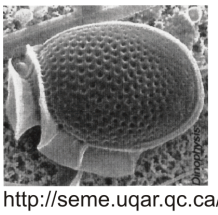
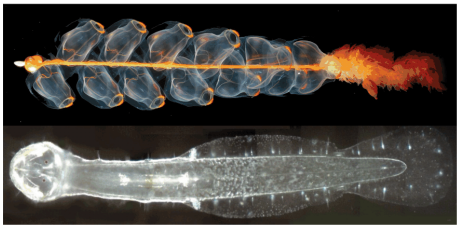
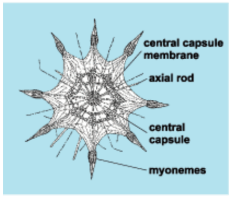
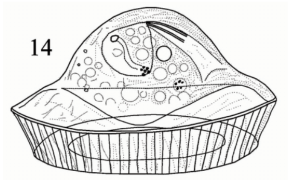
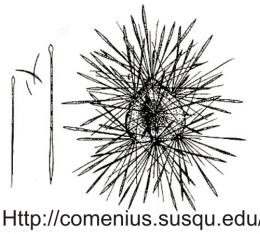
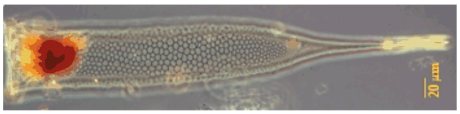
Espèce	Hôtes	Photographie de l'hôte
A. ceratii Koeppen 1903	Dinoflagellés	 http://seme.uqar.qc.ca/02_etude_cas/images/dinomeb.jpg
A. grassei Cachon 1964	*Oodinium* sp.	L'hôte est lui même un ectoparasite d'appendiculaire
A. rosei Cachon 1964	Siphonophore et Chaetognathe	 1. K. Raskoff / Monterey Peninsula College (http://biocurious.com/images/natureSiphonophore.jpg) 2. Jean Marie Cavanihac (http://forum.mikroscopia.com/uploads/post-33-1086104075.jpg&imgrefurl)
A. acanthometrae Borgert 1897	*Acanthometra pellucida* (acanthaire, radiolaire)	 Http://content.answers.com/main/content/img/McGrawHill/Encyclopedia/images/CE001800FG0010.gif
A. leptodisci Cachon 1964	*Leptodiscus medusoides* (Dynophyceae)	 http://www.scielo.cl/fbpe/img/revbiolmar/v42n1/img10-14.jpg
A. sticholonchae Koeppen 1894	Sticholonche (radiolaire)	 Http://comenius.susqu.edu/bi/202/CERCOZOAE/Radiolaria/Sticholoncheprotozoa-guide.jpg
A. tintinni Cachon 1964	Chez un seul tintinide, *Xystonella lohmanni*	 John R. Dolan (Plancton*net)

TABLE 1.2 – Tableau représentant tous les *Amoebophrya* décrit avec leurs hôtes respectifs ainsi que les photos de ces derniers.

formations d'efflorescences [Nishitani *et al.*, 1985].

La morphologie de *A. ceratii* infectant différentes espèces de dinoflagellés est relativement homogène. Cette faible variabilité phénotypique a longtemps été reliée à la faible spécificité d'infection, avec la capacité d'infecter un large choix d'hôtes [Cachon, 1964, Nishitani *et al.*, 1985]. Des cultures ont pu depuis être isolées, apportant des réponses bien souvent contradictoires. Coats et ses collaborateurs ont montré qu'une souche d'*A. ceratii* infectant initialement *Akashiwo sanguinea*, n'était pas capable d'infecter d'autre dinoflagellés comme *Ceratium furca, Gyrodinium uncatenum* ou encore *Scrippsiella trochoidea* [Coats *et al.*, 1996]. L'analyse de la physiologie de souches provenant d'hôtes différents a permis de montrer des variabilités aussi bien au niveau du temps de génération que du temps de survie des dinospores [Coats and Park, 2002]. D'autre part, certaines souches d'*Amoebophrya* sont capables d'infecter des dinoflagellés toxiques. La tolérance du parasite aux toxines de l'hôte n'est possible que grâce à une composition lipidique membranaire adaptée [Bai *et al.*, 2007]. Enfin, des études moléculaires basées sur l'analyse du 18S ont révélé une grande diversité génétique permettant de décrire *A. ceratii* plutôt comme un « complexe d'espèces » [Coats *et al.*, 1996] composé de plusieurs parasites hôte-spécifiques [Gunderson *et al.*, 2001]. La spécificité d'infection chez cette espèce reste donc encore problématique, d'autant plus que la spécificité d'infection mesurée au laboratoire est souvent plus permissive que les observations faites sur le terrain, dans un environnement plus complexe et plus restrictif [Poulin and Keeney, 2007].

1.2.2.2 Cycle de vie, exemple d'*Amoebophrya ceratii*

Amoebophrya ceratii est comme pratiquement tous les Syndiniales, un parasitoïde, c'est-à-dire qu'il tue obligatoirement son hôte pour accomplir son cycle de vie. Il est capable d'infecter de nombreuses espèces (si ce n'est toutes les espèces) de dinoflagellés, ainsi que d'autres *Amoebophrya* [Cachon, 1964]. L'infection est tout d'abord initiée par l'entrée dans l'hôte d'une (rarement plusieurs) cellule biflagellé infectante, appelée dinospore (Figure 1.10a). Après plusieurs cycles actifs de réplication nucléaire, ces dinospores produisent une structure multi–nucléée, appelée trophonte (stade endocellulaire). Ce trophonte, en forme de nid d'abeille (« beehive stage » en anglais), est caractéristique du genre *Amoebophrya* (Figure 1.10d). La pression du trophonte à la dernière étape de maturation est telle que la cellule hôte se déforme. A maturité, le trophonte rompt la thèque du dinoflagellé laissant derrière lui une thèque vide (Figure 1.10e). Ce dernier se transforme en vermiforme mobile (Figure 1.10f et g). Cette structure temporaire, composée de multiples cellules, va s'individualiser en quelques heures pour redonner de nouveaux dinospores (Figure 1.10h et i).

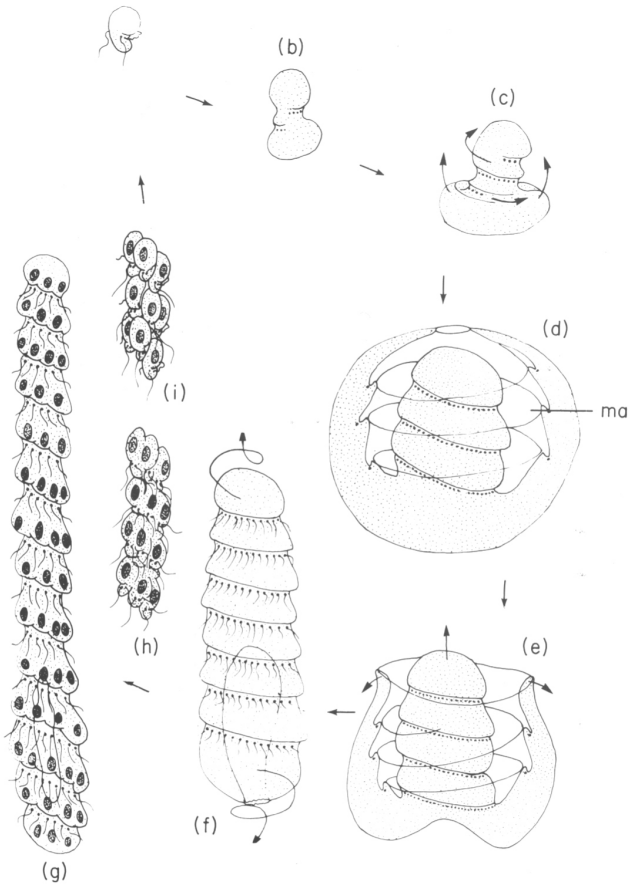


FIG. 1.10 – Diagramme du cycle de vie d'*Amoebophrya* : (a) stade libre du parasite, le dinospore ; (b,c,d) Développement du stade trophonte intracellulaire dans l'hôte (Ma=magistocoel) ; (e,f) evagination du trophonte et formation du parasite en forme de ver, le « vermiforme » ; (g) structure allongée et motile du vermiforme ; (h,i) individualisation successive du vermiforme jusqu'à donner des dinospores [Cachon, 1964].

1.2.2.3 Impact du parasite sur les populations hôtes (d'après Park) [Park *et al.*, 2004]

En 1964, Cachon remarqua qu'un grand nombre d'espèces de dinoflagellés était infecté par *A. ceratii*. D'autre part, il est apparu que le maximum de cellules infectées coïncidait avec le déclin de l'efflorescence [Cachon, 1964]. Ces observations ont été confirmées, quelques années plus tard, par différents auteurs. Sur les côtes nord-Américaines, il est apparu que lors de périodes d'efflorescences, 30 à 40 % de *Alexandrium catenella* et 80 % de *Ceratium fucus* étaient infectés [Taylor, 1968, Nishitani *et al.*, 1985]. La présence du parasite *A. ceratii* fut donc liée au rapide déclin de la population hôte, et suspectée d'être un facteur important de régulations des efflorescences. Cependant en 1992, Fritz et Nass (1992) trouvèrent une prévalence très faible (2 %) sur des populations de *Dinophysis norvegica*, et argumentèrent que ce parasite avait peu

d'effet sur la dynamique de cet hôte [Fritz and Nass, 1992]. Cependant, pour estimer l'impact du parasite sur les populations hôtes, certaines valeurs, telles que le temps de génération, ainsi qu'une estimation de la prévalence, sont nécessaires. D'autre part, la méthode classiquement utilisée (coloration nucléaire) ne permet d'observer que les stades d'infection mature. En effet, les premiers stades d'infection sont très difficiles à détecter, conduisant bien souvent à sous-estimer les prévalences.

Grâce à la première mise en culture d'un *Amoebophrya* infectant *Akashiwo sanguinea* par Coats et Bockstahler [Coats and Bockstahler, 1994], le temps de génération a été estimé entre 2 et 4 jours. Cette valeur a été confirmée depuis par la mise en culture d'autres souches d'*Amoebophrya* spp. infectant différents dinoflagellés [Coats and Park, 2002, Park *et al.*, 2002]. En utilisant les prévalences observées en milieu naturel, associées au temps de génération du parasite, Coats et Bockstahler ont calculé que le parasite induisait la mortalité de moins de 2 % de la population hôte, ce qui est loin de permettre le déclin de celle-ci [Coats and Bockstahler, 1994]. Des valeurs similaires (0,5 à 2 %) ont été observées en mer Baltique pour *Dinophysis norvegica* [Gisselson *et al.*, 2002]. A l'opposé, Coats et ses collaborateurs, en 1996, examinèrent l'impact du parasite sur un bloom de *Gymnodinium uncatenum* dans la baie de Chesapeake (USA), où la prévalence était supérieure à 80 %, et corrélée au déclin de l'efflorescence [Coats *et al.*, 1996]. Ils observèrent que *Amoebophrya* spp. était capable d'éliminer 54 % de l'espèce dominante par jour. Ainsi *Amoebophrya* spp. est reconnue pour avoir un impact significatif sur les populations de dinoflagellés, plus particulièrement sur les populations ayant la capacité de former des efflorescences.

Chapitre II

Résultats : de la génétique du parasite à la sexualité de l'hôte

CHAPITRE 2

RÉSULTATS : DE LA GÉNÉTIQUE DU PARASITE À LA SEXUALITÉ DE L'HÔTE

Sommaire

2.1 Diversité génétique des Alvéolés du Groupe II et biogéographie

Contexte et objectifs

Jusqu'à une époque récente, la description de la diversité génétique des plus petites classes de taille du plancton marin était largement sous-estimée, car limitée aux espèces cultivables. L'utilisation de la biologie moléculaire a offert, à partir de 2001, une alternative à l'isolement et permis de mieux comprendre les communautés *in situ*. Ces études consistent à extraire l'ADN génomique d'un échantillon environnemental, puis d'étudier la diversité génétique d'un gène cible, en général celui codant la petite sous-unité de l'ARN ribosomal (ARNr 18S). Ainsi, le séquençage à haut débit d'une grande variété d'environnements a permis l'accumulation dans les bases de données d'un nombre important de séquences.

De nouvelles séquences ont ainsi pu être découvertes, appartenant majoritairement à de nouveaux groupes de Straménopiles ou d'Alvéolés. Les nouveaux Alvéolés marins (MALV) décris grâce à ces techniques se répartissent principalement en deux groupes, Groupes I et II [Lopez-Garcia *et al.*, 2001, Moon-van der Staay *et al.*, 2001]. Des analyses phylogénétiques ont permis d'affilier ces séquences environnementales à certains parasites appartenant à l'ordre des Syndiniales (dinoflagellés), tels que *Amoebophrya* spp., *Hematodinium* sp. ainsi que *Syndinium* sp. rattachés dans un premier temps aux Alvéolés du groupe II [Groisillier *et al.*, 2006]. Parallèlement, les séquences appartenant aux Alvéolés du groupe I se sont révélées proches d'*Ichtyodinium* sp., parasite d'œuf de poisson [Gestal *et al.*, 2006], de *Duboscquella* sp., parasite de ciliés [Harada *et al.*, 2007], et de parasites de radiolaires [Dolven *et al.*, 2006].

Le séquençage systématique de différents environnements a permis de retrouver les séquences appartenant à ces groupes I et II dans tous les écosystèmes marins étudiés : de la surface des océans jusqu'à 3 000 mètres de profondeur ainsi qu'au niveau des sources hydrothermales [Díez *et al.*, 2001, Moon-van der Staay *et al.*, 2001, Lopez-Garcia *et al.*, 2001, Edgcomb *et al.*, 2002,Lopez-Garcia *et al.*, 2003]. Ces groupes pourraient être d'une importance écologique considérable au vu de leur abondance dans les banques de clones. En effet, elles représentent en moyenne près de 50 % des séquences environnementales récupérées au sein des librairies génétiques 18S [Lopez-Garcia *et al.*, 2001, Moon-van der Staay *et al.*, 2001]. Ainsi, chaque année, le nombre de séquences environnementales affiliées à ces lignées s'accumule dans les banques de gène publiques. Il paraissait important de comparer et d'essayer de mettre à jour la taxonomie actuelle des Syndiniales avec la diversité génétique environnementale de ces groupes. Enfin, l'origine des séquences et leur comparaison pouraient permettre de formuler des hypothèses intéressantes quant à leur distribution géographique.

Dans le cadre de cette thèse, une analyse biogéographique couplée à une étude de la diversité génétique de toutes les séquences dans GenBank a donc été réalisée (séquences de longueur supérieure à 1 600 bp pour l'analyse phylogénétique et en intégrant des séquences plus courtes pour les analyses de biogéographie). L'analyse phylogénétique des séquences environnementales issues de GenBank (soit 43 312 en Avril 2008) a permis de caractériser la diversité génétique des Syndiniales. D'autre part, cette étude a permis, entre autre, de préciser la phylogénie inter- et intra-groupe des Syndiniales.
Ce travail a été réalisé dans le cadre d'une collaboration. Dans cette optique, mon rôle a été d'obtenir des séquences issues de l'estuaire de la Penzé (France) utilisées (238 séquences) dans la publication.

Résumé

Les Syndiniales forment un ordre composé uniquement de parasites de protistes eucaryotes. Dans cette étude, nous avons recensé la taxonomie et la diversité génétique de ce groupe à partir de 43 312 séquences disponibles dans GenBank provenant soit de cultures soit d'études environnementales (6 874 séquences provenant de la mer Méditerranée ou d'Atlantique). Bien que supportée par de faibles valeurs de bootstrap, les analyses phylogénétiques montrent pour la première fois, par la méthode bayesienne ainsi que par la méthode du maximum de vraisemblance, la monophylie des Syndiniales à la base des dinoflagellés. D'après ces analyses, l'ordre des Syndiniales est composé de cinq principaux groupes (I à V). Les deux premiers groupes, Groupe I et II, présentent une grande diversité génétique (8 et 44 clades respectivement), et sont les plus représentés dans les études environnementales. Néanmoins, aucun phénomène de coévolution entre les Syndiniales et leurs hôtes potentiels n'a pu être mis en évidence. Ce résultat souligne des capacités de changement d'hôtes au cours de l'évolution.
A travers un profil de distribution global, nous avons confirmé que ces parasites sont exclusivement marins. Néanmoins, chaque groupe semble avoir un environnement préférentiel, l'analyse phytogéographique ayant montré que les groupes II et III sont fortement présents dans la zone photique, alors que le groupe I domine plus particulièrement les écosystèmes anoxiques et suboxiques. Du fait de la présence ubiquiste de ces Syndiniales, et considérant que toutes les espèces actuellement décrites sont des parasitoïdes, notre travail a donc souligné la présence probablement constante de parasites eucaryotes dans tous les écosystèmes marins étudiés. La grande diversité génétique de cet ordre peut également faire penser que le spectre d'hôtes est bien plus important que celui décrit dans la littérature. Ainsi, le rôle écologique des Syndiniales pourrait alors avoir été largement sous-estimé et négligé dans la conceptualisation du cycle du carbone marin.

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Widespread occurrence and genetic diversity of marine parasitoids belonging to *Syndiniales* (*Alveolata*)

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Summary

Syndiniales **are a parasitic order within the eukaryotic lineage** ***Dinophyceae*** **(*Alveolata*). Here, we analysed the taxonomy of this group using 43655 18S rRNA gene sequences obtained either from environmental data sets or cultures, including 6874 environmental sequences from this study derived from Atlantic and Mediterranean waters. A total of 5571 out of the 43655 sequences analysed fell within the** ***Dinophyceae*****. Both bayesian and maximum likelihood phylogenies placed** ***Syndiniales*** **in five main groups (I–V), as a monophyletic lineage at the base of 'core' dinoflagellates (all** ***Dinophyceae*** **except** ***Syndiniales*****), although the latter placement was not bootstrap supported. Thus, the two uncultured novel marine alveolate groups I and II, which have been highlighted previously, are confirmed to belong to the** ***Syndiniales*****. These groups were the most diverse and highly represented in environmental studies. Within each, 8 and 44 clades were identified respectively. Co-evolutionary trends between parasitic** ***Syndiniales*** **and their putative hosts were not clear, suggesting they may be relatively 'general' parasitoids. Based on the overall distribution patterns of the** ***Syndiniales*****-affiliated sequences, we propose that** ***Syndiniales*** **are exclusively marine. Interestingly, sequences belonging to groups II, III and V were largely retrieved from the photic zone, while Group I dominated samples from anoxic and suboxic ecosystems. Nevertheless, both groups I and II contained specific clades preferentially, or exclusively, retrieved from these latter ecosystems. Given the broad distribution of** ***Syndiniales*****, our work indicates that parasitism may be a major force in ocean food webs, a force that is neglected in current conceptualizations of the marine carbon cycle.**

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Introduction

The *Alveolata*, one of the major eukaryotic lineages, is composed of four protist classes: the *Ciliophora*, the *Apicomplexa*, the *Perkinsea* and the *Dinoflagellata*. *Alveolata* have adopted a large range of trophic modes and habitats. They can be important marine primary producers. For instance, about half of dinoflagellates are photosynthetic (Lessard and Swift, 1986), with some being responsible for toxic algal blooms. Ciliates and dinoflagellates can also be active predators; others are coral symbionts (e.g. the dinoflagellate *Symbiodinium* spp.) and some ciliates even reside in mammalian guts (Williams and Coleman, 1992). Finally, a large number of species are parasites, broadly distributed throughout these *Alveolata* classes. For instance, *Apicomplexa* is composed solely of obligate parasites. In addition to phylogenetic relatedness based on gene sequence comparisons, *Alveolata* are unified by specific morphological characters. These include the presence of membrane-bound flattened vesicles, termed alveoli (Cavalier-Smith, 1993; Patterson, 1999), distinct pores called micropores that pierce the outer membrane and the presence of a more or less developed apical complex apparatus used by alveolate parasites to enter their host (reviewed by Leander and Keeling, 2003).

The discovery of novel marine alveolate (MALV) lineages in marine planktonic communities by culture-independent techniques, specifically MALV groups I and II (Díez *et al*., 2001a; López-García *et al*., 2001; Moon-van der Staay *et al*., 2001), has raised questions regarding functional roles of these diverse populations. Phylogenetic analyses showed MALV Group II belongs to the *Syndiniales*, a dinoflagellate order exclusively composed of marine parasites. This assignment was based on the close phylogenetic relatedness of the environmental 18S

rRNA gene sequences (Moon-van der Staay *et al*., 2001; Skovgaard *et al*., 2005) with three previously described genera: *Amoebophrya* spp., *Hematodinium* spp. and *Syndinium* spp. More recently, 18S rRNA gene sequences derived from *Ichthyodinium chaberladi* (Gestal *et al*., 2006) and *Duboscquella* sp. (Harada *et al*., 2007), two parasitic *Syndiniales* genera, showed an affiliation with MALV Group I.

MALV groups I and II have been retrieved from various marine habitats, mainly from the picoplankton fraction (< 2 or < 3 μm size fractionated samples). These groups frequently form the majority of sequences within marine environmental clone libraries (see López-García *et al*., 2001; Moon-van der Staay *et al*., 2001; Massana *et al*., 2004; Romari and Vaulot, 2004; Not *et al*., 2007). This has led to a large increase in the number of MALV sequences deposited in GenBank over the last few years. In a previous study, Groisillier and colleagues (2006) detected five distinct clades within MALV Group I, and 16 clades within MALV Group II. However, in that study, many sequences could not be clearly assigned to a specific clade, suggesting further discrete lineages might exist. Interestingly, some clades comprised sequences retrieved only from very specific habitats, such as anoxic environments or deep sea hydrothermal vents, while other clades contained sequences obtained from widely varying habitats.

In the present work our aims were (i) to broaden representation of the different environments in which members of the *Alveolata* are potentially encountered by constructing and sequencing 18S rRNA gene libraries; (ii) to clarify the phylogeny of the *Alveolata* using recently published and the new 18S rRNA gene sequences for this group; (iii) to undertake a rigorous review of clade organization within the above mentioned MALV groups I and II; (iv) to compare the genetic diversity of environmental sequences derived from culture-independent PCR surveys (targeting the 18S rRNA gene) to our present knowledge of the taxonomy of *Syndiniales*; and (v) to extract general information on the preferred habitats and distribution of specific members of the *Syndiniales*.

Results

Sample sites and environmental conditions

Most of the novel environmental sequences obtained in this study were retrieved from coastal and oceanic waters in the Atlantic Ocean and Mediterranean Sea (Table 1). The collection sites varied in terms of season, depth and level of oligotrophy. For example, the Bermuda Atlantic Time-series Station (BATS) is relatively oligotrophic, and at the time of sampling was already strongly stratified. In contrast, the northern Sargasso Sea Station, although relatively close to BATS, was likely still influenced by deep winter mixing that occurs in this area, bringing nutrients to surface waters (see Cuvelier *et al*., 2008 for further discussion). The Florida Straits sites represent three distinct water types. Station 1, on the western side of the Florida Straits, was relatively coastal. Station 4 represents the core of the Gulf Stream Current-forming waters, which are highly oligotrophic, while Station 14 is also oligotrophic but a shallow setting on the eastern side of the Florida Straits (see Cuvelier *et al*., 2008). Other environmental sequences from the Atlantic Ocean were obtained during the Atlantic Meridional Transect (AMT 15, see also Zwirglmaier *et al*., 2007), which extended from 48°N [south-west (SW) of the UK] to 40°S (SW of Cape Town, South Africa). Environmental sequences from the Mediterranean Sea were collected in late summer along a Mediterranean transect sampled during the PROSOPE cruise in 1999 (Garczarek *et al*., 2007), from high nutrient levels in the Morocco upwelling to strong phosphorus limitation in the eastern most basin. Although half of the genetic libraries were built using a PCR approach biased towards green algae, dinoflagellate sequences were retrieved in both data sets (the majority of them were retrieved using general eukaryotic primers). We also used a PCR approach biased towards MALV Group II (using the specific primer ALV01) that we compared with the use of general eukaryotic primers from a coastal site (English Channel, France). Most of genetic libraries were built on very small size fractions (less than 2–3 μm), although some were processed on larger size fractions and even after incubations (see genetic libraries from coastal sites from the Mediterranean Sea). This heterogeneous data set offered us a very large range of environmental sequence origins. In total, 6874 new environmental sequences were generated and screened for dinoflagellate sequences.

Alveolate 18S rRNA gene sequence data set

The completed data set comprised 43655 eukaryotic 18S rRNA gene sequences obtained either from GenBank or from the environmental clone libraries described above. From marine environments, 351 environmental clone libraries were analysed with major contribution from sites (Table 2) in the Atlantic (Díez *et al*., 2001a; Countway *et al*., 2007; Not *et al*., 2007), Indian (Not *et al*., 2008), Arctic (Lovejoy *et al*., 2006; Stoeck *et al*., 2007) and Pacific (Moon-van der Staay *et al*., 2001) Oceans, Mediterranean Sea (Viprey *et al*., 2008), Antarctic (López-García *et al*., 2001), as well as several coastal sites (Massana *et al*., 2004; Romari and Vaulot, 2004; Medlin *et al*., 2006; Worden, 2006). These encompassed a range of marine habitats including the photic zone, sediments, hydrothermal vents and anoxic ecosystems (Table 2). Terrestrial and continental ecosystems were also included

Table 1. Description of the sampling sites and characteristics of clone libraries constructed for this study.

Environment and cruise	Station and Site	Coordinates	Date	Code library	Depth (m)	Primer	Sample details (μm)	Total number of clones with insert screened
Mediterranean Sea	St 1	38°08′N	14-Sep-99	C1-30	30	Euk328f-CHLO02	< 3	246
PROSOPE	Strait of Gibraltar	05°18′W		E1-30	30	Euk328f-Euk329r	< 3	135
Oceanic euphotic zone				C1-80	80	Euk328f-CHLO02	< 3	195
Accession numbers from EU792921				E1-80	80	Euk328f-Euk329r	< 3	69
to EU793941 + EU848494 to	St 3	37°98′N	16-Sep-99	C3-5	5	Euk328f-CHLO02	< 3	165
EU848495	Algerian Basin	03°83′E		E3-5	5	Euk328f-Euk329r	< 3	46
See also Viprey *et al.* (2008)				C3-25	25	Euk328f-CHLO02	< 3	23
				E3-25	25	Euk328f-Euk329r	< 3	56
				C3-95	95	Euk328f-CHLO02	< 3	173
				E3-95	95	Euk328f-Euk329r	< 3	27
	St 5	36°47′N	18-Sep-99	C5-25	25	Euk328f-CHLO02	< 3	151
	Strait of Sicily	13°32′E		E5-25	25	Euk328f-Euk329	< 3	168
				C5-55	55	Euk328f-CHLO02	< 3	185
				E5-55	55	Euk328f-Euk329r	< 3	131
	St MIO	33°98′N	20-Sep-99	CM-5	5	Euk328f-CHLO02	< 3	173
	Ionian Basin	22°02′E		EM-5	5	Euk328f-Euk329r	< 3	62
				CM-50	50	Euk328f-CHLO02	< 3	171
				EM-50	50	Euk328f-Euk329r	< 3	102
				CM-110	110	Euk328f-CHLO02	< 3	134
				EM-110	110	Euk328f-Euk329r	< 3	115
	St 9	41°88′N	28-Sep-99	C9-5	5	Euk328f-CHLO02	< 3	101
	Tyrrhenian Basin	10°43′E		E9-5	5	Euk328f-Euk329r	< 3	144
				C9-65	65	Euk328f-CHLO02	< 3	223
				E9-65	65	Euk328f-Euk329r	< 3	119
	St DYF	43°38′N	30-Sep-99	CD-15	15	Euk328f-CHLO02	< 3	172
	Ligurian Basin	07°82′E		ED-15	15	Euk328f-Euk329r	< 3	118
				CD-50	50	Euk328f-CHLO02	< 3	201
				ED-50	50	Euk328f-Euk329r	< 3	196
English Channel, France	Astan	48°45′N	21-Aug-01	RA1	Surface	Euk528f-Euk329r	> 12	183
Coastal euphotic zone		04°00′W		RA2	Surface	ALV01- Euk329r	0.2–3	96
Accession numbers from EU785054				RA3	Surface	ALV01- Euk329r	> 12	96
to EU785255								
Catalan, Mediterranean Sea, Spain	Barcelona Harbour	41°23′N	28-Feb-01	BAFRACTpico	Surface	EukA-EukB	0.6–1	38
Coastal euphotic zone		2°09′E		BAFRACTnano	Surface	EukA-EukB	1–10	29
Accession numbers from EU785256				BAFRACTmicro	Surface	EukA-EukB	10–50	27
to EU785301								
Catalan, Mediterranean Sea, Spain	Blanes Bay	41°40′N	06-Nov-01	BLENRinf2	Surface	EK-1A-516-GC	< 2	15
Coastal euphotic zone		02°48′E		BLENRD2T4	Surface	EK-1A-516-GC	Incubation after filtration < 2	15
Accession numbers from EU785302								
to EU785340				BLENRL2T4	Surface	EK-1A-516-GC	Incubation after filtration < 2	21
See also Massana *et al.* (2006)								
Atlantic Ocean	St UPW Morocco upwelling	31°02′N	09-Sep-99	CU-30	30	Euk328f-CHLO02	< 3	53
PROSOPE		10°03′W						
Coastal euphotic zone								
Accession numbers from EU793942				EU-30	30	Euk328f-Euk329r	< 3	126
to EU793987								
See also Viprey *et al.* (2008)								
Atlantic Meridional Transect (AMT 15)	Northern coastal	48°74′N	19-Sept-04	AMT15-1	10	Euk328f-Euk329r	< 3	217
Oceanic euphotic zone	Station 1	7°08′W						
Accession numbers from EU780589 to EU780636	Upwelling	17°83′N	04-Oct-04	AMT15-15	10	Euk328f-Euk329r	< 3	207
See also Zwirglmaier *et al.* (2007)	Station 15	20°89′W						
	Southern gyre	23°56′S	18-Oct-04	AMT15-27	30	Euk328f-Euk329r	< 3	119
	Station 27	17°49′W						
	Southern temperate	37°83′S	24-Oct-04	AMT15-33	5	Euk328f-Euk329r	< 3	234
	Station 33	1°23′E						

Table 1. *cont.*

Environment and cruise	Station and Site	Coordinates	Date	Code library	Depth (m)	Primer	Sample details (μm)	Total number of clones with insert screened
Florida Straits, Atlantic Ocean Coastal euphotic zone Accession numbers from EU818567 to EU818691 and EU836584 to EU836597	Station 1	25°30′N 80°04′W	31-Mar-05	FS01B	5	Euk328f-Euk329r	< 2	90
		25°30′N 80°04′W	31-Mar-05	FS01C	70	Euk328f-Euk329r	< 2	85
		25°30′N 80°03′W	01-Aug-05	FS01A	5	Euk328f-Euk329r	< 2	85
		25°30′N 80°03′W	01-Aug-05	FS01D	65	Euk328f-Euk329r	< 2	85
Florida Straits, Atlantic Ocean Oceanic euphotic zone Accession numbers from EU818078 to EU818566 and EU836598 to EU836612	Station 4	25°30′N 79°57′W	31-Mar-05	FS04E	5	Euk328f-Euk329r	< 2	81
		25°30′N 79°57′W	31-Mar-05	FS04F	85	Euk328f-Euk329r	< 2	88
		25°30′N 79°57′W	01-Aug-05	FS04G	5	Euk328f-Euk329r	< 2	90
		25°30′N 79°57′W	01-Aug-05	FS04H	89	Euk328f-Euk329r	< 2	169
	Station 14	25°29′N 79°20′W	30-Mar-05	FS14I	70	Euk328f-Euk329r	< 2	74
		25°29′N 79°20′W	30-Mar-05	FS14J	5	Euk328f-Euk329r	< 2	90
		25°29′N 79°20′W	31-Jul-05	FS14K	5	Euk328f-Euk329r	< 2	69
		25°29′N 79°20′W	31-Jul-05	FS14L	80	Euk328f-Euk329r	< 2	83
		25°29′N 79°20′W	Dec-08	FS14M	58	Euk328f-Euk329r	< 2	83
		25°29′N 79°20′W	Dec-08	FS14N	58	Euk328f-Euk329r	≥ 2	81
Sargasso Sea Atlantic Ocean Oceanic euphotic zone Accession numbers from EU817966 to EU818077 and EU836613 to EU836615	Bermuda Atlantic Time-Series Station	31°39′N 64°37′W	29-May-05	OC413BATS_O	75	Euk328f-Euk329r	< 2	90
			01-Jun-05	OC413BATS_P	15	Euk328f-Euk329r	< 2	87
Sargasso Sea, Atlantic Ocean Oceanic euphotic zone Accession numbers from EU817882 to EU817965 + EU836616 to EU836617		35°09′N 66°33′W	05-Jun-05	OC413NSS_Q	15	Euk328f-Euk329r	< 2	84
			08-Jun-05	OC413NSS_R	70	Euk328f-Euk329r	< 2	83
Total number of clones								6874

For further information on the primers used, see also Table S2. Accession numbers are provided for dinoflagellate sequences only.

Table 2. Environment types represented by 18S rRNA gene sequences considered herein, and the proportion falling within the *Dinoflagellata*.

Origin of the sequences (number of clone libraries considered)	Number of 18S rRNA gene sequences	Number of sequences belonging to dinoflagellates	Number of sequences per dinoflagellate group			
			Syndiniales Group I	*Syndiniales* Group II	*Syndiniales* Others	'Core' dinoflagellates
Cultures and isolates[a]	21 795	727 (3.3%)	13 (1.8%)	24 (3.3%)	9 (1.2%)	681 (93.7%)
Env. Continental (150)	5 711	35 (0.6%)	0	0	35 (100%)	
Plankton from the water column (42)	1 213	35 (2.9%)	0	0	0	35 (100%)
Biofilm/microbial mats (16)	392	0	0	0	0	0
Soil (39)	3 361	0	0	0	0	0
Sediment (30)	438	0	0	0	0	0
Others: aerosol, fermentor, gut communities . . . (23)	307	0	0		0	0
Env. Marine (347)	16 149	4809 (29.8%)	1151 (23.9%)	2411 (50.1%)	156 (3.2%)	1091 (22.7%)
Plankton from the water column (262)	13 528	4494 (33.2%)	1073 (23.9%)	2315 (51.5%)	154 (3.4%)	952 (21.2%)
Sediment (46)	1 624	139 (8.6%)	54 (38.8%)	18 (12.9%)	0	67 (48.2%)
Others: biofilm, incubation, gut communities . . . (39)	997	176 (17.7%)	24 (13.6%)	78 (44.3%)	2 (1.1%)	72 (40.9%)
Total	43 655	5571 (12.8%)	1164 (20.9%)	2435 (43.7%)	165 (3.0%)	1807 (31.5%)

a. Including analysis of single cells.
Env. means environmental sequences. The number of clone libraries screened for each environment is given in brackets in the first column. In columns 4–7, brackets indicate, as a percentage, the number of sequences obtained for a particular dinoflagellate group compared with the corresponding environment (column 3). 'Core' dinoflagellates means all dinoflagellates except *Syndiniales*.

(150 clone libraries, mostly from freshwater, sediments and soils). Taken together, this resulted in 4844 dinoflagellate sequences from these environmental data sets, with 1164 and 2435 belonging to MALV groups I and II respectively (Table 2). An additional 727 sequences were derived from dinoflagellate cultures including a few from the amplification of single cells (see Table 2), most of these belonging to marine photosynthetic dinoflagellates (a bias already pointed out by Murray *et al*., 2005). The mean length of the dinoflagellate sequences in the database was 950 nucleotides (range 108–1841 bp).

Phylogenetic analyses

Dinoflagellate sequences longer than 1600 bp (1018 sequences in total, 291 retained for the final tree, see the complete list of sequences in the Table S1) were used to perform phylogenetic analyses. Sequences belonging to the major *Alveolata* lineages were also included (Fig. 1). In order to avoid long-branch attraction artefacts, highly divergent alveolate groups were removed after preliminary phylogenetic analyses by neighbour joining (NJ). These divergent groups included *Haemosporida* (*Apicomplexa* including the human parasite *Plasmodium*), two ciliate groups, Mesodiniidae (*Myrionecta* and *Mesodinium*) and *Ellobiopsidae* (Silberman *et al*., 2004). Highly divergent dinoflagellates, such as members of *Noctilucales* and *Oxyrrhis marina*, were also excluded. Bayesian phylogeny, using 1137 positions in the 18S rRNA gene sequence alignments, delineated four primary lineages within the *Alveolata* (Fig. 1), the ciliates, the *Apicomplexa*, the *Perkinsea* and the *Dinophyceae*. As previously observed (Leander and Keeling, 2003; Groisillier *et al*., 2006), ciliates fell in the basal region of the tree, followed by *Apicomplexa* (Fig. 1). *Perkinsea* are the closest relatives of dinoflagellates. As frequently found in 18S rRNA gene phylogenies, many of these backbone nodes did not retain bootstrap support. At the basal part of *Dinophyceae*, several distinct taxa were placed in the bayesian analysis, here termed *Syndiniales* groups I–V, as a monophyletic lineage (Fig. 1). The general tree topology obtained with maximum likelihood (ML) was similar (data not shown). The existence of environmental MALV Group I and II was previously described (López-García *et al*., 2001; Moon-van der Staay *et al*., 2001), but here renamed (*Syndiniales* groups I and II) given more definitive placement within the *Syndiniales*. The genetic diversity and clade nomenclature of *Syndiniales* groups I and II are described in detail in separate analyses using partial sequences (Figs 2 and 3). For the definition of clades, we modified the general criteria chosen by Groisillier and colleagues (2006) so that a clade must (i) contain environmental sequences from at least 2 different clone libraries, and (ii) be bootstrap supported, at the defining nodes,

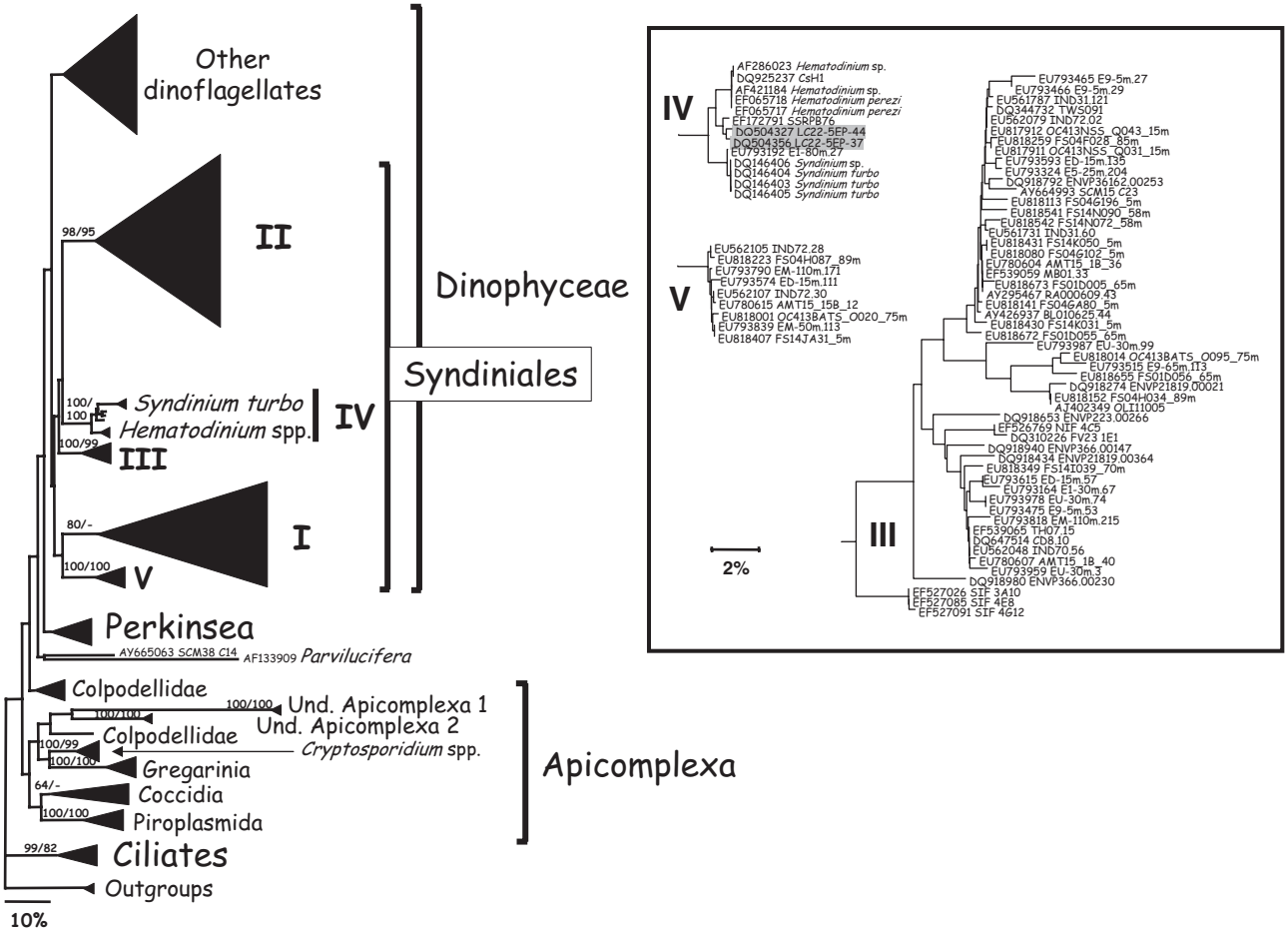

Fig. 1. Phylogeny of dinoflagellates using near-complete 18S rRNA gene sequences. Left: Bayesian phylogeny of alveolates based on analysis of 291 near full-length 18S rRNA gene sequences. Five sequences of *Bolidophyceae* (stramenopiles) were used as an outgroup. Gblock retained 1137 positions for the phylogenetic analyses. Bootstrap values, given at the principal nodes of the tree, correspond to neighbour-joining and maximum parsimony analyses respectively (1000 replicates, values > 60% shown). For neighbour-joining bootstrapping, a GTR + G + I model was selected with the following parameters: Lset Base = (0.2757 0. 1787 0.2494), Nst = 6, Rmat = (1.0746 2.9600 1.2514 1.0820 4.7079), Rates = gamma, Shape = 0.7091, Pinvar = 0.2330. The scale bar corresponds to 10% sequence divergence. Inset (at the right): Details of groups III to VI, based upon analysis of partial (500 bp) sequences (59 sequences including outgroups). The scale bar corresponds to 2% sequence divergence. The two sequences in grey belonging to Group IV are from hydrothermal vents.

by values greater than 60% in NJ and maximum parsimony (MP) analysis. Some exceptions to these rules were made as detailed below. The mean sequence identity within a clade was 87% for *Syndiniales* Group I (ranging from 76.6% for clade 5 to 91.9% for clade 7) and 93.5% for *Syndiniales* Group II (ranging from 80.8% for clade 8 to 99.4% for clades 35 and 43, see also Fig. S1). *Syndiniales* Group I contained eight different clades (Fig. 2). Clades 1–5 were described previously by Groisillier and colleagues (2006), while three new clades emerged from this study. All of these eight clades are supported by bootstrap values > 60%, except for Clade 3, which is only supported by MP, bootstrap analyses (74%). Nevertheless, the tree topology is identical with the two different methods (NJ and MP) and minimal sequence identities within this clade are inside the range of other clades belonging to this group (see Fig. S1). *Ichthyodinium chaberladi*, a parasitoid of fish eggs, belongs to Group I Clade 3, while *Duboscquella* spp., a parasitoid of tintinnides, belongs to Group I Clade 4 (Harada *et al*., 2007). *Syndiniales* sequences retrieved by single-cell PCR on radiolarian isolates (Dolven *et al*., 2007) belong to Clade 1 (DQ916408, though not in the tree because this sequence contained several nucleotide ambiguities) and Clade 2 (DQ916404–DQ916407 and DQ916410). Clades 5–8 are only composed of environmental sequences. Some sequences within clades 1–4 (highlighted in grey in Fig. 2) were retrieved exclusively from suboxic and anoxic ecosystems. Together, clades 1 and 5 were the most commonly retrieved from environmental clone libraries, representing ≥ 75% of sequences belonging to *Syndiniales* Group I.

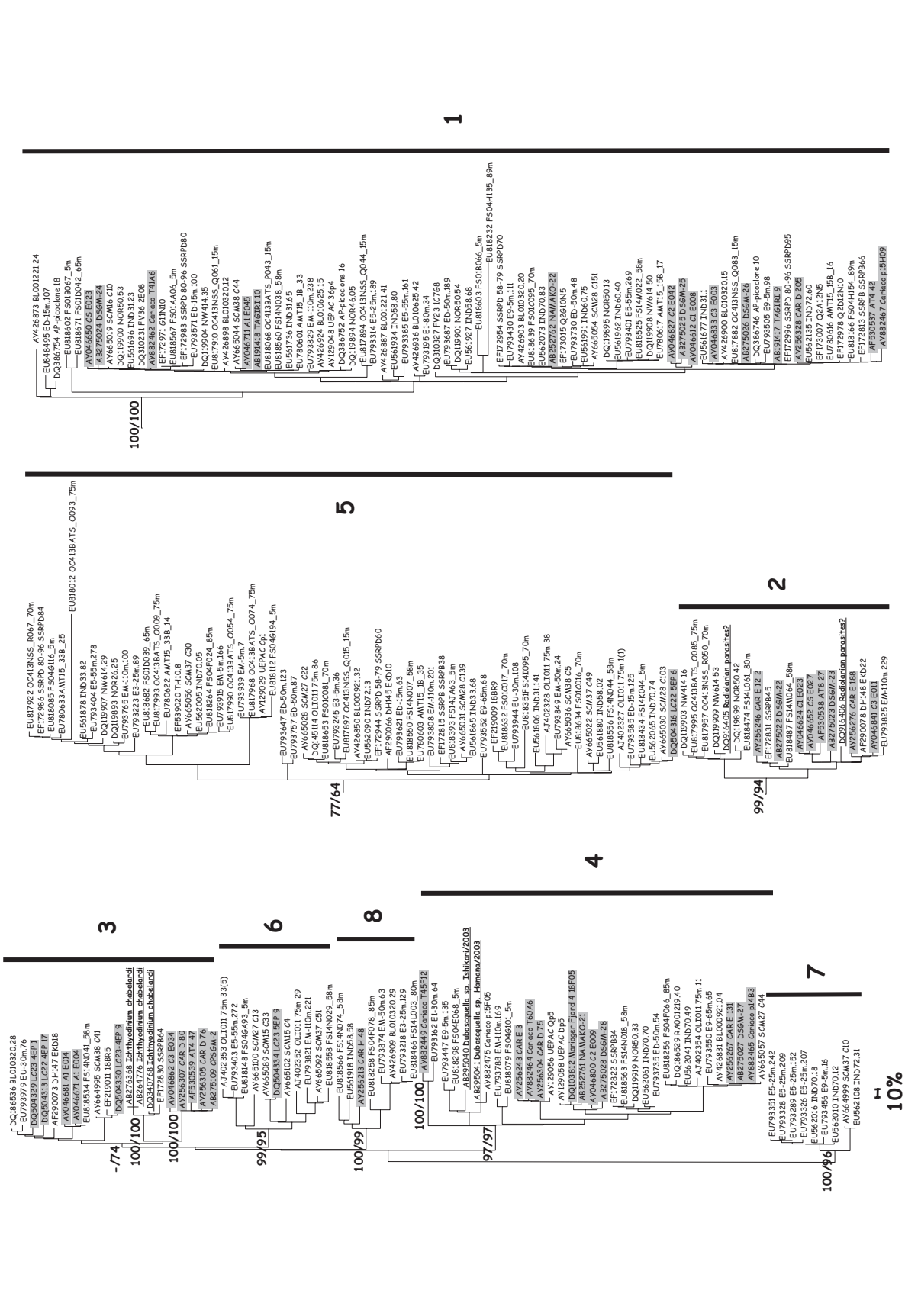

Fig. 2. Phylogeny of *Syndiniales* Group I. Neighbour-joining phylogeny of *Alveolate* Group I, based upon the analysis of 275 partial sequences, 731 bp in length (including outgroups, not shown). A GTR + G model was selected using the following parameters: Lset Base = (0.2627 0.1810 0.2622), Nst = 6, Rmat = (1.0000 2.8775 1.0000 4.6908), Rates = gamma, Shape = 0.5006, Pinvar = (0). Bootstrap values, given at the principal nodes of the different clades, correspond to neighbour-joining and maximum parsimony analyses respectively (1000 replicates, values > 60% shown). The scale bar corresponds to 10% sequence divergence. The tree was cut in order to better separate the major clades in the figure. Sequences in grey are from hydrothermal and suboxic ecosystems.

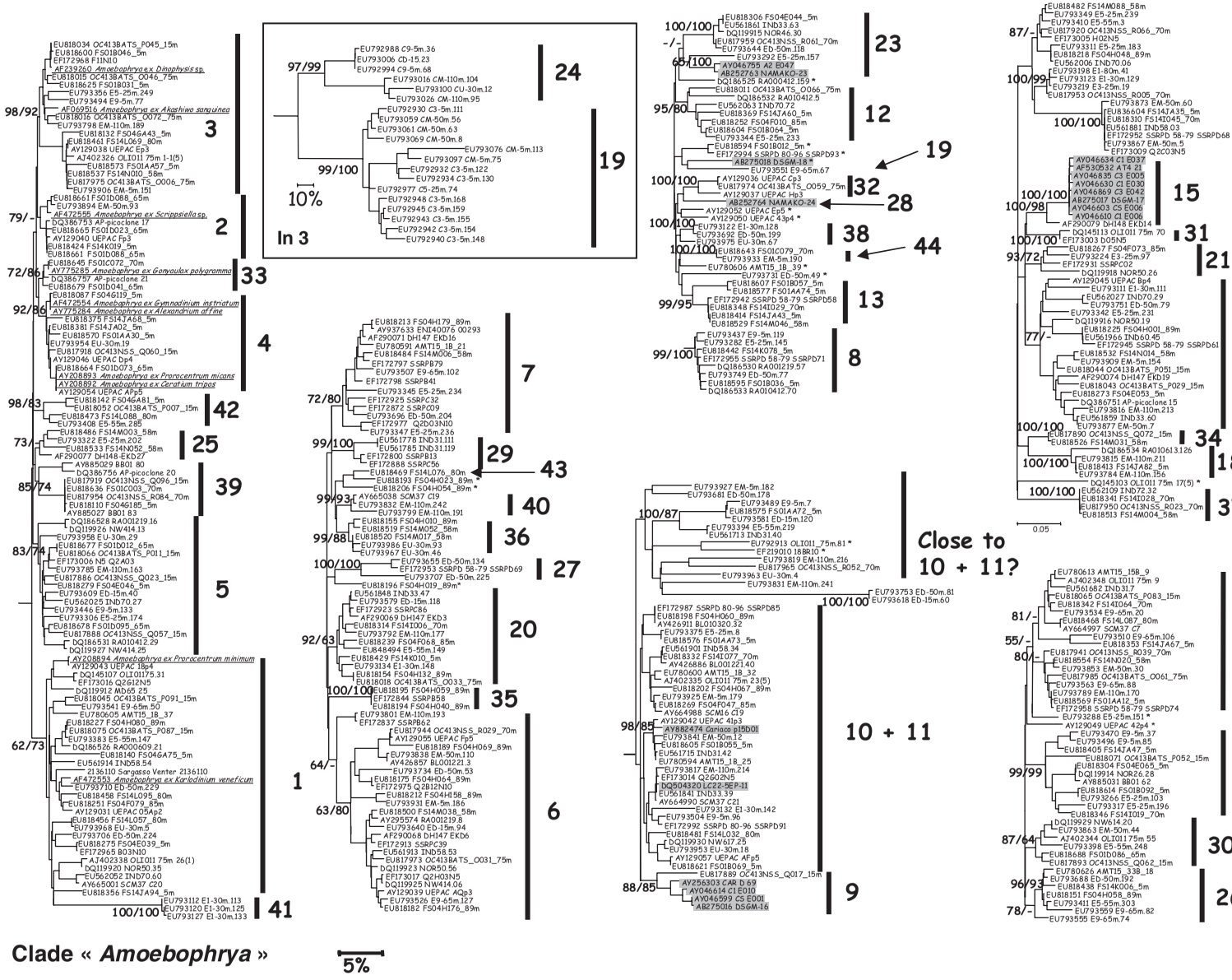

Fig. 3. Phylogeny of *Syndiniales* Group II. Neighbour-joining phylogeny of *Alveolate* Group II, based upon analysis of 423 partial sequences, 738 bp in length and centred at the 5′ end (including outgroups, not shown). A GTR + G model was selected using the following parameters: Lset Base = (0.2736 0. 1700 0.2545), Nst = 6, Rmat = (1.0000 3.3780 1.2804 1.2804 4.9683), Rates = gamma, Shape = 0.6108, Pinvar = 0.1155). Bootstrap values, given at the principal nodes of the different clades, correspond to neighbour-joining and maximum parsimony analyses respectively (1000 replicates, values > 60% shown). The asterisk '*' indicates singletons (sequences that are not included in any clades). The scale bar corresponds to 5% sequence divergence. The tree was cut in order to better separate the major clades in the figure. Inset: a small portion of the neighbour-joining tree obtained using the 3′ end of the 18S rRNA gene of *Alveolate* Group II, based upon analysis of 365 partial sequences, 617 bp in length (including outgroups). The scale bar corresponds to 10% sequence divergence. Bootstrap values correspond to neighbour-joining analyses (1000 replicates). Sequences in grey are from hydrothermal and suboxic ecosystems.

By comparison, *Syndiniales* Group II is genetically more diverse than Group I. In addition to clades 1–16 (see Groisillier *et al*., 2006), 28 new clades emerged from the present study (clades 17–44, Fig. 3). Some of these clades are not supported by bootstrap analyses obtained with one of the two phylogenetic methods tested (clades 2, 6, 10+11, 14, 16, 23, 25 and 26), but in such cases the tree topologies are identical between the two methods. Clade 10 and Clade 11 described by Groisillier and colleagues (2006) are now merged into a single clade (herein named Clade 10+11), which includes clone BL10320.32 (a singleton in the work of Groisillier *et al*., 2006). However, Clade 10+11 is not supported by bootstrap analyses. Numerous environmental sequences displaying long branches (labelled 'close to 10+11?' in Fig. 3) also appear to be closely related to Clade 10+11.

Only a single genus within *Syndiniales* Group II has been formally described, the *Amoebophrya*. All *Amoebophrya* sequences obtained from cultures and single-cell analyses formed a monophyletic group as recognized by Groisillier and colleagues (2006). This monophyletic group now also includes clades 1–5 as well as clades 25, 33, 39, 41 and 42 (Fig. 3). Recently, Kim and colleagues (2008) recognized nine different subgroups within *Amoebophrya*. Subgroup 1 (from the analysis of Kim and colleagues) corresponds primarily with our Clade 1 (except sequence AF290077 which belongs to the novel Clade 25) while subgroups 3, 4, 5 and 6 correspond to clades 4, 3, 5 and 2 respectively. The recognition of subgroup 2 (identified in Kim *et al*., 2008) is probably skewed by inclusion of a sequence we identified to be a chimera (DQ186527). Thus, sequence AY260468, obtained from the direct amplification of *Ceratium tripos* infected with *Amoebophrya*, likely remains a singleton. Sequence AY295690 (subgroup 8) is also a probable chimera, whereas sequences included within subgroups 7 and 9 belong to Clade 33 in the present study. Sequence DQ916402, obtained from the direct amplification of a cell of spumellarida *Hexacontium gigantheum* (Radiolaria), is closely related to Clade 6. This sequence was removed from our global analyses due to the high number of nucleotide ambiguities it contained. Clades 15 and 9 are primarily composed of environmental sequences from anoxic or suboxic ecosystems (highlighted in grey in Fig. 3). Both clades also include sequences retrieved from deep-sea methane cold seeps (DSGM-16 and 17, Takishita *et al*., 2007) and deep oceanic waters (Countway *et al*., 2007), but these sequences were not included in this analysis due to their short length. Clades originally containing only sequences from coastal systems (i.e. clades 2, 4, 5, 8, 12 and 13; see Groisillier *et al*., 2006) are shown here to also include clones from other oceanic regions, including oligotrophic waters. This highlights the importance of gaining sequence representation from a broader array of geographical locations. Finally, in terms of the number of sequences, the most commonly found clades in rank order were clades 10+11, 7, 6, 1, 19, 3, 22 and 16.

Other *Syndiniales* groups (III–V) also emerged from the present study (detailed in Fig. 1). *Syndiniales* Group III contains 71 environmental sequences, including clone OLI11005 (AJ402349) that was previously placed outside Group II (see Groisillier *et al.* 2006), as well as numerous environmental sequences retrieved from various oceanic surface waters (Mediterranean Sea, Indian Ocean, Sargasso Sea), coastal waters [Southern Taiwan Strait, Blanes Bay (Spain) and the English Channel] and from a supersulfidic anoxic fjord (DQ310226). *Syndiniales* Group IV contains the genera *Syndinium* and *Hematodinium*, five closely related environmental sequences, a sequence retrieved from the Mediterranean Sea, E1–80m.27, closely related to *Syndinium turbo* and a clade allied with the genus *Hematodinium* and composed of sequences from the Sargasso Sea (EF172791 and DQ918583) and from deep sea hydrothermal vents (DQ504327 and DQ504356). *Syndiniales* Group V contains 67 environmental sequences, all collected within the euphotic zone, but includes clones retrieved from very different oceanic ecosystems (Indian Ocean, Atlantic Ocean, Mediterranean Sea and Sargasso Sea). Sequence BL000921.23 from Blanes also belongs to *Syndiniales* Group V (not included here due to sequence length dissimilarities). Finally, two partial environmental sequences could not be assigned to any specific group: EU793524 (E9–65m.123) and EU818559 (F14DEC+), but phylogenetic analysis placed them closely related, although separated from these *Syndiniales* groups.

Ecological distributions

Dinoflagellate sequences represent less than 1% of environmental sequences from continental ecosystems (aquatic and terrestrial), whereas they represent close to 30% of the sequences obtained from marine ecosystems. With respect to aquatic environments (both marine and freshwater), dinoflagellates sequences as a whole were obtained primarily from the plankton, as opposed to other environments such as sediments (Table 2). Sequences belonging to *Syndiniales* groups I and II were absent in samples collected to date from continental ecosystems, however, they represent the largest portion of dinoflagellate sequences from marine systems. *Syndiniales* Group II alone represented about half of all environmental dinoflagellate sequences (Table 2).

We divided marine ecosystems included in our sequence database into various categories: (i) anoxic and suboxic ecosystems, (ii) ecosystems with hydrothermal activities, (iii) sediments, or (iv) the water column

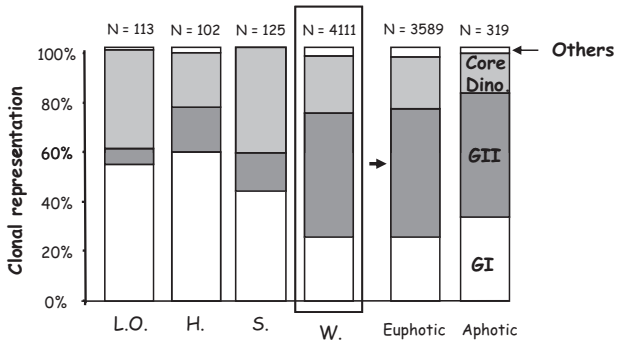

Fig. 4. The relative contribution of dinoflagellate sequences within environmental clone libraries. Left: relative contribution (in percentage) from (i) low oxygenated (L.O.) ecosystems (sediments or deep waters), (ii) Hydrothermal (H) vent ecosystems (collected directly in the hydrothermal chimney, on collectors or on sediments close to the chimney), (iii) sediments (S) that can be oxic or anoxic, deep or more coastal, and (iv) plankton from the water column (W). For this last category, two additional conditions were compared (right side of Fig. 4): whether the water sample was collected from the euphotic or the aphotic layer. N = Total number of environmental sequences considered.

(wherever possible, we also specified whether sequences were derived from the aphotic or euphotic zone; Fig. 4). In cases where these categories overlapped (e.g. anoxic sediments collected from deep hydrothermal vents, or anoxic water columns), environmental sequences were included in both categories. Considering all dinoflagellate sequences, *Syndiniales* Group II sequences were more abundant in clone libraries derived from the water column (planktonic ecosystems) than in other habitats, while the relative importance of the remaining dinoflagellate sequences (including *Syndiniales* Group I) was greater in anoxic environments, hydrothermal vents and sediments (Fig. 4). Within planktonic ecosystems, the relative contribution to clone libraries of *Syndiniales* Group I and II compared with other dinoflagellate sequences was similar between euphotic and aphotic waters (Fig. 4).

Environmental sequences derived from the euphotic zone, both coastal and oceanic waters, have generally been recovered using three different primer sets Euk328/Euk329, EukA/EukB and EukA/EukB′ (for primer references see Table S2). Although the contribution of dinoflagellate sequences to the total number of clones is very similar using either the EukA/EukB or EukA/EukB′ primer sets, the relative contribution of Group II is higher using the Euk328f/Euk329r primer set (Fig. 5). However, the distribution of *Syndiniales* Group I and II clades within the euphotic zone, as evidenced from these clone libraries, is quite similar with the different primer sets (Fig. 6). According to clone library composition, sunlit surface marine waters are dominated by *Syndiniales* Group I clades 1, 4 and 5, and Group II clades 1, 6, 7 and 10+11 (Fig. 6). Comparing sequences obtained using the same primer sets, *Syndiniales* clade distributions within the aphotic zone are quite different from surface waters, mainly dominated by *Syndiniales* Group I clades 1, 2 and 3 and by *Syndiniales* Group II clades 6 and 7 (Fig. 6).

Discussion

Our phylogenetic analysis of available 18S rRNA gene sequences from described parasitic genera (*Amoebophrya*, *Ichthyodinium*, *Duboscquella*, *Syndinium*, *Hematodinium*) and many environmental sequences, resolved for the first time the *Syndiniales* as a monophyletic lineage at the base of the more classical dinoflagellates. The basal position is not supported by bootstrap analyses, but the same topology was generated by Bayesian and ML phylogenies. This topology agrees with historical descriptions of the *Duboscquodinida* as a tribe (a taxonomic category between a genus and a subfamily) by Cachon (1964), and *Syndiniales* as an Order by Loeblich (1976). However, at higher taxonomic levels, *Syndiniales* are still difficult to place. Historically, *Syndiniales* have been retained within dinoflagellates because their short-lived dispersal stages, called dinospores, have a classic naked gymnoid morphology while also their nucleus has the chromatin condensed during part of the life cycle, like the dinokaryon of dinoflagellates. *Syndiniales* have trichocysts, a typical feature for *Dinophyceae*. However, Loeblich (1976) separated them from the rest of *Dinophyceae* (named also 'core' dinoflagellate) and placed *Syndiniales* inside the class Syndiniophyceae due to peculiarities of nuclear division in *Syndiniales* (Ris and Kubai, 1974; Soyer, 1974). Nevertheless, for all *Dinophyceae* and *Syndiniales*, chro-

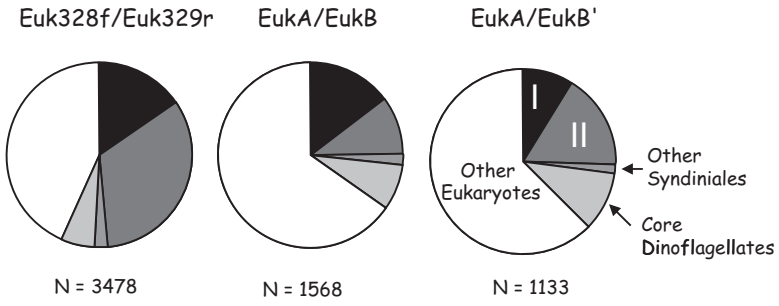

Fig. 5. Proportion of dinoflagellate sequences retrieved using different primer sets. Relative proportion of environmental sequences belonging to *Syndiniales* (I, II and others = III–VI) and the rest of dinoflagellates (= Core Dinoflagellates) compared with other eukaryotes retrieved using different primer sets during PCR amplification. For description of primers, see Table S2. Only clone libraries constructed from the euphotic zone of marine waters have been considered. N = the total number of sequences.

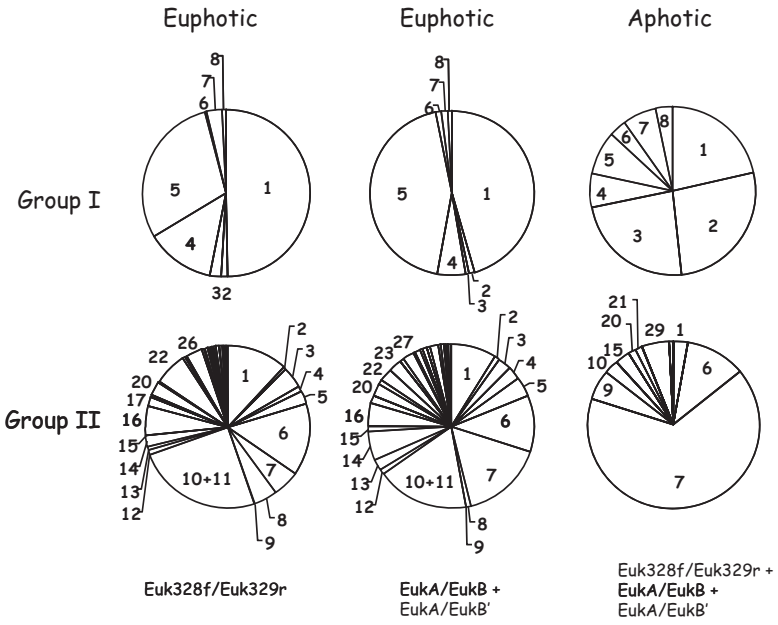

Fig. 6. *Syndiniales* clades retrieved using different primer sets, in the euphotic and aphotic layers of marine waters. The relative proportion of clades belonging to *Syndiniales* (groups I and II) using different sets of primers is indicated. Only clone libraries constructed from the euphotic zone of marine waters have been included (as in Fig. 5). N = the total number of sequences.

mosomes stay anchored to the persistant nuclear envelope during mitotic division, but their segregation is driven by microtubules through the nuclear envelope in *Dinophyceae* while centrioles participate in the formation of the mitotic apparatus in *Syndiniales* (Hollande, 1974; Ris and Kubai, 1974). Another *Syndiniales* peculiarity is their low chromosome number, 4–10 for *Syndiniales* compared with >100 for some *Dinophyceae* members such as *Gonyaulax* and *Ceratium* (see Loeblich, 1976). Taking all this together, it seems reasonable that these last two features (peculiarities in nuclear division and low chromosome number) are derived from an initial dinoflagellate model (Xiao-Ping and Jing-Yan, 1986), being forced by the obligately parasitic lifestyle of *Syndiniales*. Controversially, some recent phylogenies using 28S rRNA gene sequences, have placed the *Syndiniales Ichthyodinium chaberladi* (Group I Clade 3) within dinoflagellates (Gestal *et al*., 2006) and an environmental sequence belonging to Group I clade 1 close to *Perkinsus* (Massana *et al*., 2008). More sequences are needed to obtain a LSU rRNA phylogeny robust enough to provide accurate affiliations for the *Syndiniales*. Protein coding genes, such as actin and β- and γ-tubulins may also be a better alternative to resolve basal branches of the dinoflagellate lineage (Saldarriaga *et al*., 2003), including several deep genera such as *Perkinsus*, *Oxyrrhis* and *Noctiluca*, which are almost impossible to place using phylogenies based upon ribosomal genes.

Within *Syndiniales*, five main families have been described: the *Sphaeriparidae* and the *Coccidinidae* (no sequences available to date for both), the *Amoebophryidae*, the *Duboscquellidae* and the Syndinidae (Table S3). As *Ichthyodinium* (*Syndinidae*) and *Duboscquella* (*Duboscquellidae*) are clearly allied within Group I by 18S rRNA gene analyses, while *Hematodinium* and *Syndinium* (*Syndinidae*) are members of Group IV and quite distinct from Group I, it is likely that the taxonomy of *Syndinidae* will soon be modified. Nevertheless, it is possible that *Amoebophryidae* will remain synonymous with *Syndiniales* Group II. To date, seven different species of *Amoebophrya* have been described (see Table S3). However, this clearly does not reflect the genetic diversity observed in Group II; herein we find at least 44 distinct clades. Unfortunately, only one of the described species, *A. ceratii,* has been genetically characterized to date. *Amoebophrya ceratii* is a parasitoid of many (if not all) dinoflagellates, including heterotrophic species (for a review see Park *et al*., 2004) and was most recently reported as being a 'complex species' rather than a real species (see Coats *et al*., 1996). Currently, available SSU rRNA gene sequences from this complex species are from strains able to infect photosynthetic dinoflagellates, in accordance with the fact that this group is largely retrieved from the euphotic zone in environmental studies. Nevertheless, *A. ceratii* was also described as being able to parasitize other *Amoebophrya* species (e.g. it is frequently observed in *A. leptodisci*) or other *Syndiniales* (such as the genus *Keppenodinium*; see Cachon 1964). Consequently, despite recent additions of a substantial number of new sequences, all *Amoebophrya* SSU rRNA gene sequences fall within a monophyletic lineage, which contains at least 10 different clades. However, the taxonomic correspondence of each clade is still not clear. Within *Hematodinium* and *Syndinium*, SSU rRNA gene sequences from different species are almost identical, and species can only be distinguished using more variable genetic regions (ITS, see Hudson and Adlard, 1996;

Skovgaard *et al*., 2005). In the case of *Amoebophrya*, genetic variability is much more evident, even using conserved markers such as the SSU rRNA gene.

All described *Syndiniales* are parasitoids, and obligately kill their host. A potential exception is *Sphaeripara catenata*, which in some cases only causes castration of its host, but not death (Cachon and Cachon-Enjumet, 1964). Described *Syndiniales* generally complete their life cycle in less than 3 days, leading to the release of hundreds of free-living dispersive dinospores. Environmental sequences obtained from genetic libraries likely result from such dinospores. In general, *Syndiniales* produce two different types of dinospores, the micro and macrospores that can both be very small (from 1 to 12 μm in diameter depending on the species). There is a general agreement that dinospores can only survive a few days after their release (e.g. Coats and Park, 2002). Hence, the fact that they systematically dominate clone libraries from coastal and oceanic waters, whatever the season considered, suggests that their production is fairly constant. Alternatively, it could mean that other more resistant planktonic stages are produced. To our knowledge, cyst production has never been reported within *Syndiniales*, with the notable exception of cyst-like cells described in natural populations of *Duboscquella cachoni* infecting the tintinnid *Eutintinnus pectinis* (Coats, 1988). In this particular case, cyst-like formation was completely independent of dinospore production, and the cysts formed were nonmotile. Within dinoflagellates, permanent cysts are produced by the fusion of two gametes. Sexuality, issued from different individuals (anisogamous and heterothallic), has only been observed once in *Coccidinidae*, with the production of a planozygote-like body (Chatton and Biecheler, 1936). Nevertheless, cyst production was not observed.

The recent reports of *Amoebophrya* spp. in freshwater systems (Lefèvre *et al*., 2008) are based on placement of two environmental sequences incorrectly labelled uncultured *Amoebophrya* Clone E and F (Di Giuseppe and Dini, 2004). These sequences are in fact closely related to the genus *Cryptocaryon* (Ciliates, Protosmatea). Thus, to date, environmental sequences of *Syndiniales* have been retrieved solely from marine ecosystems. This supports the 'marine-ness' of this group, together with the fact that all described *Syndiniales* species are marine. In contrast, other unicellular alveolate parasites, such as *Perkinsea*, *Colpodellids* or even other parasitic dinoflagellates, have both marine and continental species. This is interesting given that almost all known *Syndiniales* hosts have species well adapted to continental ecosystems (within ciliates, dinoflagellates, cercozoa and crabs).

Although the distribution of *Syndiniales* is relatively homogeneous at the group level (note their similar contribution to both euphotic and aphotic zones), communities are quite different at the clade level. Some clades are uniformly distributed vertically through the water column (e.g. Group I clade 1, Group II clade 6), whereas others (e.g. Group I clades 2 and 3, Group II clade 7) are more prevalent in aphotic layers. We speculate that these 'deeper' clades are able to parasitize well-known deep planktonic organisms such as *Phaeodarea* (*Cercozoa*), *Acantharea* (*Radiolaria*) and *Polycystinea* (*Radiolaria*). Recent analyses of environmental sequences belonging to these groups highlight the increasing contribution of radiolarian environmental sequences with depth (Not *et al*., 2007), in particular sequences belonging to *Spumellarida* (*Polycystinea*). Interestingly, radiolarians are also exclusively marine, and well known to be oceanic, or blue-water, organisms. Hence, both *Syndiniales* and radiolarians may have shared a common evolutionary history inhabiting similar ecosystems as discussed by Not and colleagues (2007). Sequences retrieved from single radiolarian cells (Dolven *et al*., 2007), likely infected by *Syndiniales*, belong to both Group I and Group II (Fig. 3). Nevertheless, it is difficult to assign an identity to these sequences as *Syndiniales* able to infect radiolarians are described within three of the five described families (the *Amoebophryidae*, the *Duboscquellidae* and the *Syndinidae*). Much more information on host specificity is provided by data from the genus *Amoebophrya*. *Phylogenies* of *Amoebophrya* clades (Fig. 3) indicate that host–parasite co-evolution is not a central feature driving the observed diversity. Parasites of the same host genus, but of different species, belong to completely separate clades. In fact, within the '*A. ceratii* complex', host specificity as assessed in cultures, is highly strain dependent, with some being extremely specific (Coats and Park, 2002), while others have broader host ranges (Kim, 2006). This fact could mask clear co-evolutionary patterns. The same conclusion can be drawn when the entire *Syndiniales* order is considered. For example, all described *Syndiniales* that operate as parasitoids of metazoans are not closely related. The genus *Ichthyodinium*, a parasite of fish eggs, is more closely related to parasites that infect ciliates (i.e. *Duboscquella*) belonging to Group I than to Group IV, which contains *Syndinium* and *Hematodinium* (two genera which infect metazoans). Thus, even between groups, *Syndiniales* and their hosts do not seem to co-evolve. Consequently, we expect that parasites within *Syndiniales* are generalists or opportunists with respect to host range. This is also congruent with the detection of clades widely distributed in all explored marine ecosystems. Nevertheless, the presence of more specialized clades or subclades, related with depth or specifically retrieved from extreme environments, such as Group I clades 3 and 4, and Group II clades 9 and 15, confirms that specific interactions may exist.

Conclusions

Based on the integration of a large number of environmental sequences, we propose a new phylogeny for *Syndiniales*. This order should be considered a monophyletic sister group of the rest of *Dinophyceae* based upon 18S rRNA gene phylogenies. The *Syndiniales* are composed of five main groups, which include environmental sequences formerly referred to as novel marine alveolate groups I and II. All *Syndiniales* species described to date are parasitic marine organisms. The exclusively marine lifestyle of this order as a whole, including sequences from uncultured organisms, is confirmed by the fact that their 18S rRNA gene sequences have not been retrieved from terrestrial or freshwater PCR surveys, but rather come solely from marine ecosystems. *Syndiniales* appear to colonize all the marine habitats investigated thus far, from oceanic surface waters to sediments. Some clades are likely adapted to fairly specific habitats (and/or hosts that reside only in specific habitats). However, these parasites and their hosts do not appear to share co-evolutionary trends. This suggests that at least some *Syndiniales* are highly opportunistic, with a capacity to infect very different marine hosts, from many trophic levels within marine food webs. Even so, we cannot completely exclude that some members of the *Syndiniales* may have retained specific ancestral traits still found among the different alveolate lineages, e.g. phagotrophy and/or the inheritance of photosynthetic genes. In fact, proteins characteristic of secondary endosymbiont plastids have recently been detected from both the non-photosynthetic genera *Oxyrrhis* and *Perkinsus* (Matsuzaki *et al.*, 2008; Slamovits and Keeling, 2008). Hence, the ecological success of the *Syndiniales* may yet have a more surprising and complex explanation. That said, it is certainly still the case that the overall role of parasitism in marine systems requires further investigation and incorporation into food web and carbon flow models.

Experimental procedures

Environmental sampling and library processing

Materials and methods for each of the libraries published herein varied by site. Thus, details are provided separately for each of the regions sampled. See also Table 1 for additional details on the clone libraries constructed.

English Channel, France (RA). Ten litres of surface sea water was sampled weekly at the ASTAN site (SOMLIT station, English Channel, France, see http://www.domino.u-bordeaux.fr/somlit_national/) from the end of June through to the middle of September 2004. Water samples were size-fractionated using a peristaltic pump (with the flow rate fixed at 100 ml min^{-1}) through sequential 47-mm-diameter polycarbonate filters (12 and 3 μm, Osmonic Poretics Products) in filter housings connected in series, terminated by a 0.2-μm-pore-size Sterivex unit (Millipore). Following sample collection, filters were then submerged in DNA lysis buffer (0.75 M sucrose, 40 mM EDTA, 50 mM Tris-HCl, pH 8), immediately frozen in liquid nitrogen and stored at −80°C. DNA was extracted using a 3% (w/v) CTAB (Cetyltrimethylammonium bromide) extraction procedure (Doyle and Doyle, 1987). PCR conditions for amplifying the 18S rRNA gene were as described in Moon-van der Staay and colleagues (2001). PCR products were cloned using the TOPO-TA cloning kit (Invitrogen). Clones (96 randomly selected) were partially sequenced using the primer Euk528f for RA1 and the primer ALV01 (for primer references see Table S2) for both RA2 and RA3 on an ABI Prism 3100 (Applied Biosystems).

Barcelona Harbour, Mediterranean Sea, Spain (BAFRACT). Fifty litres of sea water was collected on 28 February 2001 from Barcelona harbour (Spain) and pre-filtered through a 50 μm mesh. On return to the laboratory (less than one hour), the sample was then fractionated through seven different pore-sizes (20, 14, 10, 5, 3, 1 and 0.6 μm), using similar filter housings connected in series as described above. Sample storage and DNA extraction were as described above. Genetic diversity in each size fraction was determined by DGGE (not shown), as described by Díez and colleagues (2001b). Clone libraries were performed for size fractions displaying the most 'contrasting' DGGE band patterns. PCR conditions were as in Díez and colleagues (2001a). Cloning was as described above. Restriction fragment length polymorphism (RFLP) analysis was used to detect polymorphisms among the retrieved clones. Briefly, PCR products were digested with 1 U μl^{-1} of restriction enzyme HaeIII (Gibco BRL) for 6–12 h at 37°C. The digested products were separated by electrophoresis at 80 V for 2–3 h in a 2.5% low-melting-point agarose gel. One clone per unique RFLP pattern was partially sequenced using the internal primer Euk528f. Sequencing was performed by Qiagen Genomics Sequencing Services (Germany).

Blanes Bay. Surface water was collected in Blanes Bay (Catalan coast, north-western Mediterranean Sea). Sea water was rapidly transported to the laboratory (less than 2 h), and gently filtered by gravity through 2- and 5-μm-pore-size polycarbonate filters. One litre of each size fraction was incubated both in the light [around 100 (μmol quanta) m^{-2} s^{-1}] and in the dark (four bottles in total). All the incubations were carried out at 20°C, close to *in situ* temperature (18°C). Samples (100 ml) were filtered through 0.2-μm-pore-size Durapore filters for DNA analysis over a course of 5 days. DNA was then extracted following the protocol described in Díez and colleagues (2001b). Abundance and genetic diversity of eukaryotes were monitored by epifluorescence microscopy and DGGE (using the same protocol as described above, data not show). 18S rRNA genes were amplified from the initial day and after 4 days of incubation both in the light and dark. PCR products were cloned using the TOPO-TA cloning kit. Clones were sequenced by the Qiagen Genomics Sequencing Services (Germany).

Atlantic Meridional Transect (AMT15). Both the sampling strategy, DNA extraction procedure and the PCR amplification protocol used have been previously published (Zwirglmaier *et al*., 2007). Cloning was as described above. Restriction fragment length polymorphism analysis was used to screen for distinct inserts by digesting with HaeIII in a total volume of 20 μl using 5 U of HaeIII at 37°C for 2 h. Fragments were resolved by electrophoresis in a 2.5% agarose gel at 60 V for 3 h. At least two representatives of each RFLP pattern were sequenced in entirety following purification of the PCR product using the QIAquick PCR Purification Kit (Qiagen), on an ABI 3130xl Genetic Analyser (Applied Biosystems).

Florida Straits and Sargasso Sea. Sea water was collected from both the surface and deep chlorophyll maximum on three transect cruises across the Straits of Florida in 2005 as described in Cuvelier and colleagues (2008). Briefly, 1 l samples were first gravity filtered through 47 mm diameter, 2-μm-pore-size filters (GE Osmonics, Minnetonka, Minnesota, USA) and then onto a 0.2-μm-pore-size Supor filter (Pall Gelman, Ann Arbor, Michigan, USA) using approximately 5 psi vacuum. Filters were placed in cryovials and frozen in liquid nitrogen. DNA was extracted using the DNeasy kit according to the manufacturer's recommendations (Qiagen, Germantown, MD, USA). PCR conditions have been published elsewhere (Cuvelier *et al*., 2008). The PCR product was ligated into vector pCR2.1 (Invitrogen, Carlsbad, CA, USA) and transformed. Clones were then sequenced on an ABI 3730xl sequencer (Applied Biosystems).

Samples were also collected on a cruise from Woods Hole, MA USA to the BATS. Two regions were sampled, BATS and a site north-west of BATS. Samples were collected and processed as for the Florida Straits clone libraries (as reported in Cuvelier *et al*., 2008).

Development of sequence database

The 18S rRNA gene database was built using all published environmental sequences deposited in GenBank up to December 2007. We also integrated the data sets generated herein and a number of other libraries which are being published independently, but without specific attention to the analysis of *Alveolata* sequences. The complete list of sequences used in this study and their annotation is available upon request (Excel file).

Sequence group assignments

The software package, KeyDNAtools, was used to (i) detect sequences belonging to dinoflagellates within a large database including more than 42000 eukaryotic SSU rRNA gene sequences; (ii) remove potential chimeras; and (iii) automatically assign sequences to a specific clade. Details of the software development will be described elsewhere. Briefly, environmental sequences were annotated using small oligonucleotides 'probes' (15 bp length), named 'keys', which were generated *in silico*. The specificity of each key, from the Order to the clade level (where possible) was automatically deduced from a reference database containing as many eukaryotic lineages as possible as outgroup and at least two sequences from each taxa created within marine dinoflagellates. In this study, more than 110000 keys were generated, each being specific at least to the level of Order. Annotations of 18S rRNA genes were deduced from the specificity of each key when they matched a sequence. As an example, sequence EF172938 was annotated by 175 different keys, providing converging annotation at the order level and covering positions 1–1289 along the sequences (Table 3). Thus, this sequence was considered as a *Syndiniales*, Dino-Group II, and belonging to Dino-Group II-Clade 7 (this last annotation is provided by 47 keys). The 18S rRNA gene sequences belonging to the majority of *Dinophyceae* were not variable enough to allow a clear separation of sequences even at the Order level. Thus, keys specific to most of the *Dinophyceae* are labelled as *Dinophyceae* at the Class and the Order levels (see annotation of sequence EU087283). Wherever possible, more precise information is given (e.g. at the Family, Genus, Species and Clade levels; see annotation of sequence EU087283). New groups/clades were often detected by conflicting annotation (caused by incorrect deduction of specificity by the keys) in a particular taxonomic field. Chimeras were detected when annotations conflicted for separate parts of an individual sequence. As an example (Table 3), sequence AJ965100 was detected as a chimera at the class level, a false gene sequence combining part of a *Ciliophora* (annotation provided by 25 keys covering positions 44–155) gene sequence with part of a *Dinophyceae* (31 keys covering positions 213–474) gene sequence. Automatic annotation of eukaryotic 18S rRNA environmental gene sequences using the final generated keys can be freely tested on the following web site: http://KeyDNAtools.com.

Phylogenetic analyses

Sequences were aligned using the slow and iterative refinement method FFT-NS-i with Mafft 5.8 software (Katoh *et al*., 2007). Using the BioEdit sequence editor (Hall, 1999) for visualization of the alignment, we deduced the secondary structures by hand using previously published studies (see for example Lange *et al*., 1996). For complete sequence alignments only, poorly aligned and highly variable regions of the alignments were automatically removed using Gblocks (Castresana, 2000) with the following parameters: allowing gaps in half positions and the minimum length of a block = 5. Different nested models of DNA substitution and associated parameters were tested using Modeltest v.3.06 (Posada and Crandall, 1998). Settings given by Modeltest were used to perform the NJ and the ML analyses. The NJ, ML and MP analyses were performed using PAUP 4.0b10 (Swofford, 2002). A heuristic search procedure using the tree bisection/reconnection branch swapping algorithm (setting as in MP) was performed to find the optimal ML tree topology (with 70 000 re-arrangements). Bootstrap values for NJ and MP were estimated from 1000 replicates. For MP, the number of re-arrangements was limited to 5000 for each bootstrap replicate. Starting trees were obtained by randomized stepwise addition (number of replicates = 20). Additionally, we used Bayesian reconstruction for the analysis of complete sequences with MrBayes, v.3.0b4 (Huelsenbeck and Ronquist, 2001). The GTR model of substitution was used, taking

Table 3. Examples of three different automatic annotations generated by the KeyDNAtools.

ID	Length	SuperGroup	Division	Class	Order	Family	Genus	Species	Clade
Case 1: EF172938	1288	*Chromalveolata* –> 175 (1–1289)	*Alveolata* –> 175 (1–1289)	*Dinophyceae* –> 175 (1–1289)	*Syndiniales* –> 175 (1–1289)	Dino-Group II –> 174 (1–1289)			Dino-Group II-Clade 7 –> 47 (1–1196)
Case 2: EU087283	886	*Chromalveolata* –> 53 (38–716)	*Alveolata* –> 53 (38–716)	*Dinophyceae* –> 53 (38–716)	*Dinophyceae* –> 53 (38–716)	*Suessiales* –> 11 (38–656)	*Symbiodinium* –> 7 (142–656)		*Symbiodinium* clade B + B′ + D –> 3 (521–656)
Case 3: AJ965100	499	*Chromalveolata* –> 56 (44–474)	*Alveolata* –> 56 (44–474)	*Dinophyceae* –> 31 (213–474)/ *Ciliophora* –> 25 (44–155)	*Syndiniales* –> 31 (213–474)/ *Oligotrichea* –> 25 (44–155)	Dino-Group II –> 31 (213–474)			*Strombidium* group –> 2 (75–99)

into account a gamma-shaped distribution of the rates of substitution among sites. The chains were run for 1000000 generations. Trees were sampled every 100 generations. The first 5000 sampled trees, corresponding to the initial phase before the chains became stationary, were discarded (burn in).

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Supporting information

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

Fig. S1. Mean sequence identities within the different clades belonging to *Syndiniales* groups I and II.

Table S1. Details of the sequences used for the phylogenetic analyses described in Fig. 1.

Table S2. Primer sequences used or cited in this study.

Table S3. Taxonomy of *Syndiniales* (= *Syndinida*) and their hosts modified from Loeblich (1976).

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Supplementary Material

Supplementary Material 1. Details of the sequences used for the phylogenetic analyses described in Figure 1.

ID	Class	Order	Family/Clade	Genus	Species	Strain/Clone
AF494059	Apicomplexa	Coccidia	*	Adelina	bambarooniae	
DQ096836	Apicomplexa	Coccidia	*	Adelina	grylli	
AY043206	Apicomplexa	Coccidia	*	Goussia	janae	
AF238264	Apicomplexa	Coccidia	Coccidia un. Symbiont	*	*	symbiont Type N
AF238265	Apicomplexa	Coccidia	Coccidia un. Symbiont	*	*	symbiont Type N
DQ136187	Apicomplexa	Coccidia	Eimeriidae	Eimeria	acervulina	Shanghai
AF120114	Apicomplexa	Coccidia	Sarcocystidae	Sarcocystis	atheridis	
M64244	Apicomplexa	Coccidia	Sarcocystidae	Sarcocystis	muris	
AJ697751	Apicomplexa	Colpodellidae	*	*	*	ex Cryptosporidium struthionis
AY919682	Apicomplexa	Colpodellidae	*	*	*	LG 02-01
AY919694	Apicomplexa	Colpodellidae	*	*	*	LG 05-01
AY919769	Apicomplexa	Colpodellidae	*	*	*	LG 25-01
AY919792	Apicomplexa	Colpodellidae	*	*	*	LG 32-03
DQ244027	Apicomplexa	Colpodellidae	*	*	*	PAA22AU2004
DQ244031	Apicomplexa	Colpodellidae	*	*	*	PAF8AU2004
DQ174732	Apicomplexa	Colpodellidae	*	Chromera	velia	RM11 ex Colpodella
AY142075	Apicomplexa	Colpodellidae	*	Colpodella	*	ATCC 50594
AY449717	Apicomplexa	Colpodellidae	*	Colpodella	*	ATCC50594
AY234843	Apicomplexa	Colpodellidae	*	Colpodella	edax	
AY078092	Apicomplexa	Colpodellidae	*	Colpodella	pontica	G-3
AF330214	Apicomplexa	Colpodellidae	*	Colpodella	tetrahymenae	
AF280076	Apicomplexa	Colpodellidae	*	Voromonas	pontica	ATCC 50640
DQ060422	Apicomplexa	Cryptosporidium	*	Cryptosporidium	andersoni	zzca
AY642591	Apicomplexa	Cryptosporidium	*	Cryptosporidium	muris	Kawatabi
AF093490	Apicomplexa	Cryptosporidium	*	Cryptosporidium	parvum	Bovine-C.-parvum-genotype-(BOH6)
AF093493	Apicomplexa	Cryptosporidium	*	Cryptosporidium	parvum	Human-C.-parvum-genotype-(GCH1)
X64340	Apicomplexa	Cryptosporidium	*	Cryptosporidium	parvum	
AY490099	Apicomplexa	Gregarines	*	*	*	
DQ462458	Apicomplexa	Gregarines	*	Ascogregarina	*	ex Ochlerotatus japonicus
AY334568	Apicomplexa	Gregarines	*	Mattesia	geminata	
DQ287951	Apicomplexa	Piroplasmida	*	Babesia	equi	
AF244911	Apicomplexa	Piroplasmida	*	Babesia	leo	
AY309956	Apicomplexa	Piroplasmida	*	Cytauxzoon	felis	Spain-1
U97056	Apicomplexa	Piroplasmida	*	Theileria	*	
AY534882	Apicomplexa	Piroplasmida	Babesia group	Theileria	equi	ET1 pCR4-984048
AB275009	Apicomplexa	Und. Apicomplexa 1	*	*	*	DSGM-9
AB275084	Apicomplexa	Und. Apicomplexa 1	*	*	*	CYSGM-1
U07937	Apicomplexa	Und. Apicomplexa 1	*	*	*	
AB275013	Apicomplexa	Und. Apicomplexa 2	*	*	*	DSGM-13

AY179976	Apicomplexa	Und. Apicomplexa 2	*	*	*	CCI 31
AF123596	Bolidophyceae	Bolidomonadales	*	Bolidomonas	mediterranea	RCC 238, MINB11E5
AF167153	Bolidophyceae	Bolidomonadales	*	Bolidomonas	pacifica var eleuthera	OLI46SE
AF167154	Bolidophyceae	Bolidomonadales	*	Bolidomonas	pacifica var eleuthera	OLI120SD
AF123595	Bolidophyceae	Bolidomonadales	*	Bolidomonas	pacifica var pacifica	OLI31SE3
AF167155	Bolidophyceae	Bolidomonadales	*	Bolidomonas	pacifica var pacifica	OLI41SA
AY007450	Ciliophora	Armophorea	*	Metopus	palaeformis	
AF300288	Ciliophora	Colpodea	*	Sorogena	stoianovitchae	ATCC 50031
EF014286	Ciliophora	Karyorelictea	*	Epalxella	antiquorum	
DQ232761	Ciliophora	Nassophorea	*	Orthodonella	apohamatus	
X65151	Ciliophora	Nassophorea	*	Pseudomicrothorax	dubius	
AF100301	Ciliophora	Oligohymenophorea	Peniculia	Paramecium	calkinsi	Woods-Hole
AF194409	Ciliophora	Oligotrichea	Protocruziida	Protocruzia	*	
DQ834370	Ciliophora	Phyllopharyngea	Suctoria	Ephelota	gemmipara	
U97109	Ciliophora	Protosmatea	*	Coleps	hirtus	
AB191414	Dinophyceae	Dinophyceae	*	*	*	TAGIRI 6
AB191415	Dinophyceae	Dinophyceae	*	*	*	TAGIRI 7
AF530534	Dinophyceae	Dinophyceae	*	*	*	IN24 2
AJ402340	Dinophyceae	Dinophyceae	*	*	*	OLI011_75m_27(5)
AY180045	Dinophyceae	Dinophyceae	*	*	*	CCW 109
AY238479	Dinophyceae	Dinophyceae	*	*	*	ex Thecadinium dragescoi
AY434687	Dinophyceae	Dinophyceae	*	*	*	
AY590484	Dinophyceae	Dinophyceae	*	*	*	sp. Shepherd's Crook
AY664934	Dinophyceae	Dinophyceae	*	*	*	SCM15 C30
AY664994	Dinophyceae	Dinophyceae	*	*	*	SCM15 C25
AY664996	Dinophyceae	Dinophyceae	*	*	*	SCM15 C84
AY665051	Dinophyceae	Dinophyceae	*	*	*	SCM38 C24
AY665052	Dinophyceae	Dinophyceae	*	*	*	SCM38 C55
AY665053	Dinophyceae	Dinophyceae	*	*	*	SCM16 C13
AY665063	Dinophyceae	Dinophyceae	*	*	*	SCM38 C14
AY665079	Dinophyceae	Dinophyceae	*	*	*	SCM37 C55
AY665080	Dinophyceae	Dinophyceae	*	*	*	SCM38 C40
AY665081	Dinophyceae	Dinophyceae	*	*	*	SCM37 C25
AY827954	Dinophyceae	Dinophyceae	*	*	*	Lake Tovel H
AY827955	Dinophyceae	Dinophyceae	*	*	*	Lake Tovel I
AY829524	Dinophyceae	Dinophyceae	*	*	*	Lake Tovel A
AY829528	Dinophyceae	Dinophyceae	*	*	*	Lake Tovel LaTo3
DQ103861	Dinophyceae	Dinophyceae	*	*	*	Mariager_Fjord_3_18A04
DQ103863	Dinophyceae	Dinophyceae	*	*	*	Mariager_Fjord_3_18B06
DQ103871	Dinophyceae	Dinophyceae	*	*	*	Mariager_Fjord_3_18H06
DQ504315	Dinophyceae	Dinophyceae	*	*	*	LC22_5EP_3
DQ916409	Dinophyceae	Dinophyceae	*	*	*	
EU780612	Dinophyceae	Dinophyceae	*	*	*	AMT15_15B_8
EU780618	Dinophyceae	Dinophyceae	*	*	*	AMT15_27B_4
EU780631	Dinophyceae	Dinophyceae	*	*	*	AMT15_33B_23
AF274249	Dinophyceae	Dinophyceae	*	Adenoides	eludens	CCCM 683
AF276818	Dinophyceae	Dinophyceae	*	Akashiwo	sanguinea	ex Gymnodinium sanguineum

AY883006	Dinophyceae	Dinophyceae	*	Alexandrium	minutum	
AJ535383	Dinophyceae	Dinophyceae	*	Alexandrium	ostenfeldii	BAHME136
AB088330	Dinophyceae	Dinophyceae	*	Alexandrium	tamarense	ULW9903
AB088332	Dinophyceae	Dinophyceae	*	Alexandrium	tamarense	ULW9903
AJ535389	Dinophyceae	Dinophyceae	*	Alexandrium	taylori	AY4T
AB107845	Dinophyceae	Dinophyceae	*	Amphidinium	*	Y42
AF274250	Dinophyceae	Dinophyceae	*	Amphidinium	asymmetricum	CCCM 067
AF274251	Dinophyceae	Dinophyceae	*	Amphidinium	carterae	CCMP 1314
AF274254	Dinophyceae	Dinophyceae	*	Amphidinium	longum	
AF274255	Dinophyceae	Dinophyceae	*	Amphidinium	massartii	CCCM 439
AF274256	Dinophyceae	Dinophyceae	*	Amphidinium	semilunatum	
AF080096	Dinophyceae	Dinophyceae	*	Amyloodinium	ocellatum	
AF274265	Dinophyceae	Dinophyceae	*	Cachonina	niei	CCMP 447, ex Heterocapsa niei
DQ487192	Dinophyceae	Dinophyceae	*	Ceratium	*	HCB-2005
AF022192	Dinophyceae	Dinophyceae	*	Ceratium	tenue	MUCC248
DQ915169	Dinophyceae	Dinophyceae	*	Cochlodinium	*	isolate UJ
AY421782	Dinophyceae	Dinophyceae	*	Cochlodinium	polykrikoides	
AJ415509	Dinophyceae	Dinophyceae	*	Coolia	monotis	
DQ322643	Dinophyceae	Dinophyceae	*	Crypthecodinium	*	CAAE-CL2
M64245	Dinophyceae	Dinophyceae	*	Crypthecodinium	cohnii	
AB261512	Dinophyceae	Dinophyceae	*	Diplopsalis	lebourae	
AB261513	Dinophyceae	Dinophyceae	*	Diplopsalopsis	bomba	
AF231803	Dinophyceae	Dinophyceae	*	Durinskia	baltica	ex Peridinium
DQ388460	Dinophyceae	Dinophyceae	*	Exuviaella	cassubica	LB1596
AB195668	Dinophyceae	Dinophyceae	*	Galeidinium	rugatum	
AF274257	Dinophyceae	Dinophyceae	*	Glenodiniopsis	steinii	NIES 463
AF274258	Dinophyceae	Dinophyceae	*	Gonyaulax	cochlea	CCMP 1592
AJ833631	Dinophyceae	Dinophyceae	*	Gonyaulax	polygramma	
AF022155	Dinophyceae	Dinophyceae	*	Gonyaulax	spinifera	CCMP 409
AF052190	Dinophyceae	Dinophyceae	*	Gonyaulax	spinifera	GSTL1
AB261514	Dinophyceae	Dinophyceae	*	Gotoius	excentricus	
AF022193	Dinophyceae	Dinophyceae	*	Gymnodinium	catenatum	MUCC273
AF274262	Dinophyceae	Dinophyceae	*	Gyrodinium	galatheanum	CCCM 555
AF022197	Dinophyceae	Dinophyceae	*	Gyrodinium	impudicum	MUCC276D
AY421786	Dinophyceae	Dinophyceae	*	Gyrodinium	instriatum	
DQ388457	Dinophyceae	Dinophyceae	*	Gyrodinium	uncatenatum	CCMP 1310
AY443016	Dinophyceae	Dinophyceae	*	Hemidinium	nasutum	NIES 471
AF274267	Dinophyceae	Dinophyceae	*	Heterocapsa	rotundata	CCCM 680
AF009131	Dinophyceae	Dinophyceae	*	Karenia	mikimotoi	GMKUSJAP-CAWD05 ex Gymnodinium
AF172713	Dinophyceae	Dinophyceae	*	Karenia	mikimotoi	KT-77D ex Gyrodinium aureolum
AF231804	Dinophyceae	Dinophyceae	*	Kryptoperidinium	foliaceum	ex Peridinium
AF022199	Dinophyceae	Dinophyceae	*	Lepidodinium	viride	MUCC247D
AF274269	Dinophyceae	Dinophyceae	*	Lingulodinium	polyedrum	CCCM 202
AB273722	Dinophyceae	Dinophyceae	*	Oblea	acanthocysta	
AB273725	Dinophyceae	Dinophyceae	*	Oblea	torta	
AF244939	Dinophyceae	Dinophyceae	*	Ostreopsis	cf. ovata	OvPR04
AF274270	Dinophyceae	Dinophyceae	*	Pentapharsodinium	*	CCMP 771
AF022202	Dinophyceae	Dinophyceae	*	Peridinium	*	

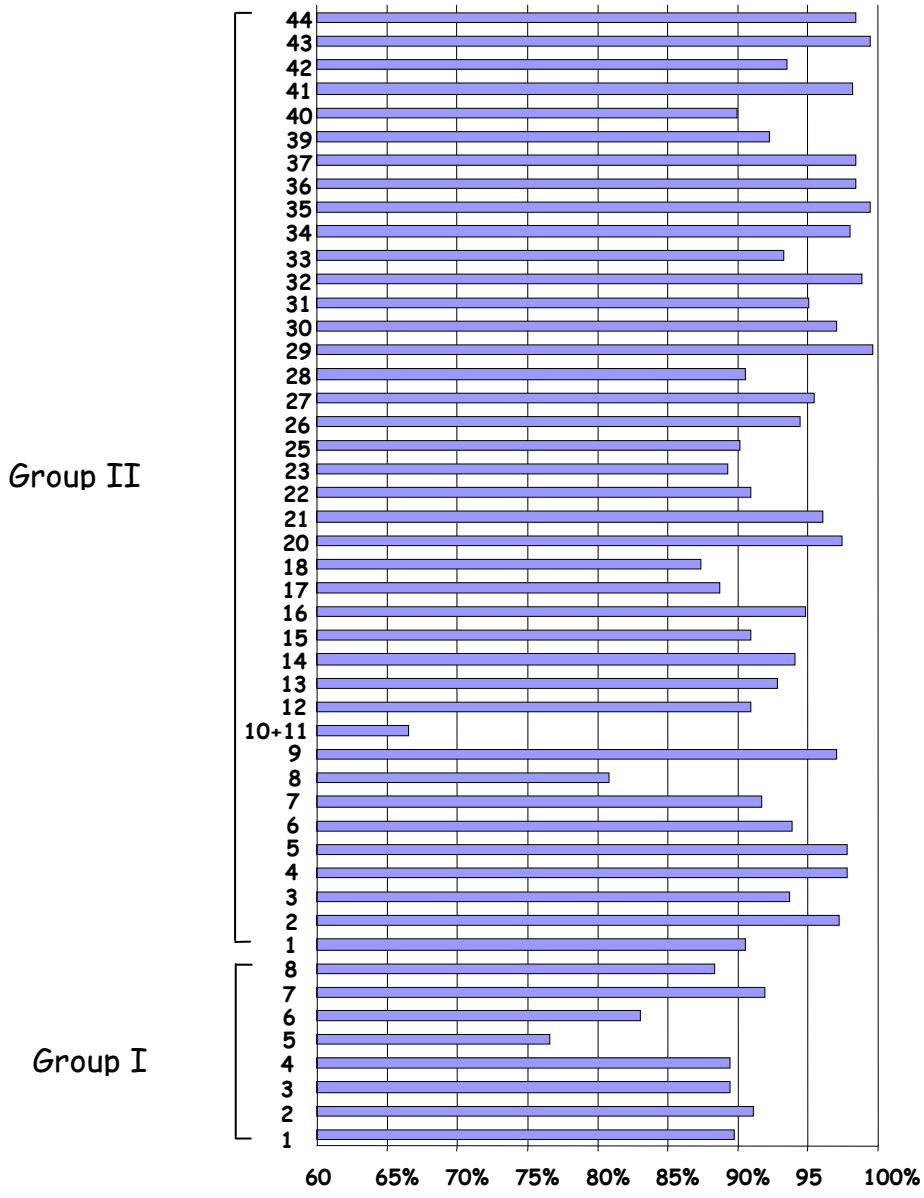
AY970653	Dinophyceae	Dinophyceae	*	Peridinium	aciculiferum	
AB246744	Dinophyceae	Dinophyceae	*	Peridinium	quinquecorne	
AF274271	Dinophyceae	Dinophyceae	*	Peridinium	umbonatum	UTEX LB 2255
AY421789	Dinophyceae	Dinophyceae	*	Pheopolykrikos	hartmanii	
Y16239	Dinophyceae	Dinophyceae	*	Prorocentrum	emarginatum	PREU2
AB189777	Dinophyceae	Dinophyceae	*	Prorocentrum	lima	USSP-F8
Y16236	Dinophyceae	Dinophyceae	*	Prorocentrum	maculosum	PPAN20
AY585526	Dinophyceae	Dinophyceae	*	Prorocentrum	micans	CCMP 1589
M14649	Dinophyceae	Dinophyceae	*	Prorocentrum	micans	LB113614
Y16233	Dinophyceae	Dinophyceae	*	Prorocentrum	panamensis	PPAN06
AF274273	Dinophyceae	Dinophyceae	*	Protoceratium	reticulatum	CCCM 535
DQ217789	Dinophyceae	Dinophyceae	*	Protoceratium	reticulatum	PRA0414
AB181882	Dinophyceae	Dinophyceae	*	Protoperidinium	abei	
AB284159	Dinophyceae	Dinophyceae	*	Protoperidinium	bipes	
AB255833	Dinophyceae	Dinophyceae	*	Protoperidinium	claudicans	
AB181886	Dinophyceae	Dinophyceae	*	Protoperidinium	conicum	
AY443020	Dinophyceae	Dinophyceae	*	Protoperidinium	conicum	
AB261515	Dinophyceae	Dinophyceae	*	Protoperidinium	crassipes	
AB181891	Dinophyceae	Dinophyceae	*	Protoperidinium	denticulatum	
AB255834	Dinophyceae	Dinophyceae	*	Protoperidinium	depressum	
AB275355	Dinophyceae	Dinophyceae	*	Protoperidinium	excentricum	
AB261517	Dinophyceae	Dinophyceae	*	Protoperidinium	punctulatum	
AB261519	Dinophyceae	Dinophyceae	*	Protoperidinium	thulesense	
AF274274	Dinophyceae	Dinophyceae	*	Pyrocystis	lunula	CCCM 517
AF022156	Dinophyceae	Dinophyceae	*	Pyrocystis	noctiluca	CCMP 732
AF521101	Dinophyceae	Dinophyceae	*	Roscoffia	capitata	
AB183671	Dinophyceae	Dinophyceae	*	Scrippsiella	*	MBIC11143
AY800130	Dinophyceae	Dinophyceae	*	Takayama	cf. pulchellum	TPXM
AY238478	Dinophyceae	Dinophyceae	*	Thecadinium	kofoidii	
AY238477	Dinophyceae	Dinophyceae	*	Thecadinium	mucosum	CCMP 1890
DQ388458	Dinophyceae	Dinophyceae	*	Thecadinium	mucosum	CCMP 1890 ex T. inclinatum
AF274278	Dinophyceae	Dinophyceae	*	Thoracosphaera	heimii	CCCM 670
AY443010	Dinophyceae	Dinophyceae	*	Togula	britannica	CCCM 081
AF274252	Dinophyceae	Dinophyceae	*	Togula	jolla	UTEX LB 1562 ex Amphidinium corpulentum
AY443025	Dinophyceae	Dinophyceae	*	Woloszynskia	leopoliensis	NIES 619
DQ317538	Dinophyceae	Dinophyceae	Blastodiniales	Blastodinium	navicula	
AB073119	Dinophyceae	Dinophyceae	Dinophysiales	Dinophysis	norvegica	
AF080097	Dinophyceae	Dinophyceae	Pfiesteriales	Cryptoperidiniopsis	brodyi	
AY456118	Dinophyceae	Dinophyceae	Pfiesteriales	Cryptoperidiniopsoid	*	CCMP1827
AF080098	Dinophyceae	Dinophyceae	Pfiesteriales	Pfiesteria	shumwayae	
L13716	Dinophyceae	Dinophyceae	Suessiales	*	*	ex Gloeodinium viscum
AB016577	Dinophyceae	Dinophyceae	Suessiales	Symbiodinium-clade A	*	PLTD-1
L13718	Dinophyceae	Dinophyceae	Suessiales	Symbiodinium-clade A	meandrinae	130
AB191418	Dinophyceae	Syndiniales	Dino-Group I-Clade 1	*	*	TAGIRI 10
AY665054	Dinophyceae	Syndiniales	Dino-Group I-Clade 1	*	*	SCM28 C151
EF172954	Dinophyceae	Syndiniales	Dino-Group I-Clade 1	*	*	SSRPD70
AB275022	Dinophyceae	Syndiniales	Dino-Group I-Clade 2	*	*	DSGM-22
AB275023	Dinophyceae	Syndiniales	Dino-Group I-Clade 2	*	*	DSGM-23

DQ916406	Dinophyceae	Syndiniales	Dino-Group I-Clade 2	*	*	
EF172831	Dinophyceae	Syndiniales	Dino-Group I-Clade 2	*	*	SSRPB45
AF530539	Dinophyceae	Syndiniales	Dino-Group I-Clade 3	*	*	AT4 47
EF172830	Dinophyceae	Syndiniales	Dino-Group I-Clade 3	*	*	SSRPB64
AB264776	Dinophyceae	Syndiniales	Dino-Group I-Clade 3	Ichthyodinium	chabelardi	
AB276368	Dinophyceae	Syndiniales	Dino-Group I-Clade 3	Ichthyodinium	chabelardi	PL
AB275027	Dinophyceae	Syndiniales	Dino-Group I-Clade 4	*	*	DSGM-27
AJ402354	Dinophyceae	Syndiniales	Dino-Group I-Clade 4	*	*	OLI011_75m_11
AB295040	Dinophyceae	Syndiniales	Dino-Group I-Clade 4	Duboscquella	*	
AJ402327	Dinophyceae	Syndiniales	Dino-Group I-Clade 5	*	*	OLI011_75m_1(1)
AY665056	Dinophyceae	Syndiniales	Dino-Group I-Clade 5	*	*	SCM37 C30
EF172944	Dinophyceae	Syndiniales	Dino-Group I-Clade 5	*	*	SSRPD60
EF172950	Dinophyceae	Syndiniales	Dino-Group I-Clade 5	*	*	SSRPD66
EF172986	Dinophyceae	Syndiniales	Dino-Group I-Clade 5	*	*	SSRPD84
DQ145114	Dinophyceae	Syndiniales	Dino-Group I-Clade 5	*	*	OLI011_75m_86
EU780610	Dinophyceae	Syndiniales	Dino-Group I-Clade 5	*	*	AMT15_15B_5
EU780622	Dinophyceae	Syndiniales	Dino-Group I-Clade 5	*	*	AMT15_33B_14
AY665100	Dinophyceae	Syndiniales	Dino-Group I-Clade 6	*	*	SCM27 C13
AY665102	Dinophyceae	Syndiniales	Dino-Group I-Clade 6	*	*	SCM15 C4
AY664999	Dinophyceae	Syndiniales	Dino-Group I-Clade 7	*	*	SCM37 C10
EU793218	Dinophyceae	Syndiniales	Dino-Group I-Clade 8	*	*	E3_25m_129
AB275018	Dinophyceae	Syndiniales	Dino-Group II	*	*	DSGM-18
AY129049	Dinophyceae	Syndiniales	Dino-Group II	*	*	UEPAC 42p4
AY129050	Dinophyceae	Syndiniales	Dino-Group II	*	*	UEPAC 43p4
AY129052	Dinophyceae	Syndiniales	Dino-Group II	*	*	UEPAC Ep5
EF172994	Dinophyceae	Syndiniales	Dino-Group II	*	*	SSRPD93
DQ145103	Dinophyceae	Syndiniales	Dino-Group II	*	*	OLI011_75m_17(5)
AY208894	Dinophyceae	Syndiniales	Dino-Group II-Clade 1	Amoebophrya	ceratii	
AY664990	Dinophyceae	Syndiniales	Dino-Group II-Clade 10 and 11	*	*	SCM37 C21
EU793925	Dinophyceae	Syndiniales	Dino-Group II-Clade 10 and 11	*	*	EM_5m_179
EU792913	Dinophyceae	Syndiniales	Dino-Group II-Clade 10 and 11	*	*	OLI011_75m_81
EU780600	Dinophyceae	Syndiniales	Dino-Group II-Clade 10 and 11	*	*	AMT15_1B_32
EU780611	Dinophyceae	Syndiniales	Dino-Group II-Clade 10 and 11	*	*	AMT15_15B_7
DQ186532	Dinophyceae	Syndiniales	Dino-Group II-Clade 12	*	*	RA010412-5
EF172942	Dinophyceae	Syndiniales	Dino-Group II-Clade 13	*	*	SSRPD58
AY129045	Dinophyceae	Syndiniales	Dino-Group II-Clade 14	*	*	UEPAC Bp4
AB275017	Dinophyceae	Syndiniales	Dino-Group II-Clade 15	*	*	DSGM-17
AF290079	Dinophyceae	Syndiniales	Dino-Group II-Clade 15	*	*	DH148-EKD14
AF530532	Dinophyceae	Syndiniales	Dino-Group II-Clade 15	*	*	AT4 21
AJ402348	Dinophyceae	Syndiniales	Dino-Group II-Clade 16	*	*	OLI011_75m_9
EF172958	Dinophyceae	Syndiniales	Dino-Group II-Clade 16	*	*	SSRPD74
EU780613	Dinophyceae	Syndiniales	Dino-Group II-Clade 16	*	*	AMT15_15B_9
DQ186534	Dinophyceae	Syndiniales	Dino-Group II-Clade 18	*	*	RA010613-126
AF472555	Dinophyceae	Syndiniales	Dino-Group II-Clade 2	Amoebophrya	ceratii	
AF290069	Dinophyceae	Syndiniales	Dino-Group II-Clade 20	*	*	DH147-EKD3
EF172923	Dinophyceae	Syndiniales	Dino-Group II-Clade 20	*	*	SSRPC86
EU793224	Dinophyceae	Syndiniales	Dino-Group II-Clade 21	*	*	E3_25m_97
EF173005	Dinophyceae	Syndiniales	Dino-Group II-Clade 22	*	*	H02N5
EF173009	Dinophyceae	Syndiniales	Dino-Group II-Clade 22	*	*	Q2C03N5

EU793410	Dinophyceae	Syndiniales	Dino-Group II-Clade 22	*	*	E5_55m_3
AB252763	Dinophyceae	Syndiniales	Dino-Group II-Clade 23	*	*	NAMAKO-23
AF290077	Dinophyceae	Syndiniales	Dino-Group II-Clade 25	*	*	DH148-EKD27
EU793703	Dinophyceae	Syndiniales	Dino-Group II-Clade 26	*	*	ED_50m_219
EU780626	Dinophyceae	Syndiniales	Dino-Group II-Clade 26	*	*	AMT15_33B_18
EF172953	Dinophyceae	Syndiniales	Dino-Group II-Clade 27	*	*	SSRPD69
EU793707	Dinophyceae	Syndiniales	Dino-Group II-Clade 27	*	*	ED_50m_225
AB252764	Dinophyceae	Syndiniales	Dino-Group II-Clade 28	*	*	NAMAKO-24
EF172800	Dinophyceae	Syndiniales	Dino-Group II-Clade 29	*	*	SSRPB13
AJ402326	Dinophyceae	Syndiniales	Dino-Group II-Clade 3	*	*	OLI011_75m_1-1(5)
AY129038	Dinophyceae	Syndiniales	Dino-Group II-Clade 3	*	*	UEPAC Ep3
AF069516	Dinophyceae	Syndiniales	Dino-Group II-Clade 3	Amoebophrya	ceratii	
AJ402344	Dinophyceae	Syndiniales	Dino-Group II-Clade 30	*	*	OLI011_75m_55
EF173003	Dinophyceae	Syndiniales	Dino-Group II-Clade 31	*	*	D05N5
DQ145113	Dinophyceae	Syndiniales	Dino-Group II-Clade 31	*	*	OLI011_75m_70
AY129037	Dinophyceae	Syndiniales	Dino-Group II-Clade 32	*	*	UEPAC Hp3
AY775285	Dinophyceae	Syndiniales	Dino-Group II-Clade 33	Amoebophrya	*	GS0209a
AF472554	Dinophyceae	Syndiniales	Dino-Group II-Clade 4	Amoebophrya	ceratii	
DQ186531	Dinophyceae	Syndiniales	Dino-Group II-Clade 5	*	*	RA010412-29
EF173017	Dinophyceae	Syndiniales	Dino-Group II-Clade 6	*	*	Q2H03N5
EF172798	Dinophyceae	Syndiniales	Dino-Group II-Clade 7	*	*	SSRPB41
EU793837	Dinophyceae	Syndiniales	Dino-Group II-Clade 7	*	*	EM_50m_108
DQ186533	Dinophyceae	Syndiniales	Dino-Group II-Clade 8	*	*	RA010412-70
AB275016	Dinophyceae	Syndiniales	Dino-Group II-Clade 9	*	*	DSGM-16
AJ402349	Dinophyceae	Syndiniales	Dino-Group III	*	*	OLI011_75m_ C(5)
AY664993	Dinophyceae	Syndiniales	Dino-Group III	*	*	SCM15 C23
EU780604	Dinophyceae	Syndiniales	Dino-Group III	*	*	AMT15_1B_36
EU780607	Dinophyceae	Syndiniales	Dino-Group III	*	*	AMT15_1B_40
EF172791	Dinophyceae	Syndiniales	Dino-Group IV	*	*	SSRPB76
AF286023	Dinophyceae	Syndiniales	Dino-Group IV	Hematodinium	*	
DQ504327	Dinophyceae	Syndiniales	Dino-Group IV	Hematodinium	*	LC22_5EP_44
DQ504356	Dinophyceae	Syndiniales	Dino-Group IV	Hematodinium	*	LC22_5EP_37
DQ925237	Dinophyceae	Syndiniales	Dino-Group IV	Hematodinium	*	CsH1
EF065717	Dinophyceae	Syndiniales	Dino-Group IV	Hematodinium	perezi	
EF065718	Dinophyceae	Syndiniales	Dino-Group IV	Hematodinium	perezi	
DQ146406	Dinophyceae	Syndiniales	Dino-Group IV	Syndinium	*	
DQ146403	Dinophyceae	Syndiniales	Dino-Group IV	Syndinium	turbo	
DQ146404	Dinophyceae	Syndiniales	Dino-Group IV	Syndinium	turbo	
DQ146405	Dinophyceae	Syndiniales	Dino-Group IV	Syndinium	turbo	
EU793265	Dinophyceae	Syndiniales	Dino-Group V	*	*	E5_25m_10
EU793379	Dinophyceae	Syndiniales	Dino-Group V	*	*	E5_25m_93
EU793392	Dinophyceae	Syndiniales	Dino-Group V	*	*	E5_55m_20
EU780615	Dinophyceae	Syndiniales	Dino-Group V	*	*	AMT15_15
AF530536	Perkinsea	Perkinsida	*	*	*	AT4 98
AY919720	Perkinsea	Perkinsida	*	*	*	LG 10-12
AY919735	Perkinsea	Perkinsida	*	*	*	LG 15-08
AY919809	Perkinsea	Perkinsida	*	*	*	LG 36-11
DQ103802	Perkinsea	Perkinsida	*	*	*	Mariager_Fjord_2_18C03
DQ244020	Perkinsea	Perkinsida	*	*	*	PAB11AU2004

DQ244021	Perkinsea	Perkinsida	*	*	*	PAD10AU2004
DQ244034	Perkinsea	Perkinsida	*	*	*	PAG2AU2004
DQ244035	Perkinsea	Perkinsida	*	*	*	PAD7AU2004
DQ244037	Perkinsea	Perkinsida	*	*	*	PAF7AU2004
DQ244038	Perkinsea	Perkinsida	*	*	*	PAB5AU2004
EF675616	Perkinsea	Perkinsida	*	*	*	Rana sphenocephala pathogen
AF133909	Perkinsea	Perkinsida	*	Parvilucifera	infectans	
AY305326	Perkinsea	Perkinsida	*	Perkinsus	chesapeaki	ATCC 50807
AF042708	Perkinsea	Perkinsida	*	Perkinsus	marinus	isolate-H49
AF126013	Perkinsea	Perkinsida	*	Perkinsus	marinus	
AF324218	Perkinsea	Perkinsida	*	Perkinsus	marinus	

Supplementary Material 2. Mean sequence identities within the different clades belonging to Syndiniales Groups I and II.



Supplementary Material 3. Primer sequences used or cited in this study.

Primer name	Specificity	Sequence (5'-3')	Reference
Euk328f	Eukaryotes	ACCTGGTTGATCCTGCCAG	(Moon-van der Staay et al., 2001)
Euk329r	Eukaryotes	TGATCCTTCYGCAGGTTCAC	(Moon-van der Staay et al., 2001)
EukA (forward)	Eukaryotes	AACCTGGTTGATCCTGCCAGT	(Medlin et al., 1988)
EukB (reverse)	Eukaryotes	TGATCCTTCTGCAGGTTCACCTAC	(Medlin et al., 1988)
EukB' (reverse)	Eukaryotes	GATCCTTCTGCAGGTTCACCTAC	(Countway et al., 2007)
Euk528f	Eukaryotes	GCGGTAATTCCAGCTCCAA	(Helwood et al., 1985)
EK-1A (forward)	Eukaryotes	CTGGTTGATCCTGCCAG	(Díez et al., 2001)
516-GC (reverse)	Eukaryotes	ACCAGACTTGCCCTCC (+GC clamp)	(Díez et al., 2001)
CHLO02 (reverse)	Chlorophyta	CTTCGAGCCCCCAACTTTC	(Zhu et al., 2005)
ALV01 (forward)	Syndiniales Group II	AGAGTGTTCACGGCAGGC	This study

Supplementary Material 4. Taxonomy of Syndiniales (= Syndinida) and their hosts modified from Loeblich III (1976)

Family/Genus	Species (Type species in dark)	Host	Marine/ Freshwater
Amoebophryaceae/Amoebophryidae Cachon 1964			
Amoebophrya Cachon (1964) (ex *Hyalosaccus* Koeppen) **= Group II**	*A. ceratii* Cachon (1964)	Dinoflagellates	Marine
	A. acanthometrae Cachon (Borgert, 1897)	Acantharia (Radiolaria)	Marine
	A. sticholonchae Cachon (Koeppen, 1894)	*Sticholonche* (Radiolaria)	Marine
	A. grassei Cachon (1964)	*Oodinium poucheti* (Dinoflagellate) ectoparasite of *Oikopleura*	Marine
	A. leptodisci Cachon (1964)	*Pratjetella medusoides* (Noctilucales, Dinoflagellate)	Marine
	A. rosei Cachon (1964)	Ciliates parasites of siphonophores (Cnidaria) and *Sagitta* (Chaetognatha)	Marine
	A. tintinni Cachon (1964)	*Xystonella lohmanni* (Ciliate)	
Duboscquellaceae/Duboscquellidae Cachon 1964			
Duboscquella Chatton (1920)	***D. anisospora*** Grassé (1952) Syn: *D. tintinnicola* (Lohmann) (Chatton, 1920)	*Favella ehrenbergi* (Ciliate)	Marine
	Duboscquella spp. (Harada et al., 2007) **= Group I Clade 4**	*Favella ehrenbergi* (Ciliate)	Marine
	D. aspida Cachon (1964) Syn: *D. tintinnicola* (Lohmann) (Chatton, 1920)	Various species of tintinnids (Ciliates) *Favella ehrenbergi, Coxliella lacinosa, Tintinnopsis campanula, Tintinnus fraknoii*	Marine
	D. cnemata Cachon (1964) Syn: *D. tintinnicola* (Lohmann) (Chatton, 1920)	*Favella ehrenbergi* (Ciliate)	Marine
	D. cachoni Coats and Heisler (Coats, 1988)	*Eutintinnus pectinis* (Ciliate)	Marine
	D. caryophaga Cachon (1964)	*Strombidium, Strombilidium*, and *Prorodon*	Marine
	D. melo Cachon (1964)	*Noctiluca miliaris* (dinoflagellate)	Marine
	D. nucleocola Cachon (1964)	*Leptodiscus mesudoides* (dinoflagellate)	Marine
Dogelodinium Loeblich and Loeblich (1966)(Syn. *Collinella* Cachon 1964)	***D. ovoides*** (Cachon, 1964) Loeblich and Loeblich (1966)	Acantharia	Marine
Duboscquodinium Grassé (Grassé, 1952)	***D. collini*** Grassé (1952)	*Tintinnus fraknoii* (ciliate)	Marine
	D. kofoidi Grassé (1952)	*Codonella campanula* (ciliate)	Marine
Keppenodinium Loeblich and Loeblich (1966)(Syn. *Hollandella* Cachon 1964)	***K. mycetoides*** (Cachon, 1964) Loeblich and Loeblich (1966)	*Spongosphaera* (Radiolaria, Polycystinea, Spumellarida)	Marine
	K. lobata (Cachon, 1964) Loeblich and Loeblich	*Plegmosphaera* (Radiolaria, Polycystinea, Spumellarida)	Marine

	(1966)		
	K. piriformis (Cachon, 1964) Loeblich and Loeblich (1966) Syn. *Duboscquella* sp. from Hollande and Enjumet 1960)	*Actinosphaera* (Radiolaria, Polycystinea, Spumellarida)	Marine
Sphaeriparaceae/Sphaeriparidae			
Sphaeripara Loeblich and Loeblich (1966) (Syn. *Lohmania, Lohmanella, Neresheimeria*)	***S. catenata*** (Neresheimer, Cachon and Cachon-Enjumet, 1964) (Syn. *S. paradoxa* Neresheimer) Loeblich and Loeblich (1966)	*Fritillaria pellucida* (Appendicularia)	Marine
Atlanticellodinium Cachon and Cachon (1965)	***A. tregouboffi*** Cachon and Cachon (1965)	*Planktonetta atlantica* (Cercozoa, Phaeodaria)	Marine
Syndiniaceae/Syndinidae Chatton 1910			
Syndinium Chatton (1910) (Syn : *Atelodinium*, *Synhemidinium*, *Cochlosyndinium*)	***S. turbo*** Chatton (1910) (Syn : *S. minutum*, *Synhemidinium rostratum*) **= Group IV**	Copepods (*Paracalanus parvus, Calanus finmarchius, Clausocalanus furcatus, Clausocalanus arcuicronis, Oithona similis, Corycaeus venustrus*)	Marine
	S. corycaei Chatton 1922	*Corycaeus geisbrechti* (=*C. venustus*)	Marine
	S. gammari (Manier et al., 1971)	*Gammarus locustra* Genus Also detected in *Calanus finmarchicus*, *Clausocalanus arcuicornis*, *Oithona helgolandica*, *Eucalanus pseudattenuatas*, *Euchirella pulchra*, *Oncaea venusta* typica, and eggs of *Pandalus borealis*	Marine, see review from Shields 1994
Merodinium* Chatton (1923) (ex *Syndinium*)	***M. brandti*** Chatton (1923)	*Collozoum inerme* (Radiolaria, Polycystinea, Collodaria)	Marine
	M. insidiosum Chatton (1923)	*Collozoum pelagicum* (Radiolaria, Polycystinea, Collodaria)	Marine
	M. globiforme Hollande & Enjumet (1953)	*Collozoum fulvum* (Radiolaria, Polycystinea, Collodaria)	Marine
	M. belari Hollande & Enjumet (1953) probable syn. *S. vernale* Hovasse (1923)	*Collozoum fulvum* (Radiolaria, Polycystinea, Collodaria)	Marine
	M. mendax Chatton (1923)	*Myxosphaera coerulea* (Radiolaria)	Marine
	M. dolosum Chatton (1923)	*Sphaerozoum punctatum* (Radiolaria, Polycystinea)	Marine
	M. chattoni Hovasse & Brown (1953)	*Sphaerozoum punctatum* (Radiolaria, Polycystinea, Collodaria)	Marine
	M. astutum Chatton (1923)	*Sphaerozoum acuferum* (Radiolaria, Polycystinea, Collodaria)	Marine
	M. breve Hovasse & Brown (1953)	*Sphaerozoum punctatum* (Radiolaria, Polycystinea, Collodaria)	Marine
Solenodinium Chatton (1923)	***S. fallax*** Chatton (1923)	*Thalassicolla spumida* (Radiolaria, Polycystinea)	Marine
	S. leptotænia Hovasse & Brown (1953)	*Thalassicolla nucleata* (Radiolaria, Polycystinea)	Marine
	S. densum Hovasse & Brown (1953)	*Thalassicola pellucida* (Radiolaria, Polycystinea)	Marine
Ichthyodinium Hollande and	***I. chabelardi*** Hollande &	Fish eggs (*Sardina*)	Marine

Cachon (1952) **= Group I clade 3**	Cachon (1952)		
Hematodinium Chatton & Poisson (1931)	***H. perezi*** Chatton and Poisson (1931) **= Group IV**	*Carcinus maenas* and *Liocarcinus depurator*	Marine
	H. australis Hudson & Shields (Shields, 1994)	*Portunus pelagicus*	Marine
	H. cf perezi (Hudson and Adlard, 1996)	*Callinectes sapidus*	Marine
	Hematodinium sp. (Hudson and Adlard, 1996)	*Nephros norvegicus*	Marine
	Hematodinium sp. (Hudson and Adlard, 1996)	*Chionoecetes bairdi* and *C. opilio*	Marine
Coccidiniaceae/Coccidinidae Chatton et Biecheler 1934			
***Coccidinium* (1934)**	***C. duboscqui*** Chatton et Biecheler (1934)	*Peridinium balticum* (dinoflagellate)	Marine
	C. legeri Chatton et Biecheler (1934)	*Glenodinium sociale* (dinoflagellate)	Marine
	C. punctuatum Chatton et Biecheler (1936)	*Coolia monotis* (dinoflagellate)	Marine
	C. mesnili Chatton et Biecheler (1936)	*Cryptoperidinium foliaceum* (dinoflagellate)	Marine
Uncertain taxa			
Trypanodinium Chatton (1912). This genus is only known by its dinospores.	***T. ovicola*** Chatton (1912)	Copepod eggs (*Oithona* spp., *Clytemnestra* spp., *Oncaea media*)	Marine
Syndinium ?	*S. borgerti* Hollande, Enjumet, and Manciet (Cachon-Enjumet, 1961)	*Aulacantha scolymantha* (Cercozoa, Phaeodarea)	Marine
***Syndinium*?**	*S. caryophagum* Cachon-Enjumet (1961)	*Phaeosphaera nucleoelongata* and *P. pigmaea* (Cercozoa, Phaeodarea)	Marine
***Syndinium*?**	*S. oikopleurae* Hollande & Fenaux (Hollande, 1974)	*Oikopleura albicans* (Appendicularia)	Marine
Unknown species = Group I clade 2	Sequence DQ916410 (Dolven et al., 2007)	*Challengeron diodon* (Cercozoa, Phaeodarea)	Marine

* In 1923, Chatton placed all *Syndinium* infecting Radiolarians inside a new genus, *Merodinium*. In 1953, Hollande and Enjumet considered that all species localized inside the endoplasm of the polycystine capsule belong to the genus *Syndinium*, while the genus *Merodinium* must be restricted to species localized outside the capsule. Nevertheless, in 1953, Hollande finally considered that all these species belong to the same genus, and conserved the older one, the genus *Syndinium*. In a more recent review, Coats (1999) followed the Chatton nomenclature. The taxonomic position of *S. borgerti*, a parasite of *Phaeodarea* (Cercozoa), is still uncertain. The different species recognized inside the genus *Merodinium* is also controversial, mostly based upon their host identity rather than clear morphological features.

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2.2 Dynamique et spécificité du couplage entre Amoebophryidae et dinoflagellés hôtes, en baie de Penzé (Finistère)

Contexte et objectifs

L'analyse des séquences environnementales provenant de multiples endroits du globe suggère fortement une synonymie entre le terme « Alvéolés du groupe II » et la famille des Amoebophryidae décrite par Cachon en 1964. Cette famille contient un seul genre, *Amoebophrya*, capable d'infecter un très grand nombre d'espèces de dinoflagellés. D'autre part, il est apparu que les séquences environnementales appartenant à ce groupe étaient particulièrement abondantes au sein des librairies génétiques construites à partir d'échantillons côtiers (voir section 2.1). D'après la littérature, *Amoebophrya* est suspectée d'être lié au déclin de certaines efflorescences de dinoflagellés, qu'ils soient toxiques ou non [Coats *et al.*, 1996, Park *et al.*, 2004]. Néanmoins, l'impact d'un tel parasite sur les populations hôtes ainsi que sa spécificité sont encore controversés (voir section 1.2.2). Nous avons donc choisi de faire une étude *in situ* de ces deux caractéristiques.
Notre choix sur le lieu d'étude s'est très vite porté sur l'estuaire de la Penzé, où *Alexandrium minutum*, un dinoflagellé toxique, y produit des efflorescences de façon récurrentes. Cet estuaire se situe au Nord-Ouest de la ville de Morlaix (Finistère Nord) (Figure 2.1).

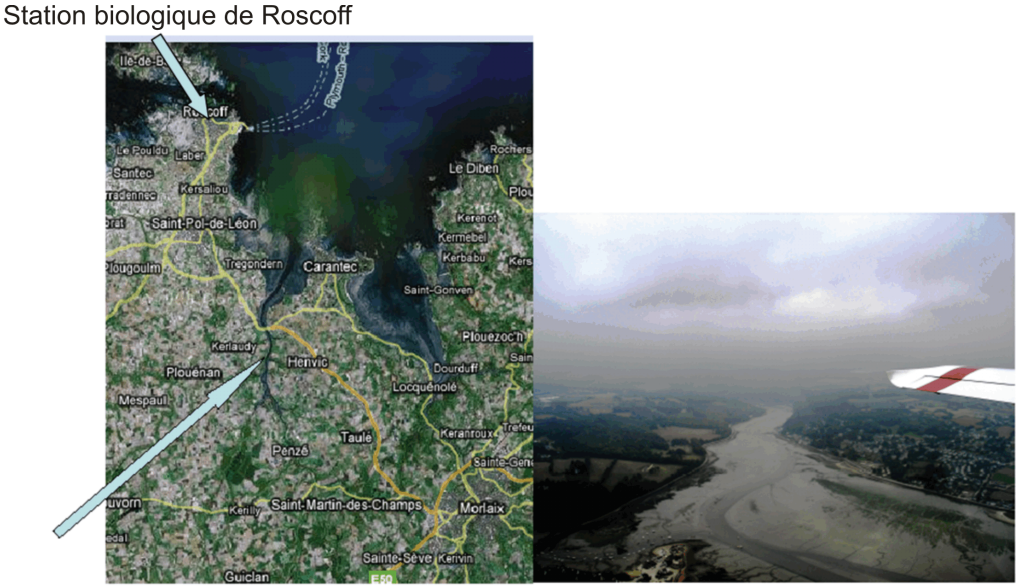

FIG. 2.1 – A gauche localisation de la rivière La Penzé (Google Earth), à droite photo vue d'avion de la rivière (Chambouvet, 2008)

Cet écosystème est caractérisé par des eaux peu profondes, soumises par conséquent à une forte influence marine. Les estuaires bretons sont un exemple malheureusement classique de zone où l'épandage (fertilisant azoté) est couramment utilisé en agriculture, aboutissant à l'en-

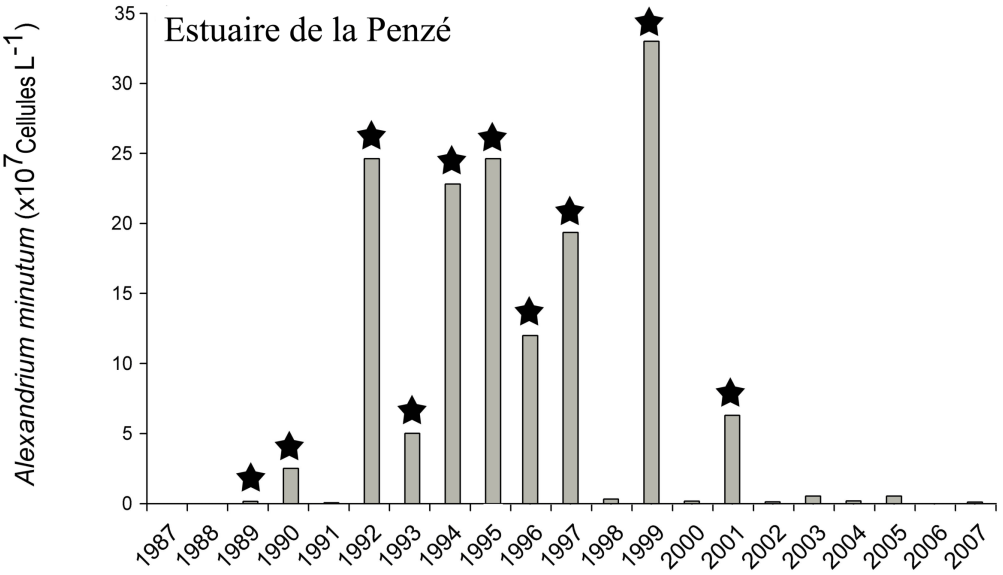

FIG. 2.2 – Concentration d'*Alexandrium minutum* entre 1994 et 2007 dans la baie de Penzé. Les étoiles représentent les fermetures des zones de conchyliculture dues à la détection de toxines dans les bivalves (>80 μg eq. STX/ 100 g de chair).

richissement des rivières en substances azotées. Le contenu en NO_3 dans la rivière peut dépasser les 500 à 600 μmol.L^{-1} avec un ratio N :P de 65 en hiver et de plus de 300 en été [Wafar, 1981]. L'excès d'azote et de phosphate permet une croissance importante du phytoplancton, et parfois la croissance de micro-algues toxiques telles que *Alexandrium minutum*. Cette dernière a été détectée pour la première fois dans la Baie de la Vilaine (Morbihan) en 1995 puis au niveau de l'Aber Wrach et de l'Aber-Benoit (Finistère Nord) lors d'une efflorescence spectaculaire (trois millions de cellules par litre) en 1988 [Desalos, 1999]. Ce dinoflagellé est capable de produire des toxines paralysantes (PSP) qui peuvent être concentrées par les bivalves filtreurs tels que les moules et les huîtres. Entre 1988 et 1997, cette espèce a été responsable de plusieurs efflorescences toxiques qui se sont traduites par l'arrêt plus ou moins long de la production conchylicole (la concentration seuil étant de 80 μg eq. STX pour 100 g de chair). Pratiquement 10 ans après les premières efflorescences, cette espèce est toujours présente mais à des concentrations cellulaires moins importantes (Figure 2.2). Aucune explication n'avait encore été apportée pour expliquer ce phénomène, sachant que la charge en nutriment de la rivière est toujours aussi élevée.

Dans le cadre de cette thèse, nous avons réalisé un suivi de l'évolution des populations hôtes potentielles (les dinoflagellés) durant trois années consécutives en début d'été. En parallèle, nous avons recherché la présence de parasites appartenant aux Amoebophryidae dans le but de décrire la dynamique parasitaire. Enfin, nous avons abordé la question de la spécificité de ces parasites dans un milieu complexe.
Avant mon arrivée, les échantillonnages de 2004 et 2005 avaient été réalisés. J'ai réalisé l'échantillonnage de 2006, participé à l'acquisition de tous les paramètres biologiques (dynamiques hôtes et parasites, évaluation de la diversité génétique) sur les trois années d'échantillonnage, ainsi qu'à l'élaboration des sondes spécifiques. L'acquisition des paramètres a été réalisée en collaboration avec Pascal Morin (Station biologique de Roscoff) pour les sels nutritifs et Dominique Marie (Station biologique de Roscoff) pour la quantification des bactéries et virus.

Résumé

L'estuaire de la Penzé (Bretagne Nord, France) est un écosystème propice à la croissance des dinoflagellés dont le dinoflagellé toxique, *Alexandrium minutum* Halim. Ce dernier a produit des efflorescences, depuis 1998, durant 10 années consécutives. Dans cet écosystème, des successions rapides d'espèces différentes de dinoflagellés ont pu être observées, l'espèce majoritaire étant différente chaque semaine. Nous avons émis l'hypothèse que cette dynamique était reliée à la présence de parasites ubiquistes du genre Amoebophryidae (Syndiniales, Groupe II).

Les eaux de cet estuaire sont également relativement riches en nutriment, calmes et stratifiées durant les périodes de mortes eaux en été. Profitant de ces conditions favorables, la microalgue toxique *Alexandrium minutum* s'y est installée durablement depuis la fin des années 1980, où elle a provoqué des marées rouges toxiques durant environ une dizaine d'années consécutives. Depuis 2001, la force de ces floraisons a nettement diminué, alors que les conditions environnementales semblaient toujours aussi favorables. Un contrôle biologique, de type parasite, a donc été suspecté. En parallèle, l'importance écologique des Syndiniales, un groupe de parasitoïdes d'un très grand nombre d'espèces planctoniques, en particulier de dinoflagellés, a été dévoilée par les travaux de diversité génétique environnementale.

Nous avons testé l'hypothèse d'une régulation de la microalgue toxique *A. minutum* en baie de Penzé par ce type de parasite, en ciblant notre étude sur la famille des Amoebophryidae (Syndiniales). Pour cela, nous avons échantillonné les eaux de surface de la rivière de Penzé durant trois années consécutives au début de l'été (de 2004 à 2006). Par FISH-TSA, nous avons retrouvé ce groupe de parasites dans tous nos échantillons. Leur présence dans la plus petite fraction de taille (dinospores, <3 μm) a rapidement pu être mise en relation avec le déclin des

différentes espèces de dinoflagellés présentes dans l'écosystème incluant *Alexandrium minutum*. L'analyse génétique de la petite sous-unité du ribosome (18S), durant ces trois années, a permis la détection de l'alternance de 4 principaux clades génétiques appartenant aux Amoebophryidae (1, 2, 3 et 14). Nous avons ensuite démontré grâce à l'élaboration de sondes spécifiques de chaque clade, la spécificité d'infection de ce parasitoïde. En effet, certains des dinoflagellés étaient infectés par des parasites génétiquement distincts et chaque clade de parasite infectait la même espèce de dinoflagellés, année après année. Leur capacité d'infecter et de contrôler une espèce toxique, suspectée d'être invasive telle qu'*A. minutum*, soulève d'intéressantes questions sur l'adaptation d'un parasite face à un nouvel hôte potentiel. Ainsi, le dérèglement d'un tel couplage hôte-parasite pourrait être à l'origine des récentes augmentations des efflorescences toxiques (HAB) dans des écosystèmes côtiers.

ulates pancreatic islet mass. Hepatic ERK activation is likely to play an important role in compensatory islet hyperplasia, although it is not yet clear how ERK signaling affects the neuronal pathway. The therapeutic effects we observed in two mouse models of insulin-deficient diabetes are especially noteworthy. Type 1 diabetes mellitus is characterized by progressive loss of pancreatic β cells, leading to a life-long insulin dependency. Recently, it was reported that β cell mass is also decreased in type 2 diabetes (*25*). Although substantial progress has been made with therapies that are based on transplantation of pancreatic islets (*26*), immune rejection and donor supply are still major challenges. In this context, therapeutic manipulation of the interorgan signaling mechanism described here may merit investigation as a potential strategy for regeneration of a patient's own β cells. Our results may open a new paradigm for regenerative medicine: regeneration of damaged tissues by targeting of interorgan communication systems, especially neural pathways.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/322/5905/1250/DC1
Materials and Methods
Figs. S1 to S9
References

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Control of Toxic Marine Dinoflagellate Blooms by Serial Parasitic Killers

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The marine dinoflagellates commonly responsible for toxic red tides are parasitized by other dinoflagellate species. Using culture-independent environmental ribosomal RNA sequences and fluorescence markers, we identified host-specific infections among several species. Each parasitoid produces 60 to 400 offspring, leading to extraordinarily rapid control of the host's population. During 3 consecutive years of observation in a natural estuary, all dinoflagellates observed were chronically infected, and a given host species was infected by a single genetically distinct parasite year after year. Our observations in natural ecosystems suggest that although bloom-forming dinoflagellates may escape control by grazing organisms, they eventually succumb to parasite attack.

Although photosynthetic dinoflagellates are important primary producers in marine ecosystems, some bloom-forming species produce toxins that can cause illness and even death in humans (*1*). These harmful algal blooming (HAB) species are particularly prevalent in warm, stratified, and nutrient-enriched coastal waters (*2*, *3*). Documented HAB events have increased substantially during recent decades as a result of extensive coastal eutrophication and, possibly, global climate change (*4*).

In 1968, Taylor proposed using specific dinoflagellate parasites, such as the Syndiniales *Amoebophrya* spp. (*5*), as biological control agents for HAB organisms. This idea was rejected because of the apparent lack of specificity of the parasites; however, the homogeneous morphology of these parasites masks extensive genetic diversity (*6*). Recently, the widespread existence of *Amoebophrya* spp. was "rediscovered" by culture-independent methods, and they were renamed "novel alveolate group II" (*7*–*9*). This eukaryotic lineage frequently forms 10 to 50% of sequences retrieved within coastal environmental clone libraries (*10*, *11*). Indeed, up to 44 distinct clusters have been detected, with extensive intraclade genetic diversity (*12*); the genetic diversity of the parasites appears to be comparable to the species richness of their hosts.

We sampled a marine coastal estuary (the Penzé River, northern Brittany, France) for 3 consecutive years (2004 to 2006), using catalyzed reporter deposition fluorescent in situ hybridization (CARD-FISH; tables S1 and S2) with probes specifically designed to detect group II alveolates. Our aim was to examine how the abundance and diversity of the parasites influenced their host populations in natural environments. In May and June of each year, we observed a rapid succession of four major species of photosynthetic

Table 1. Specificity of Syndiniales group II in the Penzé estuary in 2005 and 2006. Prevalences (percentage of infected cells) when a general oligonucleotide probe (ALV01) and clade-specific probes were used are shown (results for clades 1, 2, and 14; for description of clades see Fig. 3). Observations of a mature trophont inside the host cell are indicated by an asterisk. ND, not done. Numbers in parentheses show the percentage of the signal obtained when the general probe was used, explained by the clade-specific probes.

Host species	Dates (day/month/year)	Syndiniales group II, all clades	Syndiniales group II, clade 1	Syndiniales group II, clade 2	Syndiniales group II, clade 14
H. rotundata	03/06/2005	26*	26* (100%)	ND	ND
	29/05/2006	29*	23* (79%)	0	2 (<1%)
S. trochoidea	14/06/2005	23*	ND	11* (48%)	ND
	16/06/2006	33*	0	18* (55%)	3 (<1%)
	18/06/2006	26*	0	29* (>100%)	9 (3%)
A. minutum	14/06/2005	40*	0	0	0
	22/06/2006	19*	6 (3%)	0	0
H. triquetra	20/06/2005	10*	ND	0	11* (>100%)
	22/06/2006	14*	0	0	14* (100%)

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dinoflagellates (the dominant species changed every week; Fig. 1), namely *Heterocapsa rotundata* at the end of May, followed by *Scrippsiella trochoidea*, *Alexandrium minutum*, and *H. triquetra*. These species all have a worldwide distribution in marine coastal ecosystems, and one of them, *A. minutum*, produces paralytic shellfish poison.

No significant correlation was observed between the decline of the individual dinoflagellate species and the physicochemical environmental variables (nitrate, nitrite, phosphate, silicate, salinity, temperature, or tidal amplitude), which remained relatively constant during the 3-year study period (figs. S1 and S2). There was pervasive chronic infection of all the observed dinoflagellate species by Syndiniales; the infections occurred every year, even when host species abundance was low (Fig. 2). The prevalence of parasitized dinoflagellate cells reached 46%, with a mean value of 21% (Fig. 1) during summer. Similar values were obtained for all the species observed, including the toxic algae *A. minutum*, and are consistent with previously published data from a variety of coastal settings and several dinoflagellate host taxa (*13*–*18*). A similar average prevalence (28%) was observed for *Dinophysis norvegica* from the North Sea when a specific FISH detection approach was used (*19*). The sensitivity of the CARD-FISH analysis permitted the detection of early-stage infections as well as all the life-cycle stages of the parasite, including the very small free-living stage (3 to 5 μm in diameter). In all cases, the parasites closely corresponded to the description of *Amoebophrya* spp. provided by Cachon in 1964 (*20*).

Infections were initiated by the invasion of host cells by one or several very small infective cells called dinospores (*20*) (Fig. 2A). After several rounds of active nuclear replication, a large multinucleated trophont (the endoparasitic stage, Fig. 2, B to D) characteristic of the genus *Amoebophrya* is produced. Cultures and field observations show that this trophont matures in 2 days (*13*, *21*). The pressure of the trophont during the final stages of maturation distorts and enlarges the dinoflagellate cells (compare Fig. 2, B and C). Ultimately, the trophont ruptures the host cell wall and is elongated by a final evagination to form a swimming structure, the vermiform stage (*20*) (Fig. 2, D and E). Within a few hours, the vermiform structure fragments into 60 to 400 dinospores (*13*, *20*, *21*), each of which is able to reinfect a new host.

The amplification of newly infective parasite cells has the capacity to rapidly counter the growth rate of the host. Accordingly, the decline of the dinoflagellate populations correlated with the release of the free-living form of the parasite in the ecosystem; the dinospores were detectable in the ecosystem within 10 days of their release (a phenomenon illustrated by *H. rotundata* at the beginning of June 2005 and by *A. minutum* in 2004, Fig. 1). Although large numbers of dinospores were produced during the population decline of one dinoflagellate species, they did not prevent the growth of any other species, strongly suggesting that the parasites are host-specific. Each year, four main clades of group II Syndiniales were always detected (Fig. 1): Clade 1 was associated with the decline of *H. rotundata* in early June and clades 2, 3, and 14 appeared in late June [for clade nomenclature, see (*12*)]. Minor clades, including 32 and 42, were also detected. The parasite clades are

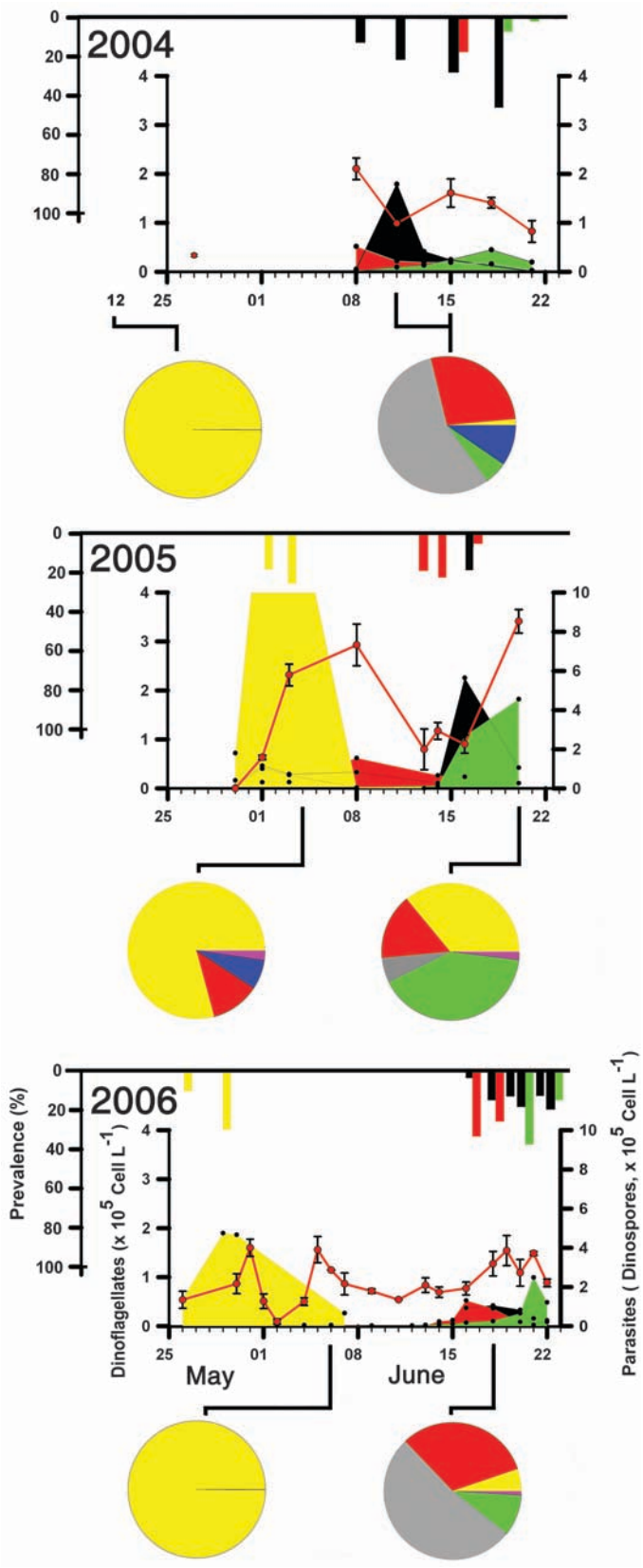

Fig. 1. Dinoflagellate-parasitoid successions and abundances in the Penzé estuary (northern Brittany, France) and the genetic clades of parasites occurring during May and June during 3 consecutive years (2004, 2005, and 2006). Dinoflagellate species are shown in the colored solid curves and by the left axis. Yellow, *H. rotundata*; red, *S. trochoidea*; black, *A. minutum*; green, *H. triquetra*. The free-living stage (dinospore) of group II Syndiniales is shown by the red line and the right axis. Prevalences (percentage of infected host) are shown in the inverted histogram at the top of each panel with the same color code as used for the host. Dinoflagellates were counted by microscopy, and dinospores and prevalence were detected with the general probe ALV01 by FISH. Error bars indicate SDs between triplicates. The pie charts represent the relative contribution, in percentages, of clones belonging to group II Syndiniales obtained from biased polymerase chain reaction amplifications made at two different dates during the monitored period. Yellow, clade 1; red, clade 2; gray, clade 3; green, clade 14; blue, clade 32; pink, clade 42.

separated from each other by at least 44 point mutations (Fig. 3).

Significant intraclade genetic microdiversity was found within environmental sequences (from the 238 sequences analyzed, 170 haplotypes were different). Clades 1 and 3 both have a starlike distribution, with an excess of rare haplotypes (Fig. 3). Furthermore, the results of Tajima's D test were significantly negative (table S3), suggesting a recent evolutionary origin and/or a rapid evolutionary divergence resulting from strong selection pressure. Clades 2 and 14 are more complex, probably indicating a more ancient evolutionary history and cryptic species (fig. S3).

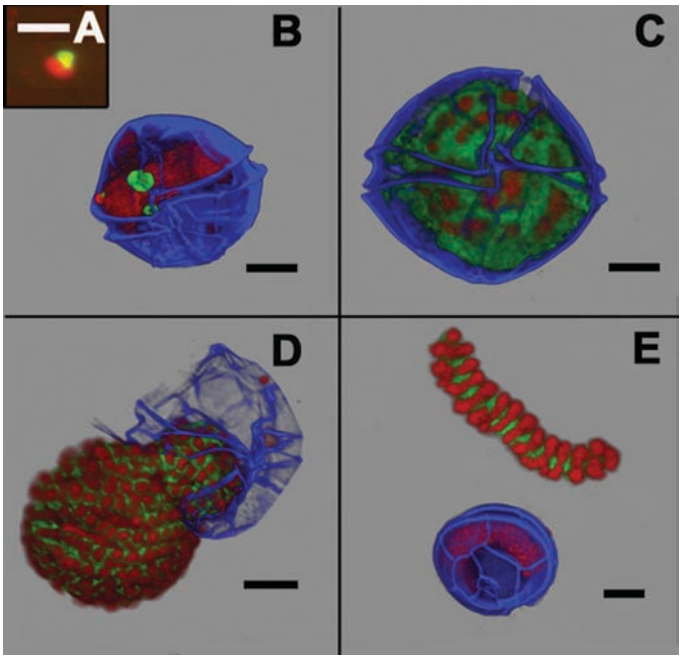

Fig. 2. Life cycle of the *A. minutum* parasitoids in the Penzé estuary. Green fluorescence shows the general oligonucleotide probe ALV01 targeting the small subunit ribosomal RNA (SSU rRNA) of the parasitoid by FISH. Red fluorescence shows nuclear genomic DNA stained by propidium iodide. Blue fluorescence shows the cellulosic theca of *A. minutum* revealed by the calcofluor. (**A**) Dinospore (free-living stage). (**B**) Early stage of *A. minutum* infection. This cell is infected by two intracellular parasites, and a third is located on the surface of the host theca. (**C**) Mature trophont inside *A. minutum*. Note the size increase of the host as compared to the previous stage. (**D**) Very late stage of trophont maturation. Note the thin ruptured host theca. (**E**) Vermiform stages, shown elongated and coiled as a ribbon. Scale bars, 3 μm in (A) and 10 μm in (B) to (E).

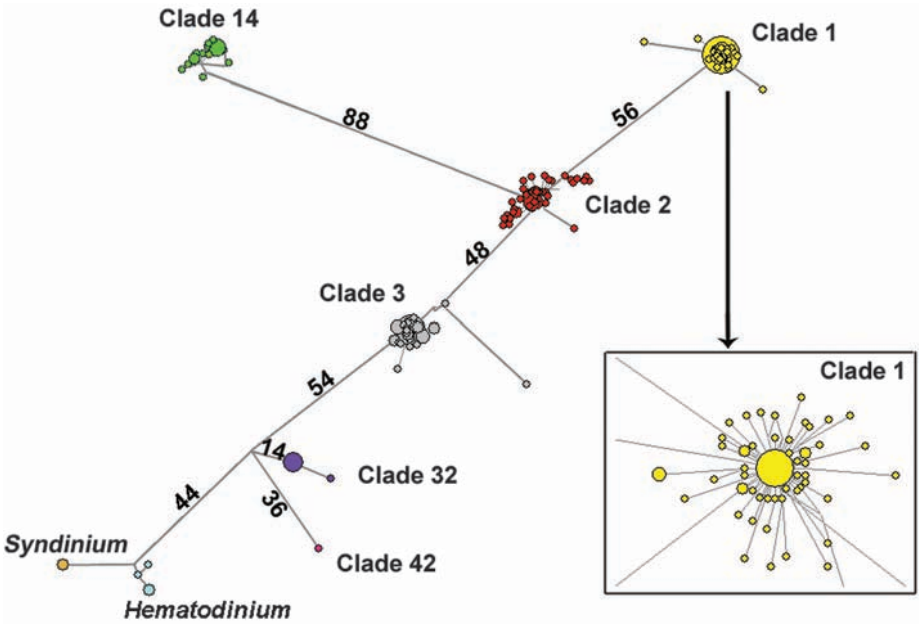

Fig. 3. Median-joining network depicting the phylogenetic relationship among environmental Syndiniales group II SSU rRNA gene sequences obtained from the Penzé estuary over 3 years (238 sequences in total). The solid colored circles follow the same color code as in Fig. 1. The size of each circle is proportional to the corresponding haplotype frequency. Each branch length is proportional to the number of mutational steps (the number of mutations between major clades is written above the branch). Four sequences of *Hematodinium* sp. and four of *Syndinium* sp. (group IV Syndiniales that are parasites of decapods and copepods, respectively) were used as outgroups.

The specificity of the four main parasite clades detected was assessed by FISH, using specific oligonucleotidic probes (tables S1 and S2). Clades 1, 2, and 14 each infected a single dinoflagellate species: *H. rotundata*, *S. trochoidea*, and *H. triquetra*, respectively (Table 1). This specificity is very stable over time; the same parasite clade infected the same species year after year. In all cases, for a given host/parasite pair, the prevalence threshold detected for a given host species when the general probe for group II Syndiniales was used was similar to the value obtained when the clade-specific probe was used (Table 1). Occasionally, parasites attacked nonoptimal host species, but mature trophonts were not observed (Table 1). Similar nonspecific and unproductive infections have been also observed in cultured strains of *Amoebophrya* (*21*, *22*). Unfortunately, we were not able to detect any cells targeted by clade 3 using the FISH technique, and none of the major clades (1, 2, 3, and 14) detected in our genetic clone libraries seemed to be specific to the toxic dinoflagellate *A. minutum*.

HABs occur when dinoflagellates escape not only predation but also parasitic infection. *A. minutum* had been suspected to be invasive after being introduced along the Atlantic coast of France, where blooms were first observed in the late 1980s (*22*). Blooms of *A. minutum* in the Penzé estuary were recorded for the first time in 1994 (fig. S4). Cell densities were reported to exceed 10×10^6 cells/liter (*22*). Over the ensuing 9 years, toxic blooms occurred with remarkable regularity in the Penzé (*22*), but although this species is still present in the ecosystem, blooms no longer occur (fig. S5). The population of *A. minutum* is now regulated by the parasitoids.

Environmental sequences belonging to Syndiniales have been detected in almost every marine ecosystem (*12*). Their host ranges are extremely diverse, extending from dinoflagellates to ciliates, radiolarians, cercozoans, chaetognaths, copepods, cnidarians, appendicularians, crabs, and even fish eggs. Thus, most marine planktonic groups are potentially affected by these parasites, which like the viruses that control bacterial populations, play a top-down control role in their host populations. However, the dinoflagellate parasites have a different impact on organic carbon transfer: In contrast to viruses, dinospores can be directly grazed by larger predators and thus are implicated in carbon transfer to higher trophic levels.

The capacity of these parasitoids to control their hosts is highly dependent on the parasitic fitness and mechanisms underlying the parasitic specificity. This also means that these natural biological controls are potentially less efficient when an exotic species is newly imported into an ecosystem (the enemy release hypothesis) (*23*) or when a rare species is promoted into abundance by substantial environmental change (such as coastal eutrophication or climate change).

Thus, the recent increase of inshore HAB events may originate in geographical and temporary disruptions between these dinoflagellates and their natural parasites.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/322/5905/[page]/DC1
Materials and Methods
SOM Text
Figs. S1 to S5
Tables S1 to S3
References

7 August 2008; accepted 14 October 2008
10.1126/science.1164387

Antimicrobial Defense and Persistent Infection in Insects

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During 400 million years of existence, insects have rarely succumbed to the evolution of microbial resistance against their potent antimicrobial immune defenses. We found that microbial clearance after infection is extremely fast and that induced antimicrobial activity starts to increase only when most of the bacteria (99.5%) have been removed. Our experiments showed that those bacteria that survived exposure to the insect's constitutive immune response were subsequently more resistant to it. These results imply that induced antimicrobial compounds function primarily to protect the insect against the bacteria that persist within their body, rather than to clear microbial infections. These findings suggest that understanding of the management of antimicrobial peptides in natural systems might inform medical treatment strategies that avoid the risk of drug resistance.

By contrast with the clinical use of antibiotics, resistance to natural antibiotics appears to be rare (*1*, *2*). Possibly, natural antibiotics play a different role in the wild than in medical applications (*3*), and our lack of understanding of their natural role results in unforeseen problems when they are used therapeutically, such as the rapid emergence of antibiotic-resistant pathogens.

Insects rely on a suite of systemic responses to combat infection (*4*) that can be classified into two main types. "Constitutive" defenses are always present and ready to act; they rely on the response of insect immune cells (haemocytes) and several rapidly activated enzyme cascades such as phenoloxidase (*5*, *6*) to defend against pathogens. Coupled with this line of defense is the "induced" response, which consists mainly of a suite of antimicrobial peptides (*7*). This component of the antimicrobial response takes at least 1 to 3 hours to generate (*8*) and 12 to 48 hours to reach peak levels (*9*). The induced response persists for weeks in a variety of insects: for example, at least 14 days in bumble bees (*10*) and mealworm beetles (*9*), and up to 44 days in dragonflies (*11*). Because immune responses bear costs [e.g., antagonistic pleiotropy (*12*), metabolic costs (*13*), and self-harm (*14*)], these slow and long-lasting antimicrobial responses, which are

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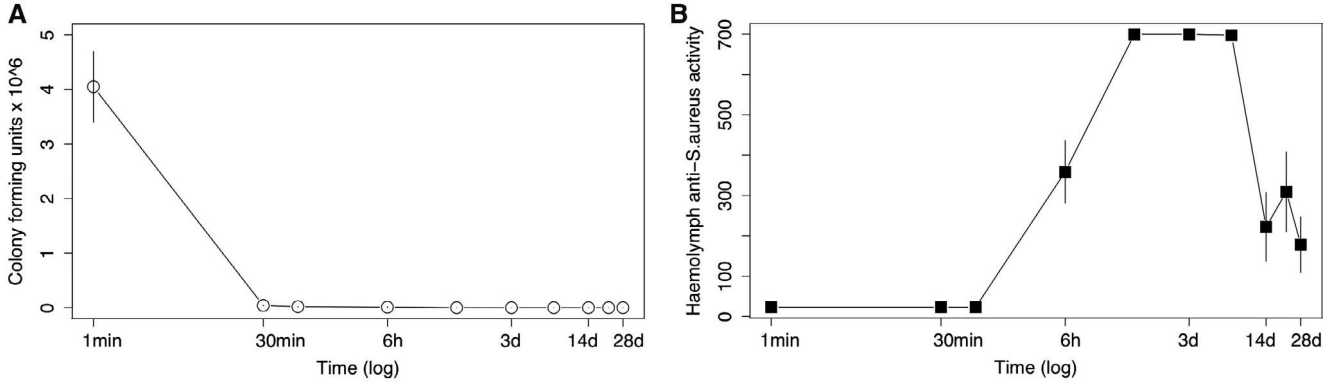

Fig. 1. The number of colony-forming units (CFU) recovered from *T. molitor* haemolymph over 28 days (**A**), and the haemolymph anti–*S. aureus* activity from the same individuals (**B**). Induced haemolymph anti–*S. aureus* activity was measured as the number of *S. aureus* CFUs killed during 2 hours of exposure to *T. molitor* haemolymph and is shown as CFU × 10^3. Each point represents the mean number of CFUs from 7 to 10 beetles (±1 SEM).

Supporting Online Material for

Control of Toxic Marine Dinoflagellate Blooms by Serial Parasitic Killers

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This PDF file includes:

Supplementary Informations.

Materials and Methods

Sampling procedure. We monitored the early summer dynamics of dinoflagellates in the Penzé estuary (North-West of France, English Channel, 48º37'N; 3º56'W) from 2004 and 2006. A portable probe WTW was used on board to measure *in situ* temperatures and salinities. Ten-liters of 27 PSU of salinity surface water were collected two hours before high tide. Salinities were further controlled back in the laboratory and samples which were not within the range of 26 to 28 PSU were rejected.

CARD-FISH. Samples were fixed with formaldehyde (prepared from paraformaldehyde 1% final concentration), during one hour at dark. They were then successively size-fractionated throughout 10 and 3 µm polycarbonate filters (100 ml per filter) by gravity and onto 0.2 µm Whatman anodisc filters (50 ml per filter) using a vacuum pump (vacuum pressure below 75 mm Hg). Each filter was dehydrated using an ethanol series (50, 80, and 100%, 3 min each), briefly dried at room temperature and stored at -80°C until further analysis.

Oligonucleotide probes were labelled with horseradish peroxidise (HRP) and *in situ* hybridizations were performed as described by Not et al. (2002)[1], with the following modifications: 1) all stages of these parasites were positively labelled following at least 12 hours of hybridization as observed by Gunderson et al. (2001)[2], 2) hybridizations were carried out at 42°C (for the general probe ALV01) and 35°C (for the specific ones), using 40 % of formamide, 0.9 M NaCl, 20 mM of TrisBase (pH=7.5), 0.01% SDS, and 2% Blocking Reagent (Roche, France), 3) two successive washing steps at 46°C (general probes) and 37°C (specific ones) during 20 min have been used to remove background fluorescent signal.

We designed a general oligonucleotide probe targeting most of the environmental sequences belonging to Syndiniales group II (Table S1). This general probe worked well on different strains of *Amoebophrya* spp. growing in cultures kindly provided by the Dr D. Wayne Coats, Smithsonian Environmental Research Center, Maryland, USA (Table S2). More specific probes were designed to target majority of environmental sequences retrieved from the Penzé

estuary. A competitor was required for three of them (Table S1). For clades 1 and 2, the corresponding probes were well working both on positive controls in culture (Table S2) and from natural samples. Any positive control was available for the clade 14, thus we directly used this probe on natural samples, with success. The ALV33, specific to clade 3 was well working on the positive control in culture (Table S2), but gave any signal from natural samples. We subsequently designed five additional probes specific to clade 3 (ALV14, ALV14 bis, ALV21, ALV24, and ALV34). All of these probes have two to three mismatches with sequence of the parasitoid infecting *Akashiwo sanguina* (sequence AF069516), the only positive control available for clade 3. Any positive signal was obtained for all of these probes using field samples.

At the end of the hybridization procedure, the cells were counterstained with propidium iodide (final concentration of 10 µg ml^{-1}) to visualize the nuclei, and calcofluor (fluorescent Brightener 28, Sigma, 1% final concentration) to stain the dinoflagellate theca. Counts were performed with an Olympus BX51 epifluorescence microscope (Olympus Optical CO, Tokyo, Japan) equipped with a mercury light source and an x100 UVFL objective. All concentrations were deduced from two independent counts obtained from at least 50 cells. Three dimensional pictures were acquired using a LSM 510 META confocal microscope (Zeiss).

Physico-chemical data. Nutrient (nitrate, nitrite, ammonium, phosphate and silicate) and chlorophyll *a* concentrations were determined from surface and bottom (3.5 meters) waters (Fig. S1). Nutrients were measured using a Bran and Luebbe autoanalyser following procedures described in Maguer et al. (2004)[3].

Flow cytometry (Fig. S2)**.** Seawater samples (1.5 ml in triplicates) were fixed with glutaraldehyde (0.25% final concentration) for at least 15 min before being frozen into liquid nitrogen and stored at –80°C until analysis. After thawing, samples were stained with SYBR

Green-I at a final dilution of 1/10,000, following the protocol described by Marie et al. (1999)[4].

Environmental sequence acquisition and analyses. For the genetic analysis, three to five liters were prefiltrated using a peristaltic pump (with a flow rate fixed at 100 ml min^{-1}) through 47-mm-diameter polycarbonate filter with a porosity of 3 µm (osmonic poretics) in filter housing connected in series to a 0.2-µm-pore-size Sterivex unit (Millipore). Filters were immediately frozen in liquid nitrogen and stored at -80°C. DNA was extracted from the 0.2 to 3 µm size fractions using a 3% (w/v) CTAB (Cetyltrimethylammonium bromide) extraction procedure[5]. During the PCR, we used the general probe ALV01 as primer (forward, 5'-AGA GTG TTC ACG GCA GGC-3'), together with the primer 329r (reverse, 5'-TGA TCC TTC YGC AGG TTC AC-3'). PCR amplification were performed as described by Romari et al. (2004)[6]. PCR products were cloned using the TOPO-TA cloning kit (Invitrogen). Clones randomly selected were partially sequenced using the forward primer ALV01 on an ABI Prism 3100 automatic sequencer (Applied Biosystems). Sequences identity and chimera detection were checked using the KeyDNAtools[7] software (http://KeyDNAtools.com). Selected clones were sequenced in both directions using the forward and reverse M13 primers. Sequences were aligned using the slow and iterative refinement method FFT-NS-I with Mafft 5.8 software[8], leading to a final alignment of 898 pb.
DNA sequence analyses (network on Fig. 3, Tajima's D tests on Table S3, and pairwise difference frequencies per site on Fig. S3) were processed using the software DnaSP Version 4.5[9]. Gaps were not considered, and a Median-joining Network was used to infer the most parsimonious branching connection between sampled haplotypes. Sequences of *Hematodinium* spp. (AF286023, EF065717, EF065718) and *Syndinium* spp. (DQ146406, DQ146403) were used as outgroup. Pairwise number of differences (mismatch distribution), were assessed under a constant population size model.
Occurrence of *A. minutum* in the Penzé estuary over last two decades. From 1994 to 2003, maximal abundance and occurrence of *A. minutum* were estimated from the compilation of data obtained from three different bi-monthly sampled sites located along the Penzé river (sampling frequencies was higher during bloom periods). These data were extracted from the IFREMER monitoring network database, publicly available online (http://www.ifremer.fr/envlit/). Data from 2004 to 2006 were obtained during this study, following the sampling procedure described initially. Cell abundances were deduced from lugol's-preserved samples sedimented in Utermöhl chambers (10 or 50 ml, depending on the concentration), and observed under an inverted microscopy (IX71 Olympus).

Text

1- Physico-chemical parameters of the Penzé estuary during the monitored period. Blooms of *Alexandrium minutum* have been known to occur mainly in nutrient-rich coastal waters such as estuaries and coastal bays. The Penzé estuary is characterized by high nutrient loadings coming from the runoff of N fertilizers resulting from intensive agricultural practice. Nitrate concentrations in this river water are in excess of 500 µmol L^{-1} during the major part of the year[10], with N: P ratios ranging from 65 to 300 corresponding to a predominance of nitrogen inputs in the estuary. These conditions are associated with increases in vertical stability of the water column by thermal and/or haline stratification during spring, creating favourable conditions for the development of phytoplanktonic blooms.

Conditions of development of *A. minutum* blooms have been studied in the Penzé estuary during 2004, 2005 and 2006 (Fig. S1). Previous observations[11] showed that the *A. minutum* blooms usually occurred in June, preferentially initiated from the low salinity waters of the upper part of the estuary. Accordingly, first detection and maximum concentrations of *A. minutum* cells were observed during neaps tide when vertical stability of the water column created optimal conditions for the phytoplankton development. Maximum cell concentrations were detected for relatively low salinity waters (in the 26-28 salinity range) of the estuary. Water temperatures were > 16°C which is known to be critical for the initiation of germination of the resting cysts. During the monitored periods, development of *A. minutum* populations occurred in an environment particularly enriched in nitrogen with N:P ratios ranging from 40 to 328. Initial nutrient concentrations (particularly nitrate and phosphate) were high in surface waters (respectively > 200 and 1.90 µmol L-1) and were probably never limiting for the *A. minutum* development. Nitrate and phosphate concentrations decreased respectively through 303 and 1.36 µmol L^{-1} in 2004 and 34.5 and 0.90 µmol L^{-1} in 2005. The rapid decrease in nitrite, nitrate, phosphate and silicate concentrations observed in late May and early June 2004 before the detection of *A. minutum* corresponded to the development of a diatom bloom. At the maximal abundances of the *A. minutum*, the high residual nitrate (in excess of 100 µmol L^{-1}) and phosphate (in excess of 0.50 µmol L^{-1} in 2004, 1.50 µmol L^{-1} in 2005 and of 1.07 µmol L^{-1} in 2006) concentrations cannot explained the rapid disappearance

of the *A. minutum* observed at the end of June. In fact, these conditions are very similar to those observed during an intense bloom on *A. minutum* (up to 33 x 106 cells L^{-1}) in the Penzé estuary in 1999[3]. Moreover, *A. minutum* exhibited a strong affinity for nitrogen (KS = 0.26 and 0.31 µmol L^{-1} for nitrate and ammonium) indicating the ability of this species, in contrast to other harmful dinoflagellates, to take up nitrogen efficiently even in waters with low dissolved inorganic nitrogen concentrations. Favourable conditions are permanently met in the Penzé estuary making highly improbable any nitrogen limitation for *A. minutum* development at any time of the year. In the case of phosphate, peak uptake rates were estimated at 4.8 µmol L^{-1} d^{-1} for the total phytoplanktonic population[3]. As any substantial changes in phosphate concentrations (< 0.2 µmol L^{-1}) and because high residual concentrations were observed during the bloom, internal inputs (adsorption-desorption between particulate and dissolved compartments, exchanges between sediments and water column) was probably sufficient for the phosphorus demand required during the peak of development in this shallow river.

2-Non-photosynthetic bacteria and virus counts by flow cytometry. Non-photosynthetic bacteria and small and large viruses were estimated by flow cytometry during the three years in the Penzé estuary (Fig. S2). Large and small viruses increased every year during the maximal abundances of *A. minutum*. Large viruses, thought to be mostly micro-algae viruses[4], are less concentrated than small ones. Non-photosynthetic bacteria only slightly increased at times of the high abundance of *A. minutum* during all three years.

3- Statistical analyses of the genetic diversity of the parasitoids from the Penzé estuary. Clades 1 and 3 have significantly negative Tajima values, which mean that both have an excess of rare haplotypes (Table S3). Two reasons may be invoked, 1- these populations are actually in expansion, or 2- they are submitted to a purifying selection pressure. Clades 1 and 3 have a typical mismatch distribution per site compared to the expected curve whereas several peacks are observed for clades 2 and 14 (Fig. S3). Presence of cryptic species can be suspected for these last two clades.

4- Occurrence of the toxic species *Alexandrium minutum* from the Penzé estuary over the two last decades.

A. minutum was detected for the first time along the French coast in July 1985 in the Vilaine Bay in southern Brittany (Fig. S4, 6 x 10^5 cells L^{-1})[12]. In August 1988, blooms of a greater magnitude occurred from two ria in the Northern Brittany (Aber Wrac'h and Aber-Benoit, Fig. S4). Subsequently, *A. minutum* proliferations were recorded in the Bay of Morlaix in July 1989.

Alexandrium minutum was the causative agent responsible for recurrent paralytic shellfish toxin contaminations of both mussels and oysters from 1994 to 2001 from the Penzé estuary (west part of the Morlaix bay). During that period, blooms with more than 5 x 10^6 cells L^{-1} regularly occurred (Fig. S5). Since 2002, both bivalve contaminations and A. minutum blooms ceased although this species is still detected in the ecosystem every year at low concentrations. *A. minutum* maximal abundances are always observed during June every year, with the notable exception of the first monitored year (1994).

Supplementary Figures.

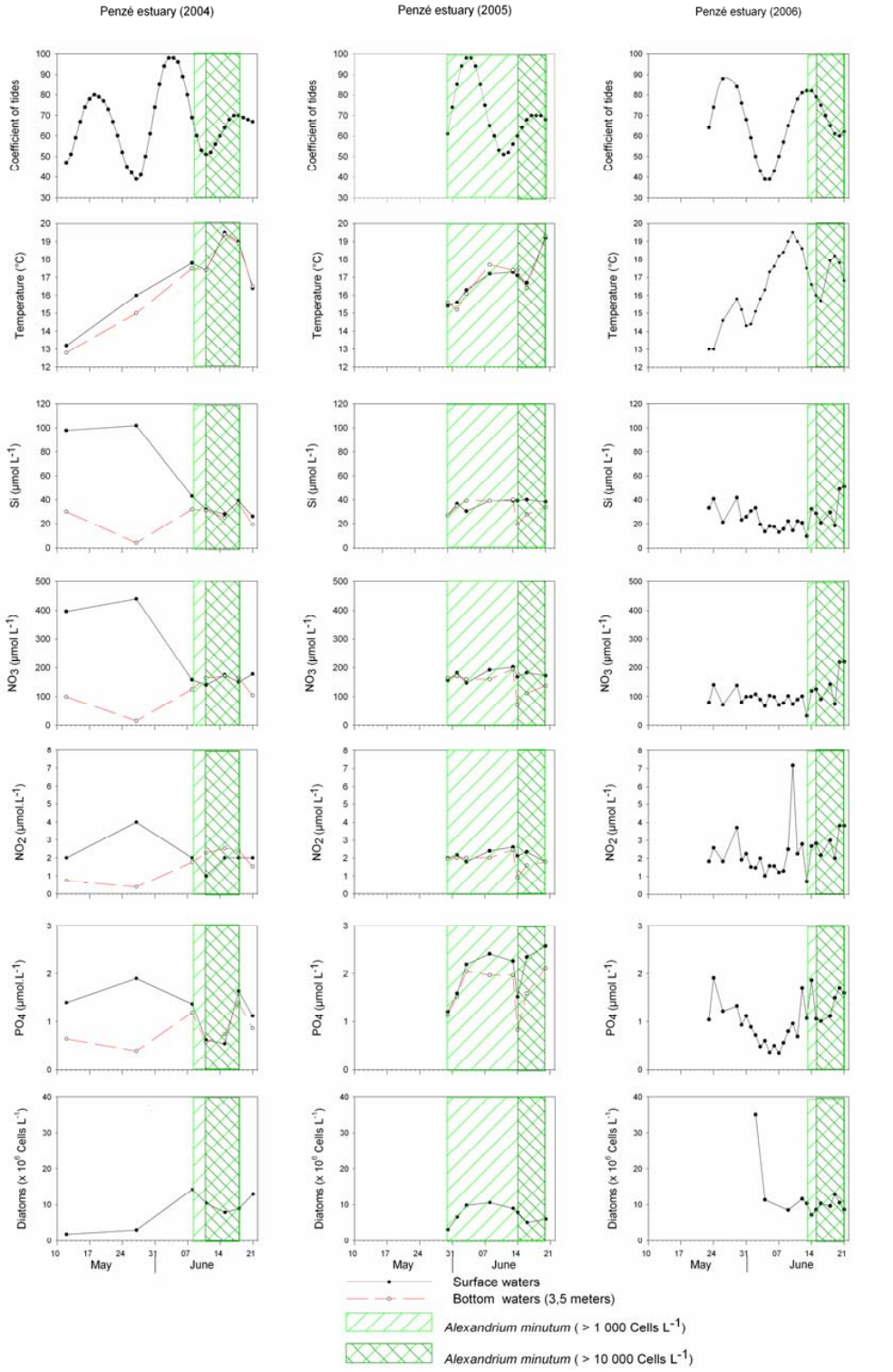

Figure S1. Physico-chemical characteristics and diatom concentration (cells L^{-1}) of samples collected in the Penzé estuary in 2004, 2005 and 2006 (tide amplitude, temperature in °C, Silicate, NO_3, NO_2, and PO_4 concentrations in µmol L^{-1}). Periods where *A. minutum* was observed are indicated in green.

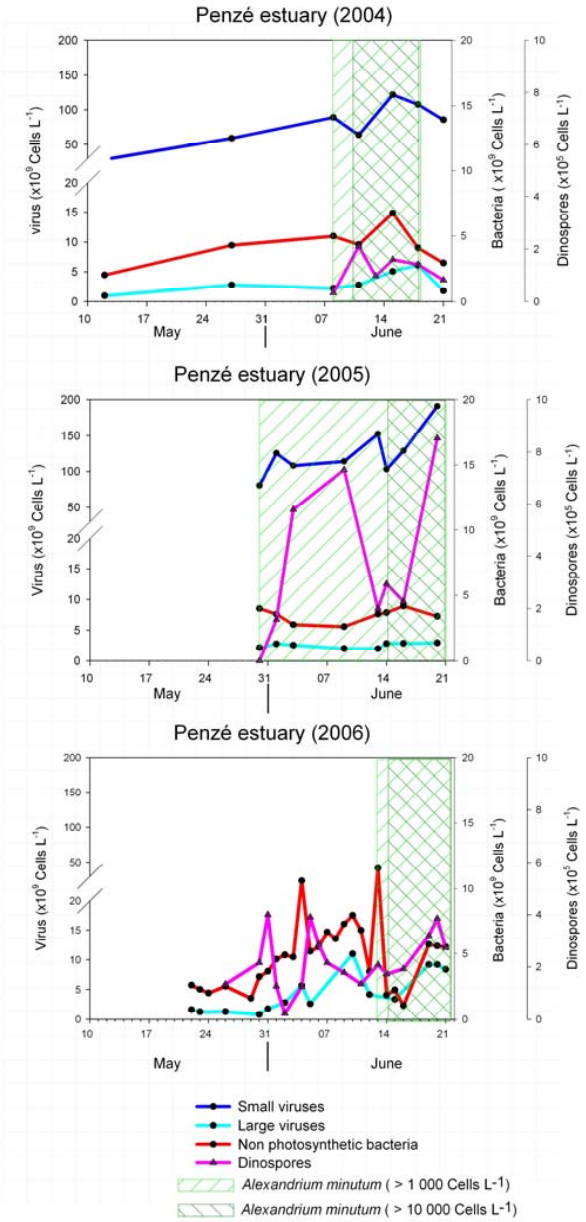

Figure S2. Virus (small and large), non-photosynthetic bacteria, and dinospores abundances at the 27 of salinity in the Penzé estuary during the monitored period in 2004, 2005, and 2006.

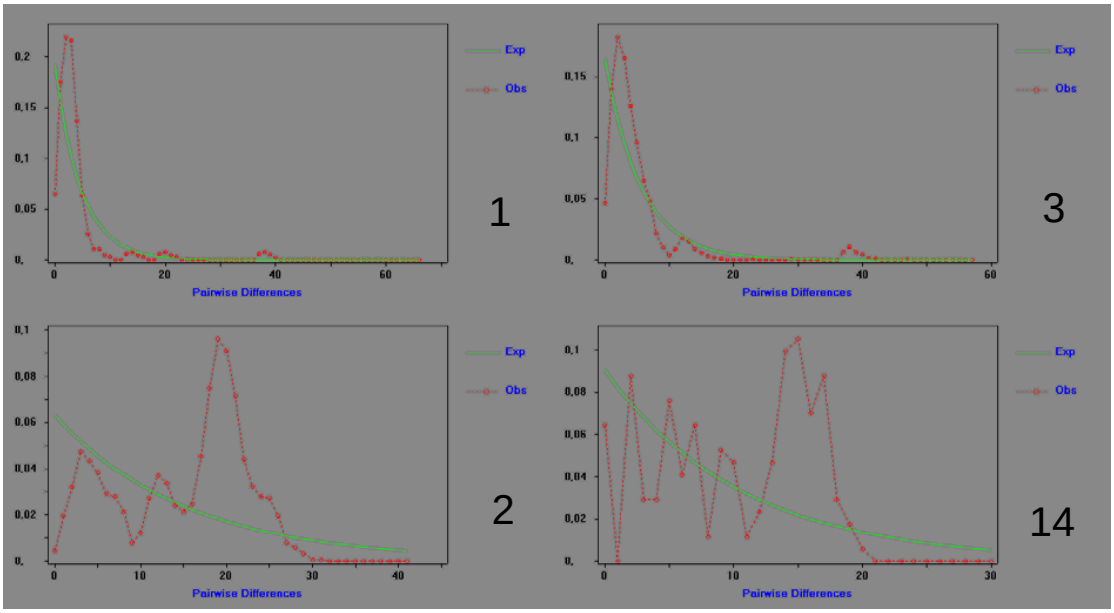

Figure S3. Pairwise difference frequencies (mismatch distribution) per site and for the four main Syndiniales Group II clades (1, 2, 3, and 14) detected in the Penzé estuary (2004-2006).

Figure S4. Localisation of first *A. minutum* blooms recorded along the French coast in late 1980s. 1- Vilaine Bay in July 1985, 2- Aber Benoit and Aber Wrac'h in August 1988, 3- Morlaix Bay in July 1989. The Penzé estuary is located in the west part of the Morlaix Bay.

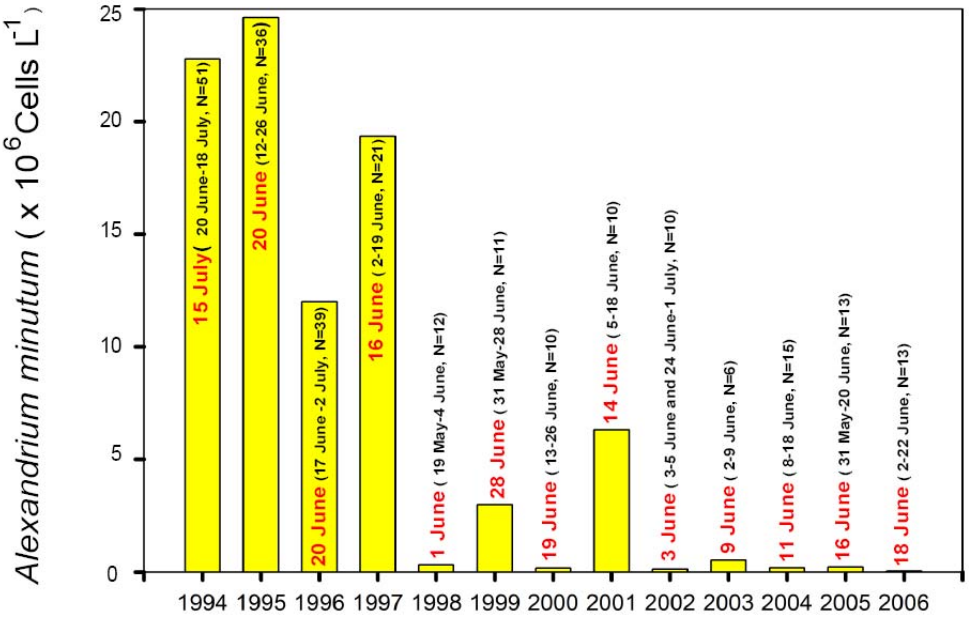

Figure S5. Maximal annual abundance and period of occurrence of the toxic dinoflagellate *A. minutum* in the Penzé estuary (North-West France, English Channel) from 1994 to 2006. Date corresponding to the annual maximal abundance in red, period of occurrence in dark (N= number of samples considered). Data were extracted from the IFREMER monitoring network database (http://www.ifremer.fr/envlit/) with exception of years 2004 to 2006 (from this study). Only concentrations superior to 1.000 cells per litre were considered.

Supplementary Tables.

Table S1: List of probes used in this study. NA: not adequate. Prob. for probe, Comp. for competitors.

Probe name	Specificity	Oligonucleotides	Specificity Penzé*	Specificity on the whole database
ALV01	Group II	Prob: 5'- GCC TGC CGT GAA CAC TCT -3'	NA	68% (on 1970 sequences)
ALV17	Group II Clade 1	Prob: 5'-TCG TCG CAA CAC AAT CCA -3' Comp: 5'-TCG TCG TAA CGC AAT CCA -3'	98%	71% (on 209 sequences)
ALV18	Group II Clade 2	Prob: 5'-TCG TCG TAA CGC AAT CCA -3' Comp: 5'-TCG TCG CAA CAC AAT CCA -3'	95%	89% (on 91 sequences)
ALV14 bis	Group II Clade 3	Prob: 5'- TCG TCG TGC AGT AAT CCA-3'	94%	69% (on 127 sequences)
ALV14	Group II Clade 3	Prob: 5'-GCA GTA ATC CAA GAA TTC C-3'	95%	89% (on 127 sequences)
ALV21	Group II Clade 3	Prob: 5'- GGT CAT GTA AGA ACG AC -3'	95%	55% (123 sequences)
ALV24	Group II Clade 3	Prob: 5'- CTC TGA CAC TAC AAT ACG-3'	89%	54% (127 sequences)
ALV33	Group II Clade 3	Prob: 5'- TCC ACA ATT CGT GCA GTT-3'	NA	92% (on 12 sequences)
ALV34	Group II Clade 3	Prob: 5'- TAA AAG GCC CTA CCT CC-3'	NA	80% (on 56 sequences)
ALV20	Group II Clade 14	Prob: 5'- TTC CAC CTC TGA CCG GTT-3' Comp: 5'- TTC CAC CTC TGA CGC GTT -3'	100%	73% (on 68 sequences)

* % of environmental sequences from the Penzé estuary belonging to Syndiniales Group II targeted by the probe and belonging to the corresponding clade.

Table S2. Origin of strains in culture used as positive controls in this study.

Name of the strain	**Host**	**Genetic clade**	**Targetting probes**	**Origin**
Amoebophrya sp.	*Karlodinium veneficum*	Group II Clade 1	ALV01, ALV17	Chesapeake Bay, USA
Amoebophrya sp.	*Akashiwo sanguinea*	Group II Clade 3	ALV01, ALV33	Chesapeake Bay, USA
Amoebophrya sp.	*Gymnodinium instriatum*	Group II Clade 4	ALV01	Chesapeake Bay, USA
Amoebophrya sp.	*Scrippsiella trochoidea*	Group II Clade 2	ALV01, ALV18	The Penzé estuary, French Atlantic coast, isolated in 2007

Table S3. Tajima's D Tests results

Syndiniales Group II clade name	**Number of haplotypes**	**Tajima's D**	**Statistical significance**
Clade 1	60	-2,91895	***, P < 0.001
Clade 2	51	-1,36672	Not significant, P > 0.10
Clade 3	40	-2,77597	***, P < 0.001
Clade 14	14	-0,88260	Not significant, P > 0.10

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2.3 Simulation par modélisation des efflorescences toxiques d'*Alexandrium minutum* : intégration du rôle du parasitisme par *Amoebophrya* sp. associé au broutage par les microprédateurs

Contexte et objectifs

A la suite de notre étude *in situ* (voir section 2.2), nous avons observé une corrélation entre le déclin d'*Alexandrium minutum* et l'augmentation de l'abondance de la forme libre du parasite appartenant aux Amoebophryidae (les dinospores). Ces observations suggèrent très fortement le contrôle des populations d'hôtes par ces parasites. Cependant, d'autres facteurs biologiques peuvent aussi interagir tels que la pression du broutage. En effet, les microprédateurs présents dans le milieu sont aussi bien capables de consommer l'hôte seul [Calbet *et al.*, 2003], que la partie libre du parasite et les hôtes infectés [Johansson and Coats, 2002]. Par exemple, certains ciliés de petite taille sont capables de consommer directement la partie libre du parasite, les dinospores. En culture, la présence de *Strombilidium* sp. consommant activement ces dinospores réduit de 70 à 80 % l'impact d'une souche d'*Amoebophrya* infectant le dinoflagellé *Akashiwo sanguinea* au bout de 24 heures [Johansson and Coats, 2002].
La capacité d'*Alexandrium minutum* à produire des efflorescences toxiques pourrait être directement liée à l'absence de pathogènes du type *Amoebophrya* (hypothèse émise dans la section 2.2). Néanmoins, la présence de microprédateurs pourrait directement affecter la croissance de la microalgue et réduire de façon drastique la virulence du pathogène, par consommation des dinospores. Nous avons construit un modèle nous permettant de faire varier ces deux paramètres, à savoir l'action du parasitisme par rapport à celui du broutage.

Pour évaluer la part du parasitisme *in situ* (dû au parasite *Amoebophrya*) dans la disparition de son hôte *Alexandrium minutum*, nous avons modélisé les interactions hôte-parasite en prenant en compte le broutage (ciliés).
Nous avons utilisé un modèle hôte-parasite du type Anderson-May [Anderson and May, 1980, Anderson and May, 1981] auquel nous avons couplé une pression de broutage par le microzooplancton.
Ce travail a été réalisé en collaboration avec David Montagnes et Andy Fenton (School of Biological Sciences, Liverpool) qui ont élaboré toute la partie modélisation. Dans le cadre de cette étude, j'ai réalisé l'acquisition des paramètres biologiques *in situ* (ciliés, parasites, dinoflagellés) et testé le modèle par la suite.

Résumé

Deux mécanismes ont été proposés pour le contrôle des efflorescences toxiques produites par des dinoflagellés : (1) le parasitisme (par exemple *Amoebophrya* sp.) et (2) le broutage par le microzooplancton. Malgré des études en culture et *in situ*, peu de données quantitatives ont été acquises. Nous avons choisi de modéliser l'impact de ces deux paramètres aussi bien sur une population invasive qu'endémique de dinoflagellés. L'utilisation de données extraites de la littérature nous a permis de compléter le modèle hôte-microparasite, de type Anderson May [Anderson and May, 1980, Anderson and May, 1981] en intégrant différents compartiments du réseau trophique microbien (nano- et microphytoplancton, nano- et microzooplancton, un dinoflagellé toxique, un dinoflagellé parasite). Les trois scénari suivants ont été examinés pour simuler l'introduction du dinoflagellé toxique et de son parasite dans un environnement :

1. Ecosystème, incluant nano- et microplanctons autotrophes, flagellés hétérotrophes, et des ciliés sans algues invasives ni parasites ;
2. Un scénario identique au cas 1 mais avec l'introduction d'un dinoflagellé invasif (*Alexandrium minutum*)
3. Un scénario identique au cas 2 mais avec un parasitoïde spécifique d'*Alexandrium minutum* de type *Amoeboprhya*. Ce modèle reproduit les observations effectuées dans l'estuaire de la Penzé où un dinoflagellé toxique a produit à partir de 1998 des efflorescences pendant 10 ans après sa probable introduction.

Après avoir confirmé le contrôle des populations par le parasite (scénario 3), nous avons fait varier des paramètres associés aussi bien au parasite qu'à d'autres compartiments du réseau trophique, pour évaluer l'impact du parasite sur le contrôle des efflorescences. Nous avons simulé les dynamiques des différentes populations sur 50 jours. Dans tous les cas, les résultats se sont montrés très concluants. En effet, pour le scénario 1, les groupes augmentent et diminuent en fonction de leur régulation par les échelons trophiques supérieurs, reproduisant parfaitement les équilibres induits par le réseau trophique microbien. Pour le scénario 2, la dynamique d'*Alexandrium minutum* introduit dans le modèle suit celle du microphytoplancton. La concentration de ce dinoflagellé est réduite par le broutage du microzooplancton, mais les efflorescences toxiques se produisent clairement. Enfin, pour le scénario 3, *A. minutum* démarre sa floraison de la même façon que le scénario 2, mais cette dernière est rapidement éliminée en une dizaine de jours environ.

L'hypothèse que le déclin des populations de dinoflagellés hôtes puisse être attribué au contrôle par un pathogène de type *Amoebophrya* est donc fortement soutenue par ce modèle. En particulier, ce modèle permet de mieux comprendre la part relative du broutage, qui ne semble pas être un facteur de régulation suffisant pour prévenir ce type d'efflorescences algales toxiques. Il est donc important d'incorporer l'impact d'un tel parasite aux modèles de réseau trophique plus complexes.

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Responsibility of microzooplankton and parasite pressure for the demise of toxic dinoflagellate blooms

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ABSTRACT: Two mechanisms proposed to control dinoflagellate blooms — parasitism by eukaryotes (e.g. *Amoebophrya* sp.) and grazing by microzooplankton — have been explored through previous laboratory and field studies but lack quantitative assessment. We modelled the relative effect of these mechanisms. We used literature values to embed an Anderson-May host-microparasite model into a microbial food web model (nano- and microphytoplankton, nano- and microzooplankton, a toxic dinoflagellate, a dinoflagellate parasite). Three scenarios were examined to simulate the introduction of a toxic dinoflagellate and its parasite into an environment: (1) a food web, including autotrophic nano- and microplankton, heterotrophic flagellates, and ciliates; (2) as for Case 1 but with a toxic dinoflagellate; (3) as for Case 2 but with a dinoparasite. This mimics observations in a French estuary, where a toxic dinoflagellate began blooming in 1988; since 1998, blooms appear to have become regulated, and numerous parasitic infections by *Amoebophrya* sp. occurred from 2004 to 2006. After supporting parasite control of dinoflagellate blooms, we assessed the effects of observed ranges of key variables associated with the parasite and other components of the food web on parasite control of the dinoflagellate population. Population dynamics were examined over 50 d. In Case 1, all taxa had 10 to 20 d blooms. In Case 2, the toxic dinoflagellate population dynamics mimicked that of the microphytoplankton, and this dinoflagellate was reduced in numbers but not extirpated by microzooplankton grazing. In Case 3 population blooms occurred, and the parasite virtually eliminated the dinoflagellate over ~10 d. Sensitivity analysis indicated that our assessment was robust. We propose that the decline in toxic dinoflagellates in the French estuary may have been due to an introduced dinoparasite. In general, we suggest that parasites may have greater impact on toxic dinoflagellate blooms than microzooplankton grazers; the parasites have the potential to eliminate the toxic dinoflagellates. We recommend that such parasites be incorporated into more complex food web models.

KEY WORDS: *Amoebophrya* · *Alexandrium minutum* · Dinospore · Harmful algal bloom · HAB · Microbial food web · Model · Plankton · Red tide

INTRODUCTION

The economic and societal impacts of harmful algal blooms (HABs) continue to motivate the study of HAB population dynamics and methods for their control. As one of the main HAB taxa, dinoflagellates have received considerable attention, and a substantial body of work exists describing the initiation, demise, and impact of their blooms (e.g. Shumway 1990, Donaghay & Osborn 1997, Smayda 1997, Turner & Tester 1997, Burkholder 1998, Anderson et al. 2002, Nagasaki et al. 2006). Two mechanisms proposed to control dinoflagellate blooms are parasitism by other eukaryotes (Coats et al. 1996), such as the dinoflagellate

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Amoebophrya sp. (Fig. 1), and grazing by microzooplankton, such as ciliates (e.g. Calbet et al. 2003, Rosetta & McManus 2003). Although both of these mechanisms have been explored through laboratory and field studies, they lack quantitative assessment. We provide a critical first step toward modelling the relative impact of these 2 potential controlling pressures on toxic dinoflagellate populations.

Our analysis was in part stimulated by historical data on the HAB dinoflagellate *Alexandrium minutum* from 2 sources. First, data from East Harbour, Alexandria, Egypt, show that this species routinely bloomed from the 1970s to 1990s, then blooms ceased, and this was attributed to removal of benthic cysts by hydrodynamically forced erosion (Ismael & Khadr 2003), but we speculate that this could have been by biological control (see below). Second, *A. minutum* also occurs in northern France (Maguer et al. 2004), especially in the Penzé estuary and the Bay of Morlaix, Finistère, where it was first seen to form blooms ($>10^6$ cells l^{-1}) in 1988, suggesting that it had recently been introduced to the region. As *A. minutum* forms resistant, resting cysts, populations became established permanently in shallow waters. However, although *A. minutum* blooms develop in this region with a predictable regularity each year in June (Maguer et al. 2004), maximal annual abundance has been decreasing since 1998 (Fig. 2), suggesting that the species is now being controlled. During *A. minutum* proliferations in the Penzé estuary between 2004 and 2007, the parasitic dinoflagellate *Amoebophrya* was detected by fluorescent *in situ* hybridization (using a general probe for the whole group); presence of this parasite appeared to be directly linked to the abrupt decline of *A. minutum* during the 3 consecutive years (A. Chambouvet unpubl. data). These data stimulated propositions that: (1) *A. minutum* was introduced to the system in the late 1980s; (2) during the 1990s *A. minutum* blooms were not controlled, or poorly controlled by grazing; and (3) in the late 1990s, the dinoflagellate parasite *Amoebophrya* entered the system and has subsequently controlled blooms. We suggest that a similar sequence of

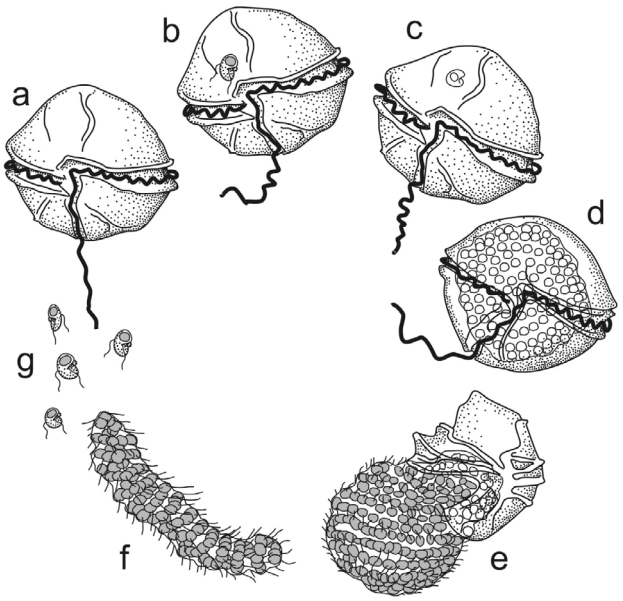


Fig. 1. *Amoebophrya*. This parasitic dinoflagellate, which infects (a) free-living dinoflagellates, has a relatively simple life cycle, consisting of (b) a dinospore, the infective dispersal stage (shown attached to dinoflagellate), (c) an intracellular trophont, (d) a growth stage, (e,f) an extracellular vermiform (a dispersal stage), and (g) individual motile dinospore cells (Cachon 1964, Cachon & Cachon 1987). Dinospores are ~10 µm long, biflagellate cells that attach to the host surface, penetrate the host pellicle, and develop into trophonts in the cytoplasm or in the host nucleus, depending on the infected species (Cachon 1964, Park et al. 2002). The trophont grows in the host for ~2 d (Coats et al. 1994, Yih & Coats 2000) and replicates to produce a multinucleate, multiflagellate stage. At maturity, the multiflagellated stage metamorphoses into the vermiform, which escapes from the host, killing it. Vermiforms are free living for a fraction of a day (Coats & Park 2002), and then undergo cytokinesis to produce hundreds to thousands of dinospores (Coats et al. 1994). Survival of dinospores outside the host is on the order of 1 to 2 d (Coats & Park 2002). Mortality of the parasite occurs through predation, either on the infected host by grazers such as large microzooplankton (e.g. ciliates) or by grazing of dinospores by large nanozooplankton or smaller microzooplankton (e.g. heterotrophic flagellates) (Fig. 3)

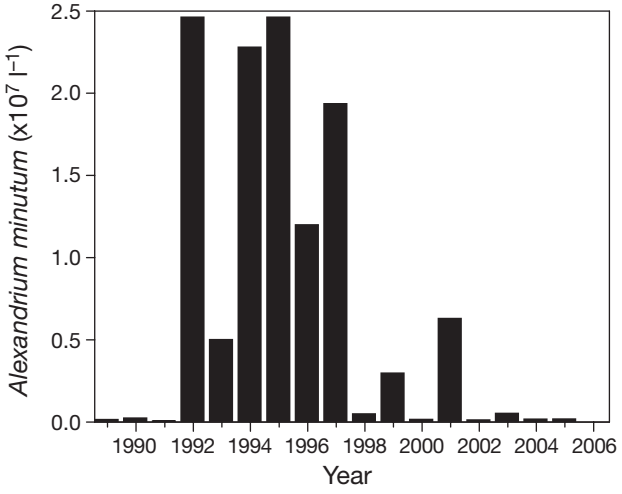


Fig. 2. *Alexandrium minutum*. Historical data on mean maximum annual abundance in the closely associated Penzé estuary and Bay of Morlaix, Finistère, France between 1988 and 2006, indicating the appearance of blooms and their subsequent decline. Note, data between 1988 and 1993 include observations from the Bay of Morlaix and from the Penzé estuary, and data for 2004 to 2006 include observations from only the Penzé estuary. Historical data from 1988 to 2003 were obtained from IFREMER (available at: www.ifremer.fr/envlit/surveillance/telechargement.htm). Data from 2004 to 2006 are unpublished and have been archived by L. Guillou

events may have occurred in East Harbour. However, we have neither established that the parasite can directly cause the decrease of the host populations *in situ*, nor can we unequivocally indicate that the parasite did not exist when *A. minutum* blooms were greatest in the 1990s. Nevertheless, based on a parameterised mathematical model, we can hypothesise that *Amoebophrya* (as opposed to grazing) can cause the demise of *A. minutum* blooms. Developing such a model is the first step towards a better understanding of the parasite–host population dynamics, which in turn may elucidate how such blooms are controlled in general.

Specifically, using existing literature parameters we have embedded a classical host-micro-parasite model (Anderson & May 1980, 1981) into the microbial food web (Fig. 3) to evaluate the relative roles of parasites and grazers in the control of dinoflagellate blooms over a short period (<50 d). Following our observations from the Finistère data, 3 progressive scenarios were examined to simulate the introduction of a toxic dinoflagellate and, subsequently, a dinoflagellate parasite into a marine environment: (1) a food web including only autotrophic nano- and microplankton, hererotrophic flagellates, and ciliates (groups that are typical of most microbial food webs); (2) as for Case 1, but with the introduction of a toxic dinoflagellate; and (3) as for Case 2, but with the addition of the parasite. After modelling indicated that the parasite (rather than microzooplankton) may control dinoflagellate blooms, we first conducted a sensitivity analysis to assess the impacts of the observed ranges of key parasite parameters on parasite control of the dinoflagellate population. We then explored, again through sensitivity analysis, whether changing other key parameters and state variables in the system would alter the bloom dynamics of parasites and their dinoflagellate host. Through these analyses we strongly suggest that parasite control of dinoflagellates may occur.

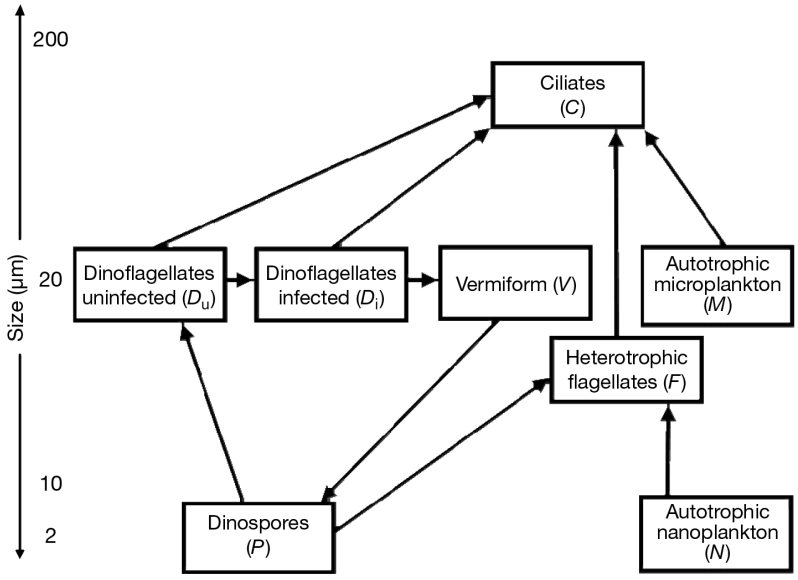


Fig. 3. The microbial food web investigated with the model included: ciliates (*C*, representing microzooplankton) as the top grazers on autotrophic microplankton (*M*), heterotrophic flagellates (*F*, representing large nanozooplankton and small microzooplankton), dinoflagellates (D_u: uninfected; D_i: infected), parasites as dinospores (*P*) and vermiform (*V*) stages, and autotrophic nanoplankton (*N*). Note, arrows denote the flow of prey to consumer. Also note that the vermiform stage produces dinospores. The size scale on the left is an indication of organism size, with the smallest organism near the bottom

METHODS

Model description. ***Overview:*** We adopted a standard population-dynamic modelling framework to describe the changes in abundance of the following components of the eukaryotic microbial community (Fig. 3): large ciliates (*C*), representing microzooplankton, as top-down control in the system; a HAB dinoflagellate (*D*), as the host of the parasite and prey for the ciliate; autotrophic microplankton (*M*), as an alternative prey pool for the ciliate; heterotrophic flagellates (*F*), as top down control on both dinospores (i.e. the free-living stage of the parasite, *P*) and the vermiform (dispersal) stage (*V*) of the parasite; and autotrophic nanoplankton (*N*), as an alternative food for heterotrophic flagellates. For this exploratory simulation of population dynamics, the food web was assumed to be in a uniform physical environment at 18°C (unless otherwise stated, all parameter values in Table 1 were obtained at this temperature) and without immigration or emigration. Carrying capacities (*K*) were imposed on the primary producers (resulting in logistic growth) to represent the impact of bottom-up nutrient control, whereby the strength of density dependence was assumed to depend on the sum of uninfected and infected dinoflagellates, microplankton, and nanoplankton ($D_u + D_i + M + N/1000$) populations (see Appendix 1).

In many population models based on the original Lotka-Volterra predator-prey models, the numerical response of the predator is determined from their functional response (assuming constant growth efficiency and metabolic loss rate; Turchin 2003), because direct measurements of growth rates are not available. However, for planktonic protozoa, there is a growing body of studies that provides direct measurements of both the functional and numerical responses within a single study of a single species

(e.g. Montagnes 1996, Kamiyama 1997, Jeong et al. 1999, Montagnes & Lessard 1999, Gismervik 2005, Kimmance et al. 2006). These measurements in turn provide the opportunity to directly combine functional and numerical responses in models, without the need to obtain independent estimates of assimilation rates or metabolic losses from the literature (e.g. Kamiyama 1997, Montagnes & Lessard 1999, Kimmance et al 2006). In fact such models have already been applied to examine the impact of the tintinnid *Favella taraikaensis* on the dinoflagellate *Alexandrium tamarense* (Kamiyama 1997). This modelling framework is similar to that of the more traditional Lotka-Volterra-style models described above, but the latter formulation makes parameterisation easier from direct measurements of functional and numerical responses. We have, therefore, adopted this latter approach and used coupled predator functional and numerical responses for both the nano- and microzooplankton. Details on the specific food web components follow below and are presented in Table 1.

Dinoflagellate (D) and dinoparasite (P): The generic dinoflagellate component of the model was parameterised primarily from data on *Alexandrium minutum*. Dinoflagellate growth was assumed to be logistic, with a maximum specific growth rate (r_d) and carrying capacity (K_d). Dinoflagellates were assumed to die following infection by the free-living infective stages of the parasite (see *P*, below) and grazing by the ciliate (see 'Ciliates [*C*]', below).

The generic parasite component was based primarily on data for *Amoebophrya* sp. The host-parasite model was structured on the Anderson-May microparasite model (Anderson & May 1980, 1981), wherein the dinoflagellate host population is divided into 2 compartments: uninfected (D_u) and infected (D_i) individuals, and comprises 4 coupled differential equations:

$$\frac{dD_u}{dt} = r_d D_u \left(1 - \frac{D_u + D_i + M + N/1000}{K_d}\right) - D_u(\beta P + CI_c) \quad (1)$$

$$\frac{dD_i}{dt} = \beta P D_u - D_i(\alpha + CI_c) \quad (2)$$

$$\frac{dV}{dt} = \alpha D_i - \alpha' V \quad (3)$$

$$\frac{dP}{dt} = \lambda\alpha' V - P(r_p + FI_f) \quad (4)$$

Table 1. Model parameters and state variables. Further details of the derivation and origin of these are presented in Appendix 1. All rates were either collected at ~18°C or have been temperature corrected, by applying a Q_{10} of 2. Values presented below were used in the 3 model-run case scenarios; ranges in parentheses were used in the sensitivity analysis

Parameter/state variable	Symbol	Value	Unit	Source
Dinoflagellate (*D*)				
Carrying capacity	K_d	3×10^6	$D\ l^{-1}$	See Appendix 1
Maximum growth rate	r_d	0.4	d^{-1}	Flynn et al. (1996)
Mortality rate due to infection	α	0.5 (0.35–0.6)	d^{-1}	Coats & Park (2002)
Initial uninfected abundance	D_u	5×10^3 ($0.5–10 \times 10^3$)	$D\ l^{-1}$	Raymont (1980)
Initial infected abundance	D_i	0	$D\ l^{-1}$	
Parasite (*P*)				
Development rate	α'	3 (2–4)	d^{-1}	Coats & Park (2002)
Number released per host	λ	200 (100 to 400)	PD^{-1}	Yih & Coats (2000)
Mortality rate	r_p	−0.55 (−0.87 to −0.23)	d^{-1}	Coats & Park (2002)
Transmission rate ($\times 10^{-7}$)	β	4 (1–7)	$d^{-1}\ l^{-1}$	See Appendix 1
Initial abundance	P_0	50	$P\ l^{-1}$	None
Nanoplankton (*N*)				
Maximum growth rate	r_n	1.0	d^{-1}	Furnas (1982)
Carrying capacity	K_n	3×10^9	$N\ l^{-1}$	See Appendix 1
Initial abundance	N_0	10^6	$N\ l^{-1}$	Raymont (1980)
Heterotrophic flagellates (*F*)				
Growth rate	r_f	See Eq. (7) (text)	d^{-1}	Kimmance et al. (2006)
Grazing rate	I_f	See Eq. (8) (text)	prey $F^{-1}\ d^{-1}$	Kimmance et al. (2006)
Initial abundance	F_0	5×10^3	$F\ l^{-1}$	Gasol (1994)
Microplankton (*M*)				
Maximum growth rate	r_m	0.4	d^{-1}	Bissinger et al. (2008)
Carrying capacity	K_m	3×10^6	$M\ l^{-1}$	See Appendix 1
Initial abundance	M_0	10^4 ($0.5–50 \times 10^3$)	$M\ l^{-1}$	Raymont (1980)
Ciliate (*C*)				
Growth rate	r_c	See Eq. (11) (text)	d^{-1}	Montagnes & Lessard (1999)
Grazing rate	I_c	See Eq. (12) (text)	prey $C^{-1}\ d^{-1}$	Montagnes & Lessard (1999)
Initial abundance	C_0	10^3 ($0.1–10 \times 10^3$)	$C\ l^{-1}$	Lynn & Montagnes (1991)

where β is the transmission rate, I_c is the ciliate grazing rate on dinoflagellates (see 'Ciliates [C]', below), α is the mortality rate due to infection, which imposes a time delay (of duration 1/α) on the system, r_p is the parasite mortality rate, and I_f is the heterotrophic flagellate grazing rate on parasites (see 'Heterotrophic flagellates [F]', below). The model incorporates the free-living vermiform stage (V) of the parasite, which lasts on average 1/α d before undergoing cytokinesis to produce λ dinospores (P). We assumed that infected dinoflagellates die without reproducing (Elbrächter 1973, Cachon & Cachon 1987), that dinoflagellates were only infected once, although multiple infections may occur (Coats & Park 2002), and that dinoflagellate infection increased linearly with parasite abundance. Furthermore, β was assumed not to decrease with dinospore age, although such a decrease can occur (Coats & Park 2002); however, the variation in β examined during our sensitivity analysis (see below) should compensate for these omissions.

Nanoplankton (N): The autotrophic nanoplankton provided prey for heterotrophic flagellates, allowing the latter to survive when there were no parasites. Nanoplankton growth was assumed to be logistic, with a maximum specific growth rate (r_n), carrying capacity (K_n), and mortality caused by heterotrophic flagellate grazing (see 'Heterotrophic flagellates [F]', below). Nanoplankton dynamics were modelled as:

$$\frac{dN}{dt} = r_n N\left(1 - \frac{D_i + D_u + M + N/1000}{K_n}\right) - \frac{FI_f N}{(N+P)} \quad (5)$$

Note that nanoplankton were grazed by heterotrophic flagellates in proportion to abundance relative to the other component of the flagellate diet, i.e. dinospores of the parasite (P).

Heterotrophic flagellates (F): Heterotrophic flagellates are grazers on dinospores and nanoplankton, and their population dynamics were modelled as:

$$\frac{dF}{dt} = r_f F - CI_c \quad (6)$$

where I_c is ciliate grazing rate (see 'Ciliates [C]', below), and growth rate (r_f) was determined from a numerical response (growth rate versus prey concentration) that includes a threshold prey concentration (6393 ml^{-1}), below which growth rate becomes negative (Kimmance et al. 2006):

$$r_f = \frac{0.94 \times (P + N - 6393)}{14160 + (P + N - 6393)} \times 0.05T \quad (7)$$

where T is temperature (°C).

Heterotrophic flagellates grazed on nanoplankton and dinospores at a rate (I_f) that followed a temperature (T) dependent functional response (Kimmance et al. 2006; note, hourly rates were converted to daily rates, assuming a constant rate over 24 h):

$$I_f = \frac{23.74 \times (P + N)}{112010 + P + N} \times 0.28(T - 7.99)^{0.10} \times 24 \quad (8)$$

We assumed that there was no preference for either nanoplankton or dinospores, so heterotrophic flagellates consumed these prey at a rate proportional to their availability.

Microplankton (M): The autotrophic microplankton population provided a prey pool for ciliates, allowing them to grow when dinoflagellates and heterotrophic flagellates were rare. Microplankton population growth was assumed to be logistic, with a maximum specific growth rate (r_m) and carrying capacity (K_m). r_m was set to equal that of the dinoflagellate, so that unequal competition between these 2 autotrophs was not an issue in the control of dinoflagellate blooms. Microplankton mortality was caused only by ciliate grazing (see 'Ciliates [C]', below) at rate I_c, and in proportion to microplankton abundance relative to the other components of the ciliate diet (dinoflagellates and heterotrophic flagellates). Hence, microplankton population growth was modelled as:

$$\frac{dM}{dt} = r_m M\left(1 - \frac{D_u + D_i + M + N/1000}{K_m}\right) - \frac{CI_c M}{(D_u + D_i + M + F)} \quad (9)$$

Ciliates (C): Ciliates grazed on autotrophic microplankton (including the dinoflagellates) and heterotrophic flagellates; their population growth was modelled as:

$$\frac{dC}{dt} = r_c C \quad (10)$$

where ciliate specific growth rate (r_c) was based on a numerical response that includes a threshold prey concentration (1308 ml^{-1}), below which growth rate is negative (Montagnes & Lessard 1999; note, these rates were established at 13°C and were temperature-corrected to 18°C, assuming a Q_{10} of 2, although this may not always be appropriate; see Montagnes et al. 2003):

$$r_c = \frac{0.622 \times (D_u + D_i + F + M - 1308)}{2384 + (D_u + D_i + F + M - 1308)} \quad (11)$$

I_c followed a functional response (Montagnes & Lessard 1999; note, rates were temperature corrected, as for Eq. 11 above, and hourly rates were converted to daily rates, assuming grazing was constant over 24 h):

$$I_c = \frac{197}{1 + 19000/(D_u + D_i + F + M) + (D_u + D_i + F + M)/3314} \times 24 \quad (12)$$

Ciliate ingestion was then determined for each of the 4 potential prey (D_u, D_i, F, M); assuming no preference, they were consumed as a proportion of the total prey available.

Note that there are indications that ciliates may have reduced growth and grazing rates when exposed to some toxic dinoflagellates (e.g. Hansen 1986, Jeong et al. 1999, Rosetta & McManus 2003). Here we are testing the potential maximum impact of microzooplankton grazing, in comparison with parasite-induced mortality. Therefore, we have not imposed an inhibition due to prey type.

Model simulations. ***Explored scenarios:*** Three distinct scenarios were examined to simulate the introduction of a toxic dinoflagellate and, subsequently, a parasite to an environment: (1) a simple food web, including only autotrophic nano- and microplankton, hererotrophic flagellates, and ciliates; (2) the food web described in Case 1, but with the introduction of a dinoflagellate; and (3) the food web described in Case 2, but with the addition of a specific parasite for the dinoflagellate introduced in Case 2. Runs of the model were conducted using parameters and state variables presented in Table 1, which are further justified in Appendix 1.

Sensitivity analysis: Our analysis indicated that the parasite has a greater impact than microzooplankton on dinoflagellate blooms (see 'Results'); consequently, our emphasis was directed to assessing the sensitivity of parameters associated with the parasite. After establishing that variation of these parameters failed to alter our results, we ran a further set of sensitivity analyses to assess whether other non-parasite parameters and state variables might alter the observed model outcome (i.e. parasite blooms co-occurring with the demise of dinoflagellate blooms, resulting in collapse of host populations; see 'Results').

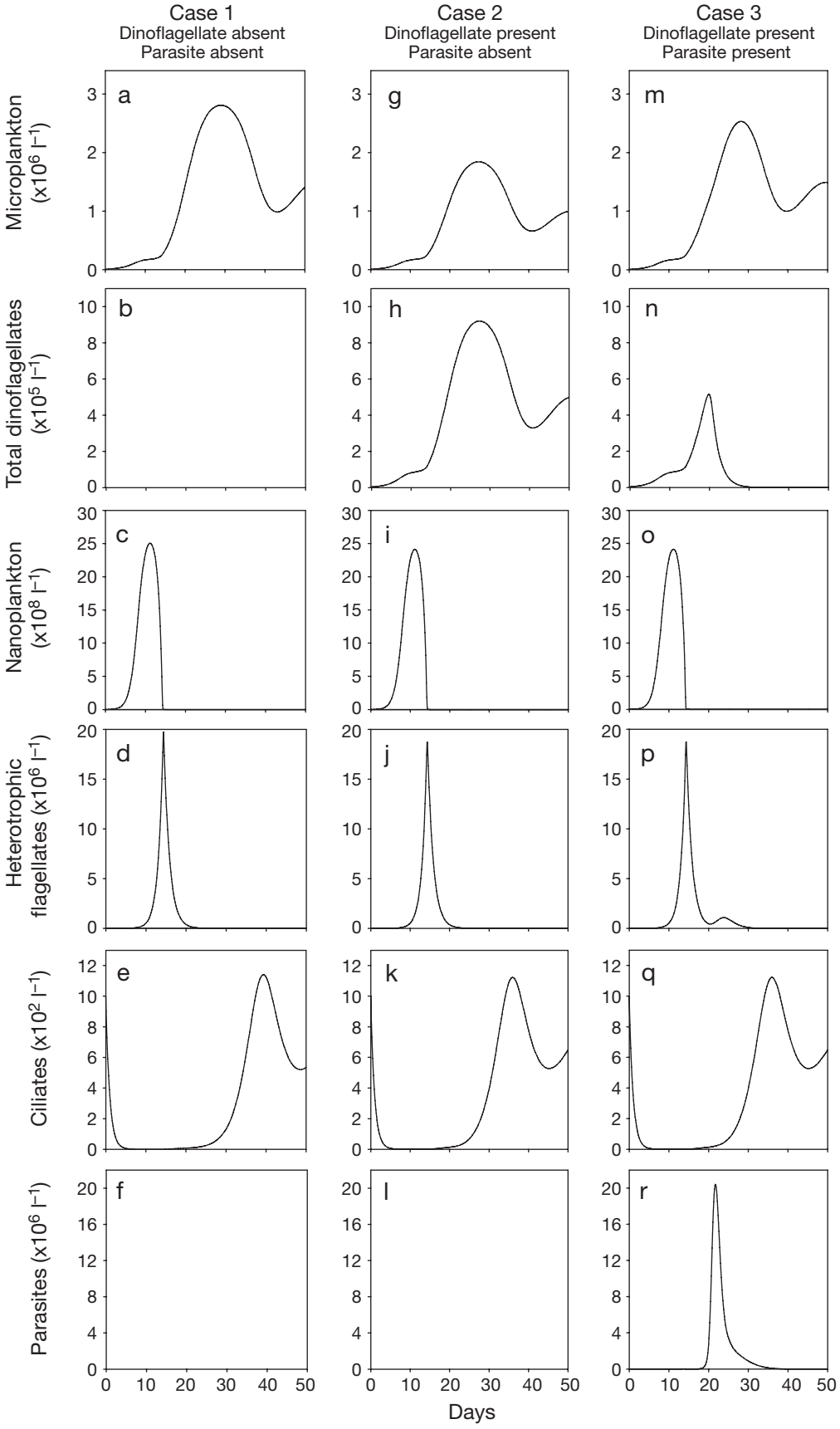
We provide ranges for 5 of the parasite parameters used in the model as estimated from the literature (Table 1), but initial observations indicated that only 4 of these altered the model output to any extent: (1) mortality rate due to infection (α); (2) the number of parasites released per host (λ); (3) parasite mortality rate (r_p); and (4) parasite transmission rate (β). We investigated how variation of these parameters altered predictions of population dynamics over observed ranges (Table 1). We also examined variation in (1) initial abundances of dinoflagellates, ciliates, and microplankton (Table 1); (2) the carrying capacity for autotrophs (K, which was altered from 0.1 to 3.0 times the value presented in Appendix 1); (3) both ciliate growth rate and ingestion rate (at the same time; i.e. the two were altered simultaneously and equally over a range spanning 0.5 to 1.5 times the literature value, Eqs. 11 & 12); (4) heterotrophic flagellate maximum growth rate and ingestion rate (concurrently, following the logic outlined above for ciliate parameters); (5) ciliate threshold concentration; and (6) heterotrophic flagellate threshold concentration (the prey concentration at which growth = 0, which was altered over a range spanning 0.5 to 1.5 times the literature values, Eqs. 7 & 11). For each parameter, model runs were conducted at 4 levels, evenly distributed across the range, from the lowest value to the highest value.

RESULTS

Explored scenarios

In Case 1 (Fig. 4a–f), in which there were no dinoflagellates or parasites, autotrophic microplankton bloomed over ~30 d and then decreased due to grazing by ciliates, which bloomed after ~30 d; nanoplankton formed a bloom after ~10 d, which was controlled by heterotrophic flagellates, which bloomed after ~15 d. In Case 2 (Fig. 4g–l), in which dinoflagellates were introduced, the population dynamics of all groups were similar to those in Case 1, but the microplankton population was depressed due to competition with the dinoflagellates; the dinoflagellate population mirrored the microplankton population dynamics (as expected, given their identical growth rates), except that dinoflagellate abundance was ~0.5 times lower than that of the microplankton, due to reduced initial dinoflagellate abundance. In Case 3 (Fig. 4m–r), in which parasites were included, population dynamics of most groups were the same as those in Case 1, except that there was a secondary, small population-pulse of heterotrophic flagellates after the initial bloom; this second pulse was due to a parasite bloom (i.e. a new prey for heterotrophic flagellates) at ~20 d (Fig. 4r). Most importantly, the peak in abundance of the dinoflagellate in the presence of the parasite was around half that in parasite absence, and the bloom, which lasted <30 d, did not recover over the 50 d simulation (Fig. 4n).

Fig. 4. Output of population dynamics from our model food web (Fig. 3): (a,g,m) autotrophic microplankton; (b,h,n) total dinoflagellates; (c,i,o) autotrophic nanoplankton; (d,j,p) heterotrophic flagellates; (e,k,q) ciliates; (f,l,r) dinospore parasites. Three distinct scenarios (Cases 1, 2 and 3; see 'Results; Explored scenarios') were examined to simulate the introduction of a toxic dinoflagellate and its parasite to the environment. Runs of the model were conducted using parameters and state variables outlined in Table 1


Case 1
Dinoflagellate absent
Parasite absent
Case 2
Dinoflagellate present
Parasite absent
Case 3
Dinoflagellate present
Parasite present
Microplankton (x10^6 l^-1)
Total dinoflagellates (x10^5 l^-1)
Nanoplankton (x10^8 l^-1)
Heterotrophic flagellates (x10^6 l^-1)
Ciliates (x10^2 l^-1)
Parasites (x10^6 l^-1)
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
Days

Sensitivity analysis

Potential variation (see Table 1) in dinoflagellate mortality rate due to infection (α), the number of dinospores released per host (λ), parasite mortality rate (r_p), and parasite transmission rate (β) revealed that under all conditions the parasite was able to control the dinoflagellate host population, exceeding that due to grazing by ciliates (cf. Figs. 4h & 5b,h,n,t). The main impacts of varying the above parameters were on peak abundance and persistence of dinoflagellates and the parasite, and this only occurred when λ and β were raised to their upper extent (Fig. 5h,l,t,x). None of the other components of the food web was substantially influenced by altering the above parameters, except for the autotrophic microplankton when they were competing with the dinoflagellate.

Variation in initial abundances of dinoflagellates, ciliates, and microphytoplankton, and the carrying capacity (K) produced many differences in the population dynamics of the entire system (Fig. 6). However, the general pattern (as indicated above) that dinoflagellate blooms were immediately followed by parasite blooms was consistent, regardless of the perturbation. Furthermore, the demise of the dinoflagellates was directly attributable to parasite infection, not ciliate grazing (results of these details are not presented here but can be explored through contact with D. J. S. Montagnes). Potential variation in ciliate and heterotrophic flagellate growth and grazing parameters also revealed marked differences in population dynamics within the food web, but again, the general indication was that dinoflagellate blooms were controlled and followed by parasite blooms (Fig. 7). The main exception to this trend was for conditions in which the ciliate threshold (i.e. the prey concentration where $r_c = 0$) was reduced to 0.5 of the literature value; in this case, ciliates survived at low prey abundances, subsequently bloomed in response to raised dinoflagellate and microplankton abundances, and then controlled both these prey populations over the first 20 d (solid lines in Fig. 7g,h,k). However, under this condition (0.5 of the ciliate threshold level), parasites controlled (and virtually removed) the dinoflagellates in the latter part of the 50 d simulation, supporting the pattern observed above for other conditions.

DISCUSSION

Using a microbial food web model, we explored 2 hypotheses proposed for the control of potentially toxic dinoflagellate blooms: microzooplankton grazing (Kamiyama 1997, Jeong et al. 1999, Rosetta & McManus 2003) and parasite-induced mortality (Coats et al. 1996, Johansson & Coats 2002). This is the first study to quantitatively test these combined hypotheses.

Here we examine, over a 50 d period, the consequences for harmful algal bloom dynamics within the microbial food web. Unlike many exploratory population models, this analysis limited the output to evaluation of ~1 cycle of the population dynamics (i.e. it would have been possible under some conditions to produce multiple population cycles, if the simulation time was extended beyond 50 d). Our logic for not extending the simulation beyond 50 d was that both environmental (e.g. climatic) and biological (e.g. metazoan zooplankton) perturbations in pelagic systems would undoubtedly occur over a period longer than 50 d and would disturb the system; thus, we have not provided longer-term (and inappropriate) simulations. Furthermore, we specifically excluded metazoan zooplankton (e.g. copepods) for the following reasons. Firstly, the focus of this study was to assess whether components of the microbial food web alone could control toxic dinoflagellates, and secondly there is evidence to suggest that microzooplankton grazing will have greater effects than metazoan zooplankton grazing on toxic dinoflagellate bloom dynamics (Calbet et al. 2003). However, we also recognise that as metazoan zooplankton generation times (>10 d, e.g. Gillooly 2000) would not result in substantial zooplankton population fluctuations, the population dynamics of these metazoans and their impact would therefore be relatively constant. Such constant grazing pressures would simply increase prey mortality rates by a constant amount (i.e. result in lower estimates of r_d and r_m). However, fast-growing smaller copepods might respond, and other modelling efforts have indicated that metazoan zooplankton grazing can control dinoflagellate blooms (Griffin et al. 2001, Gismervik et al. 2002), so this may be an area for further exploration. We have also not considered viruses, which may influence phytoplankton dynamics (Brussaard 2004), specifically that of dinoflagellates (Nagasaki et al. 2006). The influence of viruses has been included in models of plankton dynamics, with various results (e.g. Chattopadhyay & Pal 2002, Singh et al. 2004). Again, omission of viruses allowed us to specifically evaluate the potential role of microzooplankton and eukaryotic parasites. Finally, although we applied well-established, non-linear functional and numerical responses for the grazers (based on the limited data available) we assumed a linear relationship between parasite infection and abundance; non linear dynamics might reduce the impact of parasites at high abundances. Clearly, now that our findings support the prediction of Coats et al. (1996) that parasite control is possible, and also show the extent to which these dinoflagellate parasites may alter bloom dynamics, we recommend that more com-

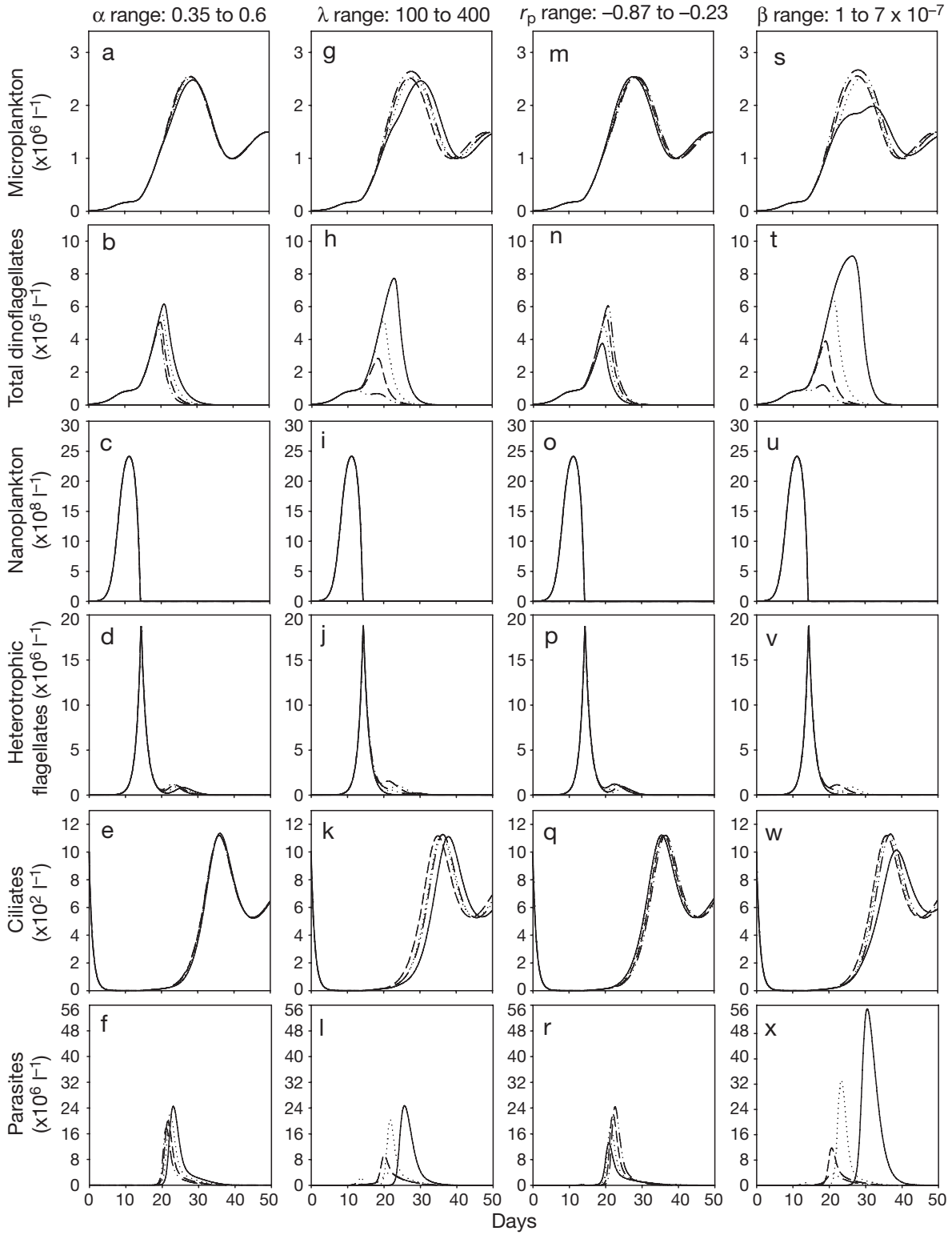


Fig. 5. Sensitivity analysis for effects of varying key parasite life-cycle parameters on population dynamics of our microbial food web (Fig. 3): (a,g,m,s) autotrophic microplankton; (b,h,n,t) total dinoflagellates; (c,i,o,u) autotrophic nanoplankton; (d,j,p,v) heterotrophic flagellates; (e,k,q,w) ciliates; (f,l,r,x) dinospore parasites. Changes in population dynamics, within a panel (i.e. different line types) are the responses of estimates for 4 parasite parameters (Table 1): mortality rate due to infection (α; a–f); parasites released per host (λ; g–l); parasite mortality rate (r_p; m–r); and parasite transmission rate (β; s–x). For each parameter, model runs were conducted at 4 levels (line types), evenly distributed across the range, from the lowest value to the highest value (solid, dots, dashes, dots & dashes, respectively)

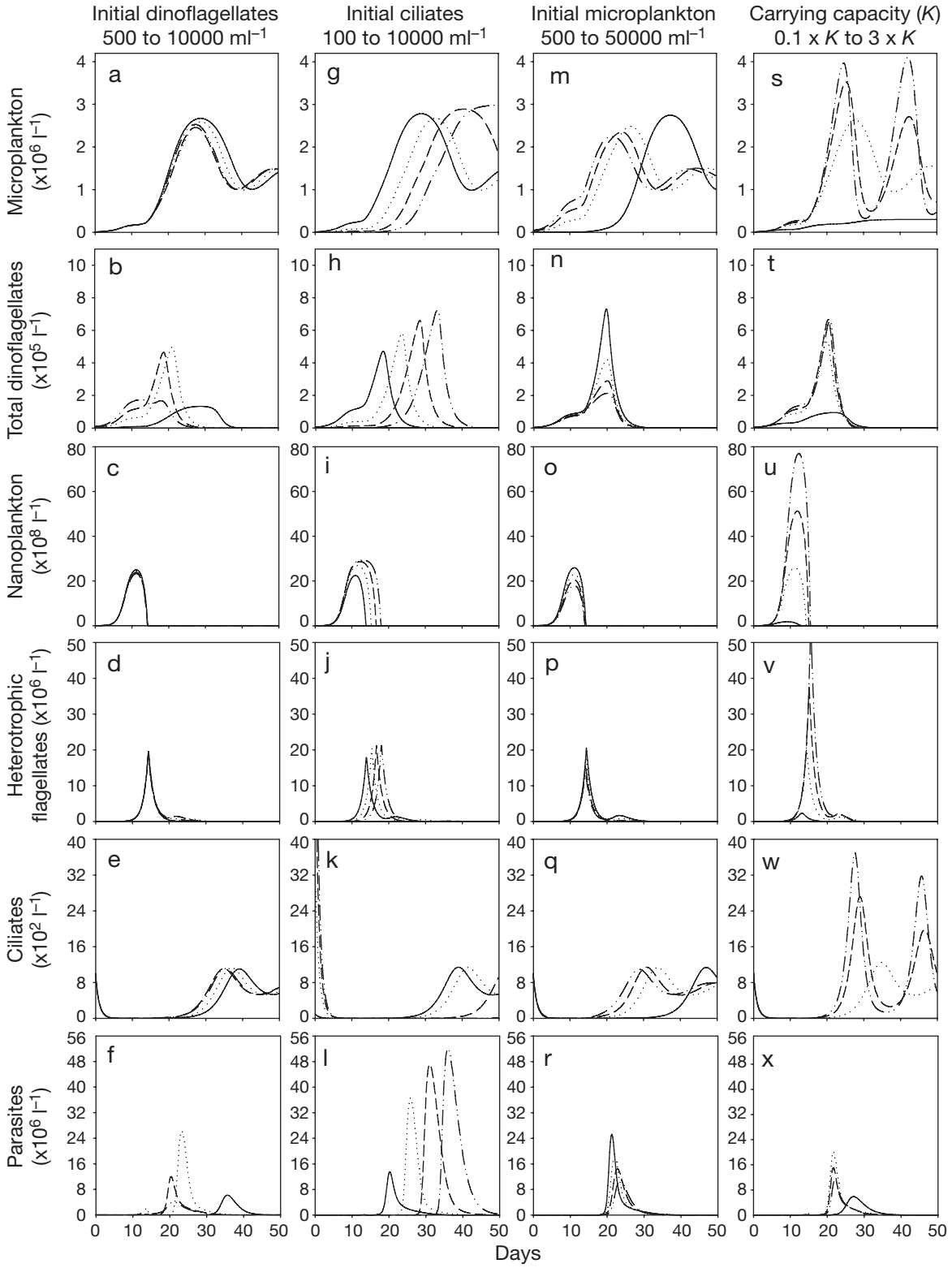


Fig. 6. Sensitivity analysis for effects of varying initial abundances of dinoflagellates (D_u), ciliates (C) and microzooplankton (M) and the combined carrying capacity (K) of the autotrophs (see Appendix 1) on population dynamics of our microbial food web (Fig. 3): (a,g,m,s) autotrophic microplankton; (b,h,n,t) total dinoflagellates; (c,i,o,u) autotrophic nanoplankton; (d,j,p,v) heterotrophic flagellates; (e,k,q,w) ciliates; (f,l,r,x) dinospore parasites. Changes in population dynamics, within a panel (i.e. different line types) are the responses of estimates for ranges presented in Table 1. For each parameter, model runs were conducted at 4 levels (line types), evenly distributed across the range, from the lowest value to the highest value (solid, dots, dashes, dots & dashes, respectively)

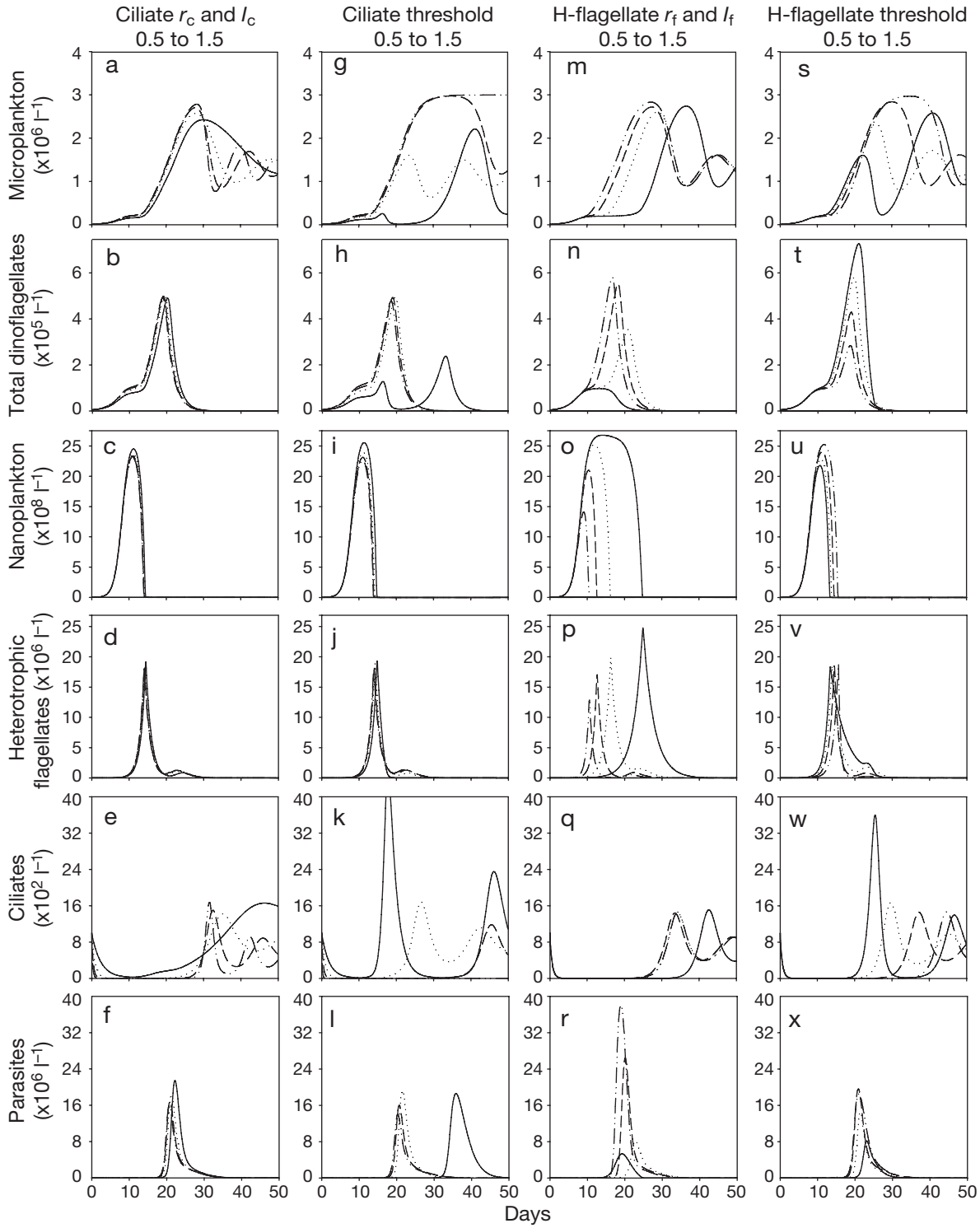


Fig. 7. Sensitivity analysis for effects of varying growth and grazing parameters of the ciliate and heterotrophic flagellate on population dynamics of our microbial food web (Fig. 3): (a,g,m,s) autotrophic microplankton; (b,h,n,t) total dinoflagellates; (c,i,o,u) autotrophic nanoplankton; (d,j,p,v) heterotrophic flagellates; (e,k,q,w) ciliates; (f,l,r,x) dinospore parasites. Changes in population dynamics, within a panel (i.e. different line types) are the responses of estimates for 4 parameters, or combined parameters: ciliate growth rate r_c and ingestion rate I_c were simultaneously altered between 0.5 and 1.5 of literature values (Table 1); ciliate threshold (the prey concentration where growth rate is zero) was varied between 0.5 and 1.5 of literature values (Eq. 11); heterotrophic flagellate growth rate (r_f) and ingestion rate (I_f) were simultaneously altered between 0.5 and 1.5 of literature values (Table 1); heterotrophic flagellate threshold was varied between 0.5 and 1.5 of literature values (Eq. 7). For each parameter, model runs were conducted at 4 levels (line types), evenly distributed across the range, from the lowest value to the highest value (solid, dots, dashes, dots & dashes, respectively). H-flagellate: heterotrophic flagellate

plex pelagic models extending over longer periods (e.g. that of Griffin et al. 2001) should include such eukaryotic parasites.

Scenarios and sensitivity analysis

In Case 1 (Fig. 4), we presented a scenario in which a body of water contains micro- and nanophytoplankton that are grazed by micro- and nanozooplankton, respectively. As can occur in nature, the autotrophic microplankton population was initially controlled by bottom-up pressure as they reached their carrying capacity, and then they were controlled by top-down microzooplankton grazing (e.g. Montagnes 1996, Glibert 1997, Strom 2002). Similarly, the autotrophic nanoplankton plankton was initially bottom-up controlled and then grazed by the nanozooplankton. The microzooplankton, which had no top-down control imposed on them, died due to starvation when prey resources dropped below their threshold level, and the nanozooplankton died from both starvation and microzooplankton grazing. Such short-term population dynamics have been described by others in field studies, laboratory studies, mesocosm experiments, and models (e.g. Stoecker & Evans 1985, Andersen & Sørensen 1986, Lynn & Montagnes 1991, Montagnes & Lessard 1999, Gismervik et al. 2002, Kimmance et al. 2006), with population levels and timing of events at a similar order of magnitude to those presented here (e.g. Raymont 1980, Andersen & Sørensen 1986, Lynn & Montagnes 1991). This congruence suggests that our initial scenario is not unrealistic. However, we recognise that there is a dearth of good data to validate our predictions, and we now strongly support further careful field, mesocosm, and microcosm monitoring to specifically assess our predictions on the occurrence and timing of these events related to parasitism.

In Case 2, we simulated the introduction of a HAB dinoflagellate into the model environment. Such introductions could be through direct human intervention (e.g. ballast water, Hallegraeff 1998), by natural dispersal, or by indirect anthropogenic dispersal induced by climate change (Gómez 2003). Regardless of the mechanism of introduction, we showed firstly that the dinoflagellate reached realistic bloom levels (e.g. Calbet et al. 2003) (as the autotrophs reached their combined carrying capacity), and secondly that the dinoflagellate was controlled, or at least reduced by microzooplankton (i.e. ciliate) grazing, as proposed by Rosetta & McManus (2003) and Calbet et al. (2003). It should be noted that our model presupposes that the microzooplankton will consume toxic species, although such toxic species can reduce growth and grazing of ciliates (Kamiyama 1997, Jeong et al. 1999); our predictions, therefore, provide a maximum potential for microzooplankton control, and it may be lower in reality. Furthermore, the dinoflagellate population was never significantly reduced, suggesting that microzooplankton grazing pressure alone may not suffice to alleviate problems associated with toxic dinoflagellate blooms.

In contrast, in Case 3, in which a dinoflagellate parasite was introduced to the system, not only was the toxic dinoflagellate controlled, it was virtually eliminated from the system, supporting the argument of researchers such as Coats et al. (1996) that parasitism is important in the control of blooms; we also suggest that *Alexandrium minutum* may have been removed from both East Harbour, Alexandria, Egypt, and the Penzé estuary, France (see 'Introduction', Fig. 2) by parasite control, although this is clearly speculative. However, there will be parasite-specific variation in virulence, and there is both experimental and environmentally induced variation in virulence, even for a single parasite species (Table 1, Appendix 1). There will also be variation in parameters associated with the ciliate and heterotrophic flagellate growth and grazing responses, and the initial abundances and carrying capacity of the autotrophs may vary. We have, therefore, explored the robustness of our predictions by altering key parameters associated with the parasite biology (Fig. 5), the abundances and carrying capacity of the autotrophs (Fig. 6), and parameters associated with the predator responses (Fig. 7).

Importantly, altering parasite-related parameters had little effect on the various components of the food web, other than the dinoflagellate and the parasite. Furthermore, except by applying the extremes of the estimates of 2 factors, i.e. the number of parasites produced per host and transmission rate (Table 1), there was little impact of changing parameters on our initial predictions for Case 3; this supports our general conclusions even when considering the potential variation of the parasite. Moreover, although varying other aspects of the model impacted a number of components of population dynamics (Figs. 6 & 7), there was a single overriding outcome: within the 50 d simulation, dinoflagellate blooms were closely followed by parasite blooms, with the parasite directly reducing the dinoflagellates almost to extirpation. Although there is clearly variation in the dynamics, these observations support our initial hypothesis that it is the parasite, and not grazing by microzooplankton, that can cause the demise of dinoflagellate HABs.

Eukaryotic parasites, feedback and food web models

For over 100 yr, there has been a growing recognition of the importance of dinoflagellate parasites

(Coats 1999), and in general there has been a recent appreciation of the potential impact of parasites on phytoplankton in a range of marine and freshwater environments (Ibelings et al. 2004, Park et al. 2004). As illustrated in this study, parasites such as *Amoebophrya* sp. may prevent dinoflagellate blooms, including those of toxic dinoflagellates, and ultimately may provide a sufficient pressure to induce a regime-shift (Lees et al. 2006) in the system, leading to removal of the HAB species. Ciliates such as tintinnids may also be parasitized by a species related to *Amoebophrya* sp. (Coats et al. 1994). If this were to occur in our proposed food web (Fig. 3), then we might expect a cascade whereby top-down control on the dinoflagellates and microphytoplankton would be removed, allowing their populations to approach carrying capacities, potentially resulting in very different population dynamics. There has been a recent trend to place viruses into pelagic food web models (e.g. Singh et al. 2004), and, in general, there has been a recognition that parasites must be incorporated into food web models (Marcogliese & Cone 1997). Our study extends this parasite-oriented development of models by indicating that parasites of dinoflagellates should be included in models of plankton population dynamics. Perhaps of greater importance is a recognition that, in general, eukaryotic parasites should be considered in microbial food web models.

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Appendix 1. Information on how parameters and state variables were obtained, beyond details presented in the text and in Table 1

Dinoflagellate, microplankton, and nanoplankton carrying capacities (K) were based on estuarine waters experiencing pulses on the order of 100 to 200 µM dissolved available nitrogen, all of the nitrogen entering the primary producers, and size-specific phytoplankton nitrogen levels as described in Montagnes et al. (1994); we also assumed that the nanoplankton, being an order of magnitude smaller, contained 3 orders of magnitude less nitrogen per cell.

Dinoflagellate mortality rate due to infection (α) was determined from data of Coats & Park (2002), who provide a range of estimates of parasite intracellular times for *Amoebophyra* sp. in 3 dinoflagellate hosts, ranging from ~35 to 60 h. These were then converted to per day rates (0.4 to 0.7 d^{-1}), and temperature corrected from 20°C to our standard 18°C, assuming a Q_{10} of 2 (0.35 to 0.6 d^{-1}). Note, Yih & Coats (2000) found similar times (~45 to 55 h) for *Amoebophyra* sp. infecting *Gymnodinium sanguineum*, under a range of nutrient conditions, at 20°C.

Parasite development rate (α'), i.e. the inverse of the time needed for a parasite to transform from the vermiform stage to dinospores (Fig. 1) was determined from data of Coats & Park (2002); they provided a range of estimates of total parasite generation time (time required to infect host, plus time spent in the host, plus extracellular maturation time) for *Amoebophyra* sp. in 3 dinoflagellate hosts, ranging from ~60 to 70 h. They also provide values of the time spent in the host (see previous paragraph) and from their Fig. 4 (op. cit.) we determined the time required to infect a host as ~10 h. From these we calculated the extracellular maturation time as being between 5 and 10 h. Temperature correcting this value (see above), we obtained a daily rate of 2 to 4 d^{-1}.

Number of parasites released per host (λ) was estimated as 100 to 400, based on data on *Amoebophyra* sp. parasitizing *Gymnodinium sanguineum*, under a range of nutrient conditions (Yih & Coats 2000).

Parasite mortality rate (r_p) was determined from estimates established for *Amoebophyra* sp. parasitizing 3 dinoflagellate hosts, and ranged from –1.0 to –0.26 d^{-1} at 20°C, which was temperature corrected to 18°C, assuming a Q_{10} of 2.

Parasite transmission rate (β) is notoriously difficult to estimate. However, Coats & Park (2002) present experimental data wherein an initial number of uninfected dinoflagellates (10^3 ml^{-1}) was challenged for 36 h with a known number of parasite dinospores (expressed as the dinospore:host ratio) within 10 ml; prevalence of infection (p) was determined and Eq. (A1) was fitted to the data,

$$p = I_{MAX}(1 - e^{-bx}) \tag{A1}$$

where I_{MAX} is the maximum observed infection level and $b = a/I_{MAX}$, where a is the slope of the initial linear portion of the fitted curve and x is the dinospore:host ratio. From this, Coats & Park (2002) estimated a = 66% and I_{MAX} = 98.2% for *Amoebophyra sanguinea*, which can be used to calculate the prevalence of infection (p) at all dinospore:host ratios (x).

Assuming no dinospore or host reproduction or mortality throughout this short experiment, the dynamics of uninfected host cells (D_u), infected host cells (D_i), and parasite spores (P) can be modelled by Eqs. (A2) to (A4):

$$\frac{dD_u}{dt} = -\beta D_u P \tag{A2}$$

$$\frac{dD_i}{dt} = \beta D_u P \tag{A3}$$

$$\frac{dP}{dt} = -\beta D_u P \tag{A4}$$

Note that, as with standard host-parasite models (Anderson & May 1980, 1981), this framework assumes that transmission increases linearly with host concentration. However, in the experiments of Coats & Park (2002), host concentration was kept constant, meaning it is not possible to determine how transmission scales by host concentration. For simplicity, we therefore make the standard assumption of a linear relationship, but acknowledge that this is an approximation; clearly, detailed experiments are needed to quantify the functional form of the parasite transmission relationship before more accurate projections can be made.

In the model given by Eqs. (A2) to (A4), the initial number of D_u (10^4 cells ml^{-1}), D_i (0) and P (given by the initial dinospore:host ratio) are known, as is the final prevalence (calculated from Eq. A1). Solving Eqs. (A2) to (A4) for the expected prevalence ($D_i/[D_u + D_i]$) at the end of the experiment (i.e. 36 h) and solving for β we obtain Eq. (A5):

$$\beta = \frac{\ln\left[1 + \frac{\ln(1-p)N}{P_0}\right]}{Nt} \times 24/100 \tag{A5}$$

where N is the initial number of hosts in the experiment (10^4 ml^{-1}), P_0 is the initial number of dinospores (which is given by xN, where x is the dinospore:host ratio) and t is time (36 h). This expression is multiplied by 24 to convert from h^{-1} to d^{-1} and divided by 100 to convert to l^{-1}. Performing this calculation for various dinospore:host ratio values (x) presented in Coats & Park (2002) provides an average estimate for β of 4.2×10^{-7} d^{-1} l^{-1}, with a range between 7.06×10^{-8} and 7.15×10^{-7} d^{-1} l^{-1}.

Microplankton maximum growth rate (r_m) was not necessarily the maximum potential growth of any phytoplankter. However, the rate chosen was appropriate for dinoflagellates, allows competition, and is well within the boundaries of this size class (see Bissinger et al. 2008)

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2.4 Etude de l'infection de la microalgue *Alexandrium catenella*, produisant des efflorescences toxiques récurrentes dans l'étang de Thau, par des Amoebophryiade

Contexte et objectifs

Historiquement, la Baie de Penzé n'est pas le premier site où une diminution brutale d'efflorescences toxiques a été observée (voir section 2.3). Pour rappel, en Egypte, dans le port d'Alexandrie, *Alexandrium minutum* a produit des efflorescences toxiques chaque année entre 1970, date de sa première observation, à 1990 [Ismael, 2003]. Cette microalgue a d'ailleurs été décrite pour la première fois à Alexandrie, d'où elle tire son nom. Cependant depuis 1990, ces efflorescences ont cessé. Cette disparition brutale a tout d'abord été attribuée à une destruction des kystes due à une forte érosion des sédiments [Ismael, 2003]. En effet, la densité maximale de kystes a été retrouvée à 7 cm en dessous de la surface neutralisant de cette manière les banques de kystes (impossibilité de germer due à l'absence de lumière) [Ismael, 2003]. Bien que l'hypothèse d'un contrôle biologique du type *Amoebophrya* n'ait jamais été testée, cette situation n'est pas sans rappeler la situation décrite en baie de Penzé.
Les observations *in situ* (voir section 2.2) et la modélisation *in silico* (voir section 2.3) ont suggéré que ce parasitoïde était en fait un contrôle efficace de la prévention des efflorescences de dinoflagellés. La dérégulation de l'interaction entre un hôte et son parasite pourrait avoir comme origine un découplage physique entre les deux partenaires, qu'il soit géographique ou temporel. Cependant cette hypothèse ne peut être vérifiée dans l'estuaire de la Penzé car (1) le caractère invasif d'*Alexandrium minutum* n'a jamais été véritablement confirmé et (2) l'interaction entre le parasite et son hôte est déjà établie.
Dans l'optique de tester cette hypothèse, l'algue toxique *Alexandrium catenella* s'est révélée un modèle intéressant car récemment introduite dans l'étang de Thau. Depuis 1998, *Alexandrium catenella* produit des efflorescences toxiques régulières parfois jusqu'à deux fois par an, au printemps et en automne. Nous avons émis l'hypothèse que cette microalgue était capable de produire des efflorescences du fait de l'absence d'infection par *Amoebophrya* spp. L'étang de Thau se situe à une cinquantaine de kilomètre au Sud-Ouest de Montpellier (Golfe du Lion, France, Figure 2.3).

Cette étendue d'eau représente la plus grande lagune de la région du Languedoc-Roussillon (75 km^2, jusqu'à 10 m de profondeur). Depuis 1880, l'activité conchylicole dans l'étang de Thau ne cesse de se développer. Aujourd'hui les tables d'exploitation conchylicoles occupent 1/5 de la surface de la lagune. Environ 13 000 tonnes d'huîtres (*Crassostrea gigas*) et 2 500 tonnes de moules (*Mytilus galloprovincialis*) sont élevées et commercialisées chaque année représentant 10 % de la production nationale [Genovesi-Giunti, 2006]. Cependant en 1998, des proliférations d'*Alexandrium catenella* avec production de toxines ont été observées, entraînant

FIG. 2.3 – A gauche, vue satellite de l'étang de Thau (Google Image). A droite, photo de l'étang de Thau (http://www.bouzigues.fr/musee/images/Ãľtang_vueaerienne.jpg&imgrefurl=http://www.bouzigues.fr/musee/francais/Ãľtang-thau-).

la fermeture des zones de pêche et d'élevage [Masselin *et al.*, 2001]. Depuis, chaque année, cette microalgue produit des efflorescences au printemps ainsi qu'en automne avec des conséquences socio-économiques majeures.

En raison de la régularité des efflorescences, nous nous sommes intéressés au cas particulier d'*Alexandrium catenella* dans l'étang de Thau. Cette microalgue est une espèce invasive génétiquement proche du clade asiatique [Lilly *et al.*, 2002]. Son histoire récente dans l'étang de Thau en fait un modèle d'étude biologique très intéressant par rapport à l'hypothèse de la régulation des efflorescences par le parasitisme.

Dans le cadre de cette étude, les échantillonnages ont été réalisés par Mohamed Laabir (UMR 5119, Université de Montpellier II) et les infections croisées par Mario Sengco (Smithsonian Environmental Research Center, Maryland, USA). Durant cette étude, j'ai acquis et analysé les données à partir des échantillons prélevés dans l'étang de Thau. L'étude de la dynamique des hôtes a été réalisée par la technique Utermölh [Utermolh, 1931]. La recherche du parasite s'est faite par détection à partir de sondes oligonucléotidiques générales des Amoebophryidae (FISH-TSA). Enfin la diversité génétique du parasite a été réalisée par PCR, clonage et analyses phylogénétiques.

Résumé

La capacité des microalgues à produire des efflorescences peut être due à une absence de contrôle biologique de type parasitaire (voir sections 2.2 et 2.3). Cette dérégulation peut être due au découplage géographique ou temporel de l'hôte et de son pathogène comme l'importation

de l'hôte dans un nouvel environnement. Dans le cas de l'estuaire de la Penzé, *Alexandrium minutum* suspectée d'être invasif, s'est propagée le long des côtes Nord finistériennes françaises. Depuis 1988, cette microalgue a produit des efflorescences spectaculaires, par exemple, dans l'estuaire de la Penzé, où *A. minutum* a atteint des concentrations supérieures à 10^5 cellules.L^{-1} [Maguer *et al.*, 2004]. Cependant, depuis 2001, cette microalgue est toujours présente dans l'écosystème mais à de plus faibles concentrations, et ne produit donc plus d'efflorescences (voir section 2.2). Un parasitoïde Amoebophryidae (Syndiniales) s'est avéré responsable de leur régulation, jouant le rôle d'un bio-contrôle naturel (voir sections 2.2 et 2.3). D'autre part, les analyses phylogénétiques ont révélé que les Amoebophryidae forment plus un complexe d'espèces, composé d'une multitude de clades génétiquement distincts (voir section 2.1) [Coats *et al.*, 1996]. Ainsi, *in situ*, les clades de ce parasite sont apparus très spécifiques (voir section 2.2). Un retour dans le passé, lors de l'établissement d'une telle interaction entre l'espèce toxique et son parasite pourrait être riche en information. Nous nous sommes donc intéressés à un autre environnent, l'Étang de Thau, où une ou plusieurs souches d'*Alexandrium catenella* invasive(s) affiliée(s) aux clades asiatiques, a (ont) été récemment introduite(s) [Lilly *et al.*, 2002, Penna *et al.*, 2005]. Depuis 1998, *A. catenella* produit des efflorescences biannuelles. Les Amoebophryidae ont été détectées infectant tous les espèces de dinoflagellés recensées, excepté *A. catenella*. La diversité génétique de ce parasite a été déterminée par séquençage du gène codant pour l'ARNr 18S après l'utilisation d'une PCR biaisée (une amorce spécifique des Amoebophryidae a été utilisée en forward). Une grande diversité génétique a été retrouvée, 10 clades sur les 44 décrits (voir section 2.1). En culture, il est apparu que plusieurs souches de *A. catenella* étaient sensibles à l'infection par un parasite de type Amoebophryidae. En effet des infections croisées ont été réalisées avec une souche de parasite isolée de l'estuaire de Chesapeake (USA) capable d'infecter de nombreuses espèces appartenant au genre *Alexandrium*. Cette sensibilité révèle l'incapacité d'infection par les parasites locaux de l'étang de Thau sur cette microalgue invasive nouvellement importée. Cette étude valide davantage le rôle régulateur de ces parasitoïdes, leur absence pouvant permettre la formation d'efflorescences toxiques.

ALEXANDRIUM CATENELLA (DINOPHYCEAE) IN THE THAU LAGOON (MEDITERRANEAN SEA, FRANCE) WAS THE ONLY DINOFLAGELLATE SPECIES TO NOT BE INFECTED BY THE PARASITOID *AMOEBOPHRYA* SPP. DURING TOXIC BLOOMS.

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Abstract

Toxic blooms may arise from a disruptive connection between toxic microalgae and its pathogens (the enemy release hypothesis, [Keane and Crawley, 2002]. Thus, non-endemic species imported into a new environment may have opportunist behaviour by producing toxic blooms causing illness and huge economic loss. This hypothesis is sustained by several empirical observations, where a toxic dinoflagellate spe-

cies was locally blooming during several years to abruptly stopped, although still present at low concentrations in the ecosystem. The perfect example is provided by *Alexandrium minutum*, which was regularly blooming during more than 10 years in the Penzé estuary (North of Brittany, French coast) from the end of the 80' at more than 10^6 cells per ml [Maguer *et al.*, 2004]. However, from 2004 to 2006, this species was observed to be well controlled by the parasitoid *Amoebophrya ceratii* [Chambouvet *et al.*, 2008]. This control was then successfully simulated under an Anderson-May model coupled with the activity of grazers able to fed on both the host and the free-living stage of the pathogen [Montagnes *et al.*, 2008, Chambouvet *et al.*, 2008]. *Amoebophrya ceratii* is considered to be a « complex species » rather than a single species [Coats *et al.*, 1996]. This hypothesis was reenforced by the genetic diversity analyses of Amoebophryidae (synonymous to alveolate group II) compiled from environmental SSU rRNA sequences extracted from marine waters [Guillou *et al.*, 2008]. At least 44 distinct genetic clades were retrieved. From the Penzé estuary, all dinoflagellate species observed were infected by Amoebophryidae in addition to *A. minutum*. These interactions were highly specific, one host dinoflagellate being infected by one genetic clade, the same parasitoid infecting the same host species year after year [Chambouvet *et al.*, 2008]. Hypothesis that toxic dinoflagellate blooms occur when the pathogens are absent was specifically suggested for Amoebophryidae.

This hypothesis can be easily tested on still blooming species. We thus checked for the presence of Amoebophryidae from the Thau lagoon (South of France) where *Alexandrium catenella* belonging to the Asian clade was recently introduced [Lilly *et al.*, 2002, Penna *et al.*, 2005]. Since 1998, *A. catenella* blooms almost every year, twice per month (during spring and autumn). The presence of this parasitoid was detected infecting all present dinoflagellates species except *A. catenella*. Genetic diversity of Amoebophryidae was assessed by the amplification of the SSU rDNA from field samples using a PCR-biased approach. A huge genetic diversity was retrieved, with 10 clades detected on the 44 already described [Guillou *et al.*, 2008]. Several strains of *A. catenella* were isolated from the Thau lagoon in (1998, 2001-2003). All of these strains were sensitive to infection by a strain of *Amoebophrya* infecting several species of *Alexandrium* from Chesapeake Bay, USA. We thus concluded that *A. catenella* from the Thau lagoon is still blooming because this strain is resistant to local parasites.

Keywords : Thau Lagoon, *Amoebophrya*, *Alexandrium catenella*, Parasitism.

Introduction

Many species of *Alexandrium* (Dinophyceae) are toxic for humans. Most of them produce potent neurotoxins (paralytic shellfish poisoning) that can rapidly be accumulated in bivalve tissues during red tides. These last two decades, *Alexandrium* toxic bloom events have considerably increased, with a lot of species suddenly emerging from previously preserved regions [Vila *et al.*, 2001, Maguer *et al.*, 2004, Penna *et al.*, 2005]. In France, a toxic red tide produced by *A. minutum* was recorded for the first time in July 1985, from the Vilaine Bay (Southern of Brittany), by the REPHY (the monitoring survey in charge by IFREMER, `http://www.ifremer.fr/envlit/surveillance/phytoplancton_phycotoxines`. In August 1988, the same species was simultaneously detected from two shallow estuaries located in the North-West of Brittany of the French coast (the Aber Wrach' and the Aber Benoît, from the Channel). Rapidly extending towards the North-East of Brittany, *A. minutum* toxic blooms were detected in the Bay of Morlaix one year after, in July 1989. Because of its capacity to form resistant diploid cysts that are locally disseminated into sediments, this toxic species was durably introduced in several estuaries of the North of Brittany, blooming almost every year in summer and becoming a recurrent disaster for local aquaculture. This was the case in the Penzé estuary (Fig2.4), where this species was blooming with a remarkable regularity during almost 10 years during summer (Fig2.4 and Fig2.5). This site rapidly became a reference study-case to understand optimal environmental conditions for bloom inductions [Maguer *et al.*, 2004]. However, after 2001, blooms suddenly ended (Fig2.5). Today, *A. minutum* is still present every June, but its growth is limited to low and non toxic concentration (around 10^5 cells per litre). A recent study identified a parasitoid, *Amoebophrya ceratii* Cachon, as the probable causing agent for this regulation [Chambouvet *et al.*, 2008]. This group of parasitoid is really widespread, and belongs to Amoebophryidae (Syndiniales), an order exclusively composed of parasites, still described inside dinoflagellate [Guillou *et al.*, 2008]. Indeed, bounder lines of this species are not well understood, masking extensive genetic diversity, with at least 44 well separated clusters based upon SSU rRNA gene phylogenies [Guillou *et al.*, 2008]. This complex-species is described to infect a wide range of dinoflagellate species, if not all [Cachon, 1964]. From the Penzé estuary, infections were host specific ; with a single parasitoid clade able to infect the same dinoflagellate species, the same genetic clade infecting the same species year after year [Chambouvet *et al.*, 2008]. Multiplication of genetic clades in Amoebophryidae can be well explained by the parasitoid specialisation for a restricted number of host species. Infections always begin by the entrance of a small infective biflagellate cell inside its host [Cachon, 1964]. After rapid nuclear replications, the trophont is finally mature in almost two days [Coats and Bockstahler, 1994, Coats and Park, 2002]. The parasitoid leaves its host as a long filament of cells, with a worm shape, named the vermiform stage [Cachon, 1964]. Within few hours, each constitutive cell of this vermiform will be liberated to produce around 60 to 400 new infective dinospores able to reinfect a new host cell [Cachon, 1964, Coats and Bockstahler, 1994, Coats and Park,

2002, Chambouvet *et al.*, 2008]. From the host, only remains a very thin theca.
Both field observations [Chambouvet *et al.*, 2008] and *in silico* modelisation [Montagnes *et al.*, 2008] suggested that this parasitoid is in fact an efficient controlling factor that could even prevent toxic bloom formations. Otherwise, the hypothesis that red tides can occur in fields because of the absence of these natural pathogens was suggested [Chambouvet *et al.*, 2008].

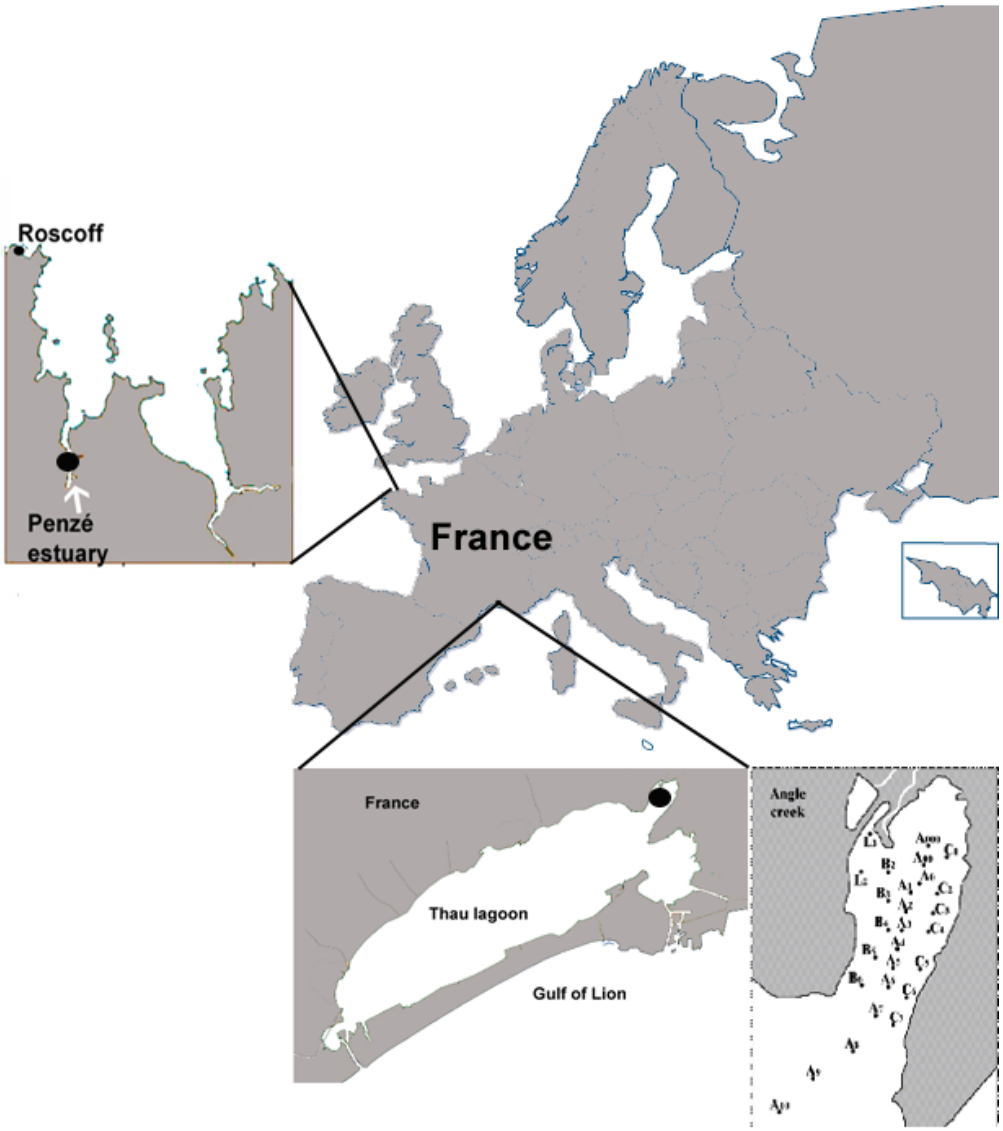

FIG. 2.4 – Location of study site showing the Thau Lagoon (South of France) where *Alexandrium catenella* blooms occurred since 1998. The black circle represents the sampling area and on the Europe map, the star represents the location of Thau lagoon.

The presence of a novel invasive species of *Alexandrium* along the French coasts offers us

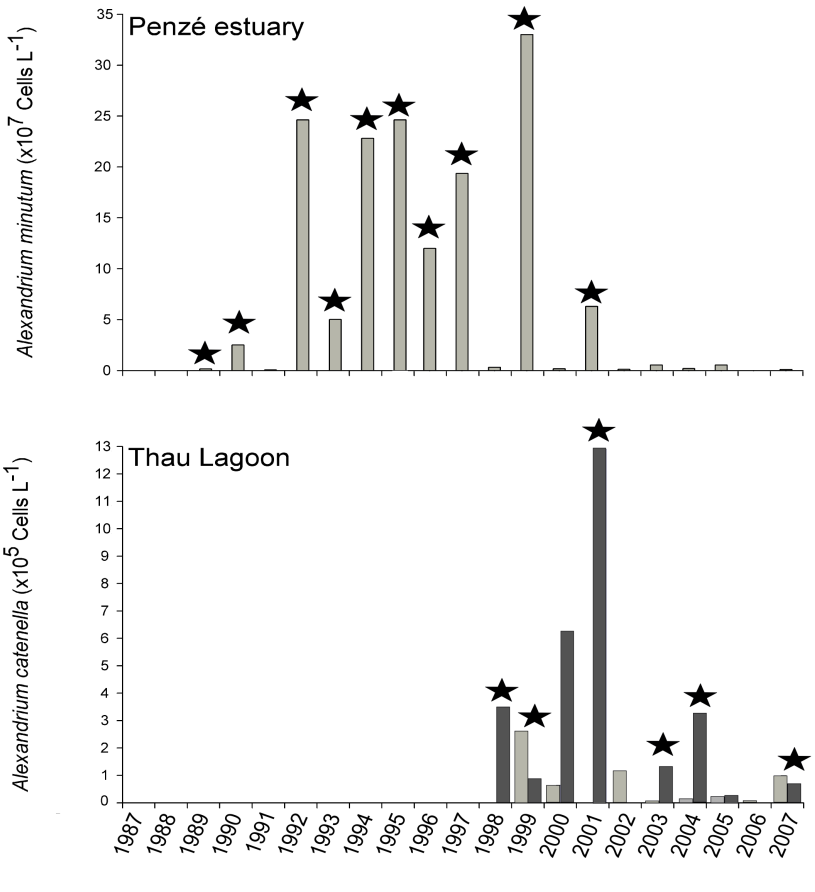

FIG. 2.5 – Occurrence of *Alexandrium catenella* in the Thau lagoon in 2007, as measured by the French monitoring network (IFREMER, France) and from the samples collected during this study. Stars represent toxic events ($>$ 80 μg eq. STX 100 g of bivalves.

the capacity to test this hypothesis from *in situ* environmental conditions. Invasions of *A. catenella* through the Mediterranean Sea have been extensively reported this last decade. This species was first detected along the Catalan coasts [Margalef and Estrada, 1987], but suspected few years after to be expanding along the northwest Mediterranean coasts [Vila *et al.*, 2001]. Along the French coasts, the first outbreak of PSP toxicity was detected in the Thau lagoon in 1998 [Abadie *et al.*, 1999]. Genetic analysis revealed than the species was a strain of *A. catenella* closely related to the Asian Pacific clade [Lilly *et al.*, 2002, Penna *et al.*, 2005].
From these first events, *A. catenella* regularly bloomed during spring and autumn in the Thau lagoon, with toxicity of bivalves regularly reported during autumn (Fig2.5). During the present work, we were interested i) to determine the presence of such parasitoid in the Thau lagoon, ii) to evaluate their capacity to infect *A. catenella*, and iii) tested the resistance capacity of different strains isolated from the Thau lagoon towards one strain of *Amoebophrya ceratii* infecting a wide range of *Alexandrium* species.

Materiel and methods

Study area

The Thau lagoon is a shallow marine lagoon from the marine French Mediterranean coast (43°24'N-3°36'36'E), covering 75 km^2 with a mean-depth of 4.5 m (10 m maximum). This lagoon receives fresh water supplies from different rivers, Vène, Aygues, Vagues, Joncas, and marine water exchanges through two different channels. Aquaculture from this zone provides one of the most important oyster markets in Europe. Since 1998, recurrent blooms of the toxic dinoflagellate *A. catenella* have periodically prevented these economic activities. IFREMER is in charge of the monitoring survey along the French coats. They weekly sampled three main sites ; the Angle Creek (corresponding to A7), Bouzigues (labeled B in Fig2.4 located outside Angle Creek and near the first row of oyster pens) and Marseillan.

Sampling strategy

During the last years, *A. catenella* blooms always originated from the Angle Creek, (Fig2.4) where the sampling for this study was carried out. This semi-enclosed area constitutes one of the main storage areas of *Alexandrium* resting cysts in sediment, and one of the most monitored site for future bloom initiations [Genovesi-Giunti, 2006].
For the survey of *A. catenella* vegetative cells in the water column, a regular monitoring program was established on a weekly basis. A sampling grid with a mesh of 100 m was applied and 27 stations located by GPS were established and distributed along the transects : transect B (stations B2 to B6) located on the West shore of the creek ; central transect (stations A00 to A10) extending from the creek bottom to the beginning of the shellfish structures, and transect C (stations C1 to C7) located on the east shore. Water samples were collected using a pump system at the depth of 50 cm at each selected station. For microplanktonic diversity, 15 ml were fixed using lugol [Utermolh, 1931]. For FISH observations, 200 ml of surface water was fixed with paraformaldehyde (SIGMA) at 4°C during 1 hour (1% final concentration). Samples were size fractionated throughout 10, 3 and 0.2 μm polycarbonate filters and processed as explained in Chambouvet et al. [Chambouvet *et al.*, 2008]. Filters were dehydrated by different successive washing steps using increasing quantities of Ethanol (50%, 80% and then 100%). After a drying step at 37°C, filters have been stored at -20°C.
For genetic diversity analysis, microbial organisms were collected by filtering through 3μm filter and the 0.2 μm Sterivex unit in succession with the peristaltic pump. The Sterivex unit was filled up with lysis buffer (EDTA 40 mM, Tris-HCl 50 mM, saccharose 0.75 M). The 3 μm pore-size filter and the Sterivex unit were kept at -80°C until the DNA extraction.

Numeration of dinoflagellates by microscopy

The diversity of dinoflagellates was determined using the Utermölh method [Utermolh, 1931]. 10 ml were put settling over night and the entire tank were counted with an inverted microscope (IX71, Olympus,).

DNA extraction

DNA extraction was performed using CTAB (hexadeCyltrimathyl Ammonium Bromide) protocol as described by Doyle with some modifications [Doyle and Doyle, 1987]. Sterivex filters were incubated in 3% (w/v) CTAB solution (0.1 M Tris-HCl pH=8, 3% CTAB (Sigma), 1.4 M NaCl, 0.2% β-mercapto-ethanol (Sigma), 20 mM EDTA pH=7.2) preheated at 60°C for two hours. Nucleic acids were extracted twice with an equal volume of chloroform-isoamyl alcohol (96 :4). After two centrifigations for 15 min at 11.000 g (4°C), the aqueous was transferred into a clean tube and two volumes of cold isopropanol were added and incubated at -20°C overnight in order to precipitate the nucleic acids. The nucleic acids were thus recovered by centrifugation (10 min 11.000 g at 4°C). Tubes were drained by gravity and the pellet was dried. Then, pellet were re-suspended in Milli-Q sterile water prior their storage at -20°C.

Validity of the primer/probe ALV01 to amplify/detect the whole group of Amoebophryidae

On the 44 genetic clusters described within the Syndiniales Group II, 11 have at least one mismatch with the primer/probe ALV01 sequence (clades 7, 18, 19, 20, 22, 29, 34, 35, 36, 37, 40). Only part of sequences of clusters 6, 14, 10-11, 21, and 26 are targeted by the ALV01 sequence (from 16% of sequence for clade 14 to 85% of sequences for clade 10-11). The rest of clusters perfectly match this primer/probe (27 clusters in total). All sequences enclosed in the Amoebophrya clade have 100% of match. In particular, this probe perfectly matches the sequence of the parasite infecting *A. catenella* tested during the cross infection experiments in this study.

Analysis of the genetic diversity of Amoebophryidae from field samples

In order to better assess the genetic diversity of Amoebophryidae, we used two different sets of primers during the PCR amplification (Table 2.1). Primers 328f and 329r were used to amplify all eukaryotes from the samples. This primer set is efficiently amplifying Amoebophryidae [Guillou *et al.*, 2008]. We also used the primer ALV01f in complement to the primer 329r, to preferentially amplify target clusters of Amoebophryidae (see chapter concerning the validity of the primer/probe ALV01 to amplify/detect the whole group of Amoebophryidae). PCR mixture using the kit GoTaq™ Flexi PCR (50 μl final concentration) was used for selected samples days to do PCR reaction and performed as described by Promega (1/5 5X Green Go-

Taq™ Flexi Buffer, 4 mM $MgCl_2$, 10 pmol of each primer, 100 μM final concentration dNTPs, 2.5U of GoTaq™ Flexipolymerase). Thirty-five cycles were used, each cycle containing a 30 s denaturation step at 95°C, a 1 min annealing step at 38°C, and a 3 min extension step at 72°C. After the final cycle, the reaction mixture was incubated at 72°C for 10 min. Sequencing reaction was checked on 1% agarose gel. Amplified PCR products were cloned into TOPO™ TA cloning vector (Invitrogene) and insert into competent One-Shot™ TOPO 10 F' E. coli. Plasmids were purified with the kit « plasmid Miniprep96 ™ » (Milliport). Sequencing were done using Sequencing kit version 3.1 (Applied Biosystems) and an ABI PRISM model 377 (version 3.3) automated sequencer using the plasmid primers M13F and M13R at the Ouest-Genopole platform™ of the station Biologique of Roscoff (France).

Primer	Sequence	Target	Lenght (pb)	Use to	Reference
Euk328f	5'-ACC TGG TTG ATC CTG CCA G-3'	Eukaryotes-All	1875	DNA amplification	Moon-van der Staay, 2000
Euk329r	5'-TGA TCC TTC YGC AGG TTC AC-3'	Eukaryotes-All		DNA amplification	Moon-van der Staay, 2000
Alv01	5'- AGA GUG UUC ACG GCA GGC-3'	Alveolate Group II	740	DNA amplification	Chambouvet *et al.* 2008
M13f	5'-GGG CAT CAC AGA CCT G-3'	pCR2.1-TOPO® vector		Sequencing reaction	Invitrogen
M13r	5'-GCT GCC TCC CGT AGG AGT-3'	pCR2.1-TOPO® vector		Sequencing reaction	Invitrogen

TABLE 2.1 – 18S primer sequences used in this study

Detection of Amoebophryidae by CARD-FISH

The Alv01 oligonucleotide probes were used with the modified CARD-FISH protocol explained by Chambouvet *et al.* [Chambouvet *et al.*, 2008] using the 10 μm pore-size filters. Hybridizations were thus carried out at 42°C during at least 15 hours, and then two successive washing steps at 46°C during 20 min were necessary to remove background. Photos were acquiring using epifluorescence microscope BX51 (Olympus). Field samples collected from the Penzé estuary (Brittany), where parasites belonging to Amoebophryidae are known to occur, were used as positive control.

Sequences analysis

Around 50 clones per genetic library were sequenced. The KeyDNAtools software (available online `http://www.pc-informatique.fr/php-fusion/news.php`), was used i) to identify putative chimera and ii) assign each environmental sequences to a clade as defined by Guillou *et al.* [Guillou *et al.*, 2008]. One representative clones per clade and per genetic libraries were selected to be entirely sequenced. These sequences were aligned with a selection of published sequences, mostly from the Penzé estuary (Brittany, [Chambouvet *et al.*, 2008], using Clustal W2 software [Larkin *et al.*, 2007]. Different nested models of DNA substitution and associated parameters were tested using the Modeltest v3.06 [Posada and Crandall, 1998]. A GTR+G model was selected using the following parameters : Lset Base= (0.2310 0.2266 0.2865), Nst=6, Rmat= (1.1243 2.3842 1.3011 1.2140 3.7847), Rates=gamma, Shape=0.7913, Pinvar=0. Settings given by Modeltest were used to perform Neighbour Joining (NJ), the maximum likelihood (ML) and bayesienne analyses.
NJ and maximum parsimony (MP) were performed using PAUP 4.0b10 [Swofford, 1993]. Bootstrap values for NJ and MP were estimated from 1,000 replicates. For MP, the number of rearrangement was limited to 5,000 for each bootstrap replicate. Additionally, we used Bayesian reconstruction for the analysis of complete sequences with MrBayes, v3.1.2 started with random tree, run for 2,000,000 generations in four chains and burn-in of 5,000 generations in order to ensure the use on stable chain [Huelsenbeck and Ronquist, 2001].

Cross infections

Several strains of *A. catenella* have been isolated during toxic bloom events occurring in the Thau lagoon at different years (1998, 2001, 2002, and 2003, Table 2.2). The strain of *Amoebophrya* was isolated by Mario Sengco and specificity of this strain will be described in a separate study. Sensitivity of host strains were tested by cross infections, using freshly produced dinospores from the *Amoebophrya* cultures growing on *A. tamarense* strain isolated from the US coats. Infections in host cultures were checked every day by microscopy, using the natural autofluorescence of the parasitoid for its detection [Coats and Bockstahler, 1994].

Results

Occurrence of *A. catenella* during 2007

During 2007, dinoflagellates and *Euglena* sp. (Euglenozoa, protist) were the main observed species along all the samples. Presence of toxic dinoflagellate *A. catenella* was reported by IFREMER mainly during late spring and early summer (from the 10 of May to the 19 of June 2007) and the autumn (from the 10 of September to the 12 of November 2007 ; Fig2.5). As previously described, these periods initiated at the Angle Creek, were maximal *A. catenella* concentrations were 10^5 Cells.L^{-1} in spring. During autumn, two picks were observed, with

Taxonomy	Strain name	Origin	Isolation year	Isolation procedure	Medium
Host					
A. catenella	ACT1	Thau lagoon	2001	Vegetative cell, monoclonal	ESAW
	ACT2	Thau lagoon	2002	Cyst germination, monoclonal	ESAW
	ACT3	Thau lagoon	2003	Vegetative cell, polyclonal	ESAW
	TL001	Thau lagoon	1998	Vegetative cell, monoclonal	ESAW
Parasitoid					
Amoebophrya sp.	GOM	ND	2003	*A. catenella* primary host in culture	F/2 + 5% soil extract

TABLE 2.2 – List of strains used in this study

maximal densities of 6.9 and 6.3 $\times$ 10^4 Cells.L^{-1}, the 17 of September and the 22 of October 2007, respectively (Table 2.3). *A. catenella* maximal concentrations slowly reached 10^4 and 10^2 Cells.l^{-1} at the Bouzigues and the Marseillan sites, respectively. During 2007, only mussels (*Mytilus edulis*) were detected with toxicity upper than 80 μg.eq.STX.hg^{-1} by IFREMER, during two consecutive sampling days at the Bouzigues site in autumn (the 5 and the 12 of November 2007) (Fig2.6).

Dates (dd/mm/yyyy)	*Alexandrium catenella*	*Peridinium quinquecorne*	*Prorocentrum micans*	*Scrippsiella* sp.	*Dinophysis* sp.	Sample site
Spring bloom						
26/04/2007*	0	0	0	0	0	A3
10/05/2007	1.5 x10^2	3 x10^1	5 x10^1	6 x10^1	1 x10^1	C5
16/05/2007	2.4 x 10^2	3 x10^1	1 x10^1	0	2 x10^1	C5
24/05/2007*	ND	ND	ND	ND	ND	A3
30/05/2007	2 x10^2	0	3 x10^1	0	0	A00
07/06/2007*	3.3 x 10^3	1 x10^2	0	1.1 x10^2	1 x10^1	C5
Autumnal bloom						
20/09/2007	1.23 x 10^5 6.4x10^4	ND	ND	ND	ND	B3 A3
11/10/2007	7.5 x 10^4 8.8x10^3	ND	ND	ND	ND	B3 A3
15/10/2007*	1.68x 10^5 6.1x10^4	ND	ND	ND	ND	B3 A3

TABLE 2.3 – List of environmental samples with corresponding concentration of different dinoflagellates found in Thau Lagoon (Cells.l^{-1}). *Select dates for genetic libraries ; ND : not determinate

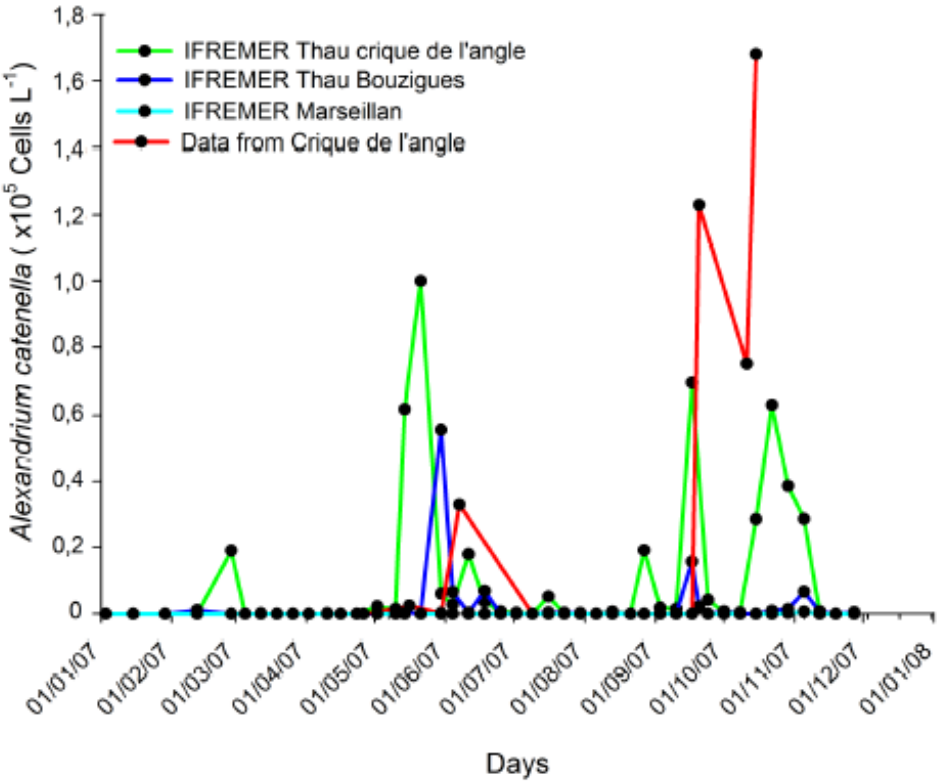

FIG. 2.6 – Monitoring of *Alexandrium minutum* concentration from Penzé estuary (North Brittany, France) (A) and *Alexandrium catenella* from Thau lagoon (South of France) (B) over two decades. (A) represents the bloom occurring once per year in summer, while (B) represent the two successive blooms of the year (in light grey, the spring bloom and in dark grey the autumn bloom). Data provide of the French monitoring of phytoplankton network, REPHY (IFREMER).

According to the IFREMER data, the *A. catenella* spring bloom was sampled at the very beginning (the 10 of May 2007), and during the decline (the 7 of June 2007) of the microalgae. Measured cell concentrations from these samples were always above the values reported by IFREMER during the bloom (Table 2.3). The *A. catenella* autumnal bloom was mostly sampled between the two picks reported by IFREMER. However, densities of *A. catenella* that we measured from our samples were always above the IFREMER data by at least a factor two, with maximal cell concentrations of 1.68×10^5 Cells.l^{-1} the 15 of October 2007 (at the same date and for the same site, IFREMER reported a concentration of 2.8×10^4 Cells.l^{-1}). *A. catenella* was the dominant dinoflagellate in all samples analyzed. Other accompanying dinoflagellate species were *Peridinium quinquecorne*, *Scrippsiella* sp., *Prorocentrum micans*, and *Dinophysis* sp., with concentrations never exceeding 2×10^2 Cells.l^{-1}.

Detection of Amoebophryidae by CARD FISH

Positive cells, targeted by the specific Amoebophryidae (Syndiniales Group II) probe, were observed from all sampled dates. Each time, trophont maturation stages were observed inside different host species (Fig2.7). All dinoflagellates (and only dinoflagellates) were infected (see examples of *Prorocentrum* sp., *Peridinium quinquicorne*, *Scrippsiella* sp. on Fig2.7, with the exception of the toxic species *A. catenella* that was never bind by the probes whatever the date considered (Fig2.7). Furthermore, the *A. catenella* nucleus, stained by the propidium iodide, was always presenting the typical ring like shape. Additional nucleus, very abundant when the parasite develops inside its host (Fig2.7), was never observed inside the *A. catenella* vegetative cells.

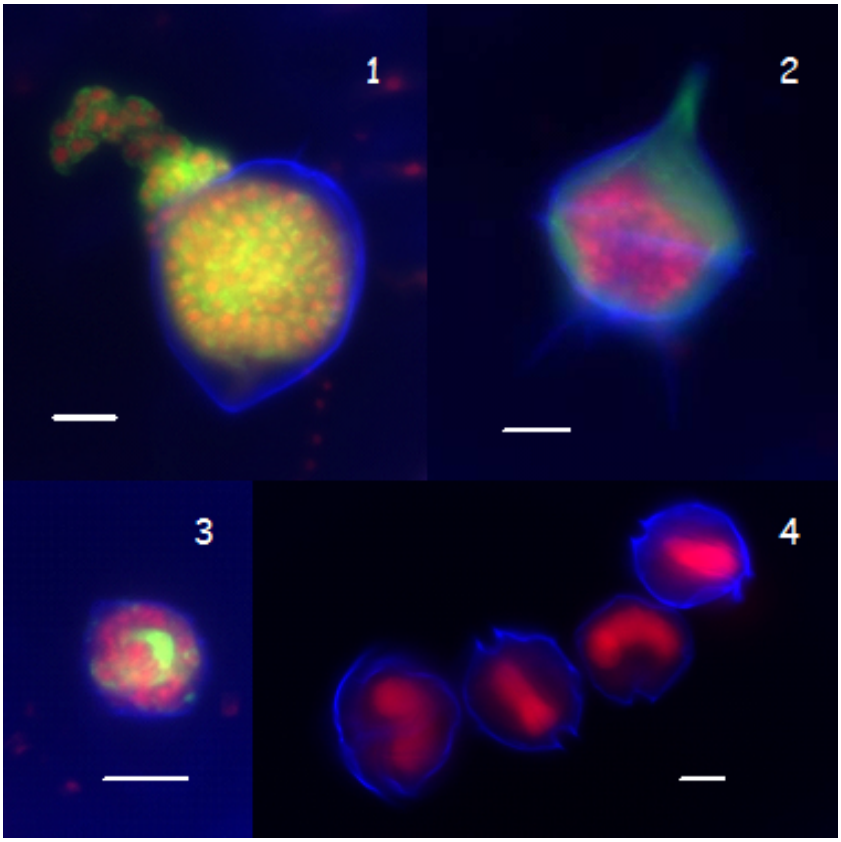

FIG. 2.7 – Detection by FISH of infected dinoflagellates by parasitoid belonging to Syndiniales Group II from the Thau lagoon. 1-Infected cell of *Prorocentrum* sp. from the 11 of October 2007, 2-Infected cell of *Peridinium quinquicorne* from the 11 of October 2007, 3-Infected cell of *Scrippsiella* sp. from the 26 of April 2007, 4-Non-infected cells of *Alexandrium catenella* from the 11 of October 2007.

Genetic diversity of Amoebophryidae during the monitored period

The analyses of the genetic diversity of Amoebophryidae were performed on 3 sampled dates during the spring bloom (26/04/2007, 24/05/2007 and 07/06/2007) and one sample during the autumnal bloom, (the 15/10/2007, Table 2.1 and Fig2.8). In total, 112 environmental sequences from Thau lagoon belonging to Amoebophryidae were collected, and 28 of them were completely sequenced (mean length of 740 bp).
Two Syndiniales, *Hematodinium* sp. and *Syndinium* sp., infecting crustaceans, were added to root the phylogenetic tree. On the 44 clades already described within Amoebophryidae, 10 were retrieved from the Thau lagoon using only 4 different samples. Several strains of *Amoebophrya* spp. infecting a wide range of dinoflagellate species are closely related to sequence retrieved from the Thau lagoon, in particular within clades 1, 2, and 4. Four clades on the 10 were also retrieved from the Penzé estuary, in the North West of France (Clades 1, 2, 14, and a new clade closely allied with clade 2). Otherwise, the remaining clades have representatives collected from coastal and oceanic marine waters, as well from hydrothermal vents and anoxic sediments (in particular clades 23 and 28). Clades 1 and 4 have been retrieved both from the spring and the autumnal periods, with very similar sequences (for clade 1, the sequences identity between spring and autumn reached 99%, while for clade 4 between in 95% to 98%). However, Clades 2, 14, 23, 28, and 44 have been only retrieved from spring (but 3 genetic libraries were performed from this period), and clades, 8, 10-11, and 12, were only retrieved from the autumnal period.

Cross-infection

Cross infections were performed using the strain infecting *A. tamarense* and isolated from the Chesapeake Bay, USA (belonging to clade 2, see Fig2.8) and four strains of *A. catenella* isolated from the Thau lagoon between 1998 and 2003.
In all case, this *Amoebophrya* strain was able to successfully infect each host culture.

Discussion

Annual observations of *A. catenella* abundances in the water column in the Angle Creek since 2002 showed that the distribution of vegetative cells was heterogeneous according to a patchy mode (Vaquer, Unpublished data). Blooms initiated usually in the Angle Creek where they reached the higher concentrations and could extend to other areas of the lagoon when favourable hydrodynamical conditions appeared. This explain why the measured *A. catenella* values in the stations located inside The Angle Creek are higher to those measured in the stations monitored by IFREMER. This heterogeneity also explains the variability of density observed between sample collections.
A. catenella was the most abundant dinoflagellate species during the two sampling periods, a presence and a domination that extend over at least one month both during the spring and the

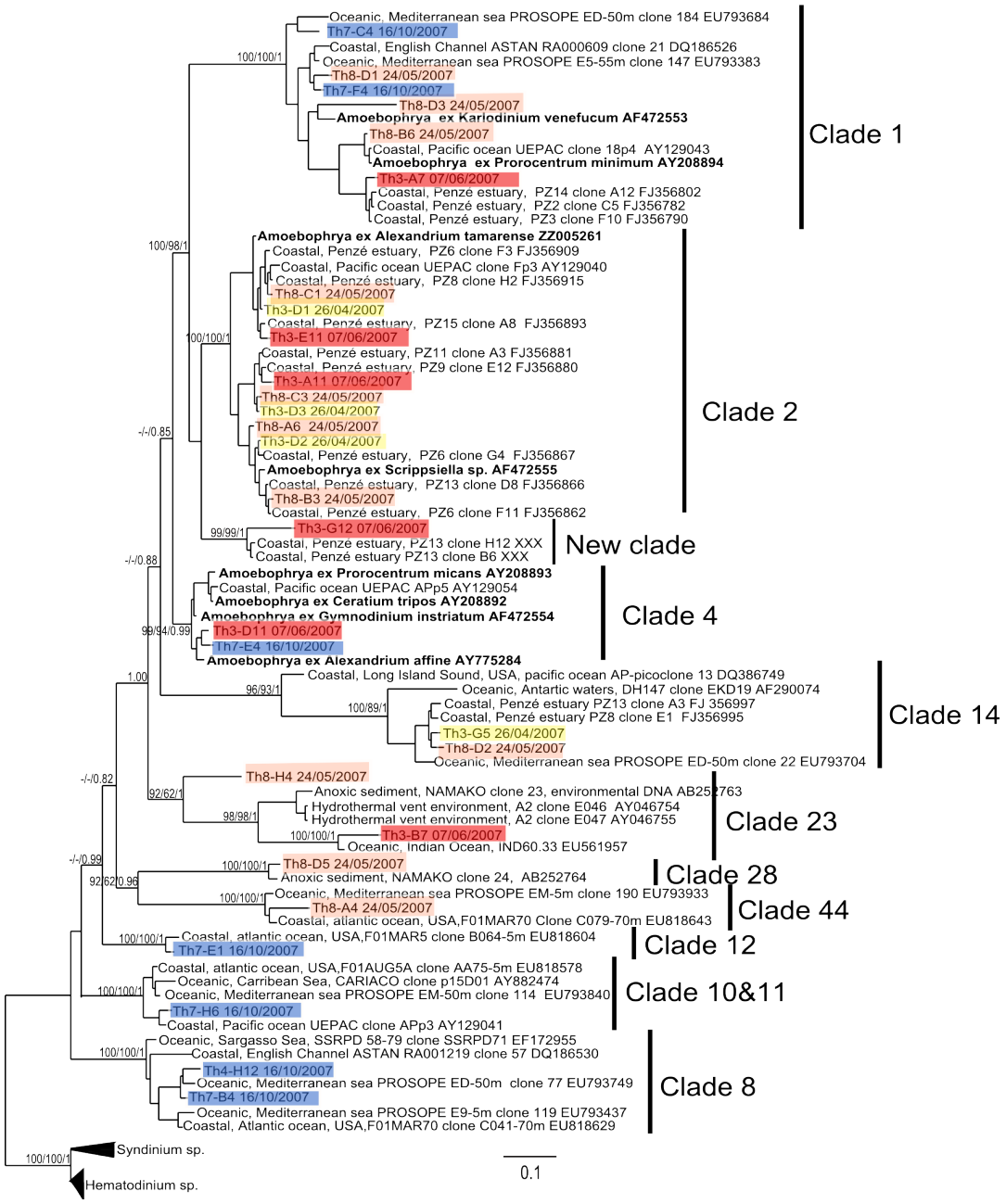

FIG. 2.8 – Environmental sequences belonging to Syndiniales Group II. Bayesian phylogeny based upon the analysis of 82 full length sequences, 740 bp in length (including outgroup). Sequences from the Thau lagoon are highlighted with a colour corresponding to the sample date ; the 26/04/07 in yellow, the 24/05/07 in pink, the 07/06/07 in red and the 15/10/07, in blue. Sequences retrieved from cultured *Amoebophrya* sp. are in bold. Neighbour-Joining and Maximum parsimony bootstrap values associated with the Bayesian posterior probability, higher to 70% are shown at the principal node of the tree. The scale bar corresponds to 0.1% sequence divergence.

autumn. Whilst all dinoflagellates were observed to be infected by Amoebophryidae in the Thau lagoon, the toxic strain of *A. catenella* is not affected. However, this strain can be well infected by a parasitic strain isolated from the American coast infecting *A. tamarense*. These results strongly suggest that the *A. catenella* strains that occurred in the Thau lagoon during 2007 was resistant to at least all Amoebophryidae retrieved from the genetic libraries performed in this study (the 10 clades observed). This resistance is well illustrated by the persistence of these species in this ecosystem (more than one month), and by the recurrence of toxic events caused by this toxic species since 1998. These results also confirm that such parasitoids are highly specific in field. However, in cultures, strain of *Amoebophrya* may have a larger host range, as illustrated by the strain tested in this study, specific of A. tamarense, but also able to infect *A. catenella*. It is well known that cross infections in laboratory represent often more permissive conditions than environmental conditions [Poulin and Keeney, 2007]. This also minds that local parasites may also adapt with time to specifically infect the *A. catenella* strain occurring in the Thau lagoon. In fact, the lack of a clear coevolution within *Amoebophyra* strains and their host indicated the capacity of these parasitoids to relatively easily jump from one host to the other [Guillou *et al.*, 2008].

Change of primary hosts is not uncommon in parasitic interactions. We can cite the example of *Gyrodactylus* sp., parasite of fishes, which have jumped between different host families. Such events often led to speciation, leading to a faster evolution for parasites than for the hosts. These mechanisms occur during long-term cohabitation between a parasite and the novel host species, time required for the parasite to adapt itself against host defence system [Zietaria and Lumme, 2002].

Importation of species into new environments involves disruptive interaction between hosts and their pathogens. This phenomenon has already been described for plants, conceptualised under the Enemy Release Hypothesis (ERH) [Keane and Crawley, 2002]. Plants introduced into an exotic area do not suffer of the pressure by herbivorous and others natural enemies such as parasite, which are not yet adapted, resulting in an increase of abundance and distribution. By comparison, it appears that the densities of *A. minutum* in the Penzé estuary, occurring from the 90', were controlled after 2001. Thus, more than 10 years were necessary for this parasitoid to efficiently control the *A. minutum* host populations. In the Thau lagoon, *A. catenella* appears for the first time in 1988, ten years ago, confirming that it takes time for this type of bio-control to emerge. The use of Amoebophryidae as a biological control can be very tempting to reduce this period. We know that the *A. catenella* from the Thau lagoon is sensible to a strain isolated from the US. However, the use of biocontrol against pests was often found in the past as worst than the pest itself. We can cite in Europe the use of *Harmonia axyridis*, a voracious coccinellid beetle native from Asia (like *A. catenella*), introduced to North America and Europe to control aphids and scale insects, and become now dangerous invaders for endemic populations.

In conclusion, the Thau lagoon offers the unique opportunity to follow the successive steps required by parasites, such as Amoebophryidae, to adapt to a new and invasive species in a semi-closed ecosystem. We probably have a very partial vision of how toxic dinoflagellates are controlled from natural samples, and many other biological factors probably interact, and we sincerely hope for oyster productions that this natural bio-control will not be too long to establish itself in the Thau lagoon.
However, with our present knowledge, we can considered this study as an evaluation of the initial conditions (a sort of T0) prevailing in the Thau lagoon, with invasive *A. catenella* strains still non-infected to date, and with a list (probably non exhaustive) of ten potential parasitoid candidates existing from the Thau lagoon. As this species was able to colonize almost all the Mediterranean Sea, it will be also interesting to compare weather this strain is infected by Amoebophryidae along the Mediterranean coasts, and compare their genetic clades.

Acknowledgements

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2.5 Couplage étroit entre *Amoebophrya* sp. et *Scrippsiella trochoidea*, relié à la sexualité de l'hôte

Contexte et objectifs

Suite à l'étude *in situ* (voir section 2.2) ainsi qu'à la modélisation *in silico* (voir section 2.3), nous avons montré que ces parasites exercent un contrôle efficace sur les populations hôtes. Chaque type génétique de parasite infecte spécifiquement une espèce hôte dans l'estuaire de la Penzé (voir section 2.2). Ainsi, année après année, chaque parasite infecte le même hôte à la même période de l'année. Néanmoins, la plupart des dinoflagellés ne sont présents que saisonnièrement et absents le reste de l'année. Par exemple, dans la baie de Penzé, *Alexandrium minutum* n'est présente dans l'année qu'entre les mois de mai et juin généralement (voir section 2.2). Durant l'hiver, les dinoflagellés forment en général des kystes de résistance diploïdes, produits grâce à la reproduction sexuée, et qui sédimentent au fond de l'estuaire [Wang *et al.*, 2007, Anderson *et al.*, 2005]. La spécificité d'infection observée *in situ* nécessite donc un couplage très fort entre le cycle de vie du parasite et celui de l'hôte. En effet, il est apparu *in situ* et en culture que le parasite ne survivait pas longtemps en dehors de son hôte. Coats et collaborateurs ont montré que l'abondance des dinospores d'*Amoebophrya* infectant *Gymnodinium instriatum* et *Karlodinium micrum* en culture, diminue exponentiellement en fonction de la souche de parasite considérée, le nombre de dinospores devenant inférieur au seuil de détection à partir du troisième jour ou du treizième respectivement (250 cellules.L^{-1}) [Coats and Park, 2002]. D'autre part, la même étude a montré que les capacités d'infection diminuaient en quelques jours en fonction de l'âge des dinospores. Les dinospores d'une souche d'*Amoebophrya* infectant *G. instriatum* et *K. micrum* perdent leur caractère infectieux respectivement à partir du 23^{me} jour et du 8^{me} jour [Coats and Park, 2002]. Il se pourrait donc qu'*in situ* le parasite soit physiquement présent à l'intérieur d'un hôte secondaire plus permanent dans la colonne d'eau, ou sous une forme de résistance jusque là inconnue, ou les deux. En effet, de nombreux Alvéolés parasites tels que *Plasmodium falciparum*, agent de la malaria, ont des hôtes secondaires. Par ailleurs, les Syndiniales restent des dinoflagellés, même si ils sont très modifiés, et sont donc peut-être capables de produire des kystes de résistante par reproduction sexuée.

Parallèlement, un hôte ne subit pas passivement l'attaque du parasite. Des réactions de défense sur du court-terme peuvent être utilisées, par exemple, des molécules chimiques peuvent être induites pour contrer le pathogène (allélopathie). Ce mécanisme est aussi bien décrit chez des organismes planctoniques [Ibelings *et al.*, 2004] que chez les organismes supérieurs, par exemple, les plantes [Dicke and Bruin, 2001]. Par exemple, quelque secondes après un stress mécanique, les diatomées *Asterionella* et *Thalassiossira*, produisent une molécule défensive qui est en fait un puissant fongicide contre des champignons parasites [White *et al.*, 2000]. Chez les dinoflagellés, en cas de stress la réaction de défense se traduit généralement par la formation de kystes temporaires (haploïdes) ou permanents (diploïdes). C'est le cas d'*Alexandrium ostenfeldii* qui,

face à un parasite, *Parvilucifera infectans* (Perkinsozoa), induit par signal moléculaire la formation de kystes temporaires [Toth *et al.*, 2004].
Dans cette étude, nous nous sommes donc intéressés à deux aspects de l'interaction hôte-pathogène : (1) quels peuvent être les mécanismes de défense de l'hôte face au parasite et (2) que devient le parasite en l'absence de son hôte.

Grace à la collaboration avec Wayne Coats (Smithsonian Environmental Research Center, Maryland, USA), pendant le suivi de 2007 dans l'estuaire de la Penzé, nous avons été capables d'isoler et de maintenir en culture une souche du parasite *Amoebophrya* infectant un dinoflagellé non toxique *Scrippsiella trochoidea*.
Dans le cadre de cette étude, mon travail avec l'aide de Valérie Cueff (Master 2 de l'Université de Bretagne Occidentale) a consisté à mettre en place et procéder à l'expérimentation en culture. La quantification des dinospores a été réalisée par cytométrie en flux en collaboration avec Dominique Marie (Station biologique de Roscoff).

Résumé

La reproduction sexuée est généralement considérée comme une réponse avantageuse au cours d'interactions antagonistes. Au cours de la méiose, des recombinaisons génétiques innovantes peuvent être produites, et de nouvelles résistances peuvent alors être créées puis sélectionnées positivement (Théorie de la reine rouge) [Jaenicke, 1978, Van Valen, 1973]. Dans le monde microbien, un changement de niveau de ploïdie, lié à une importante métamorphose morphologique et physiologique, peut aussi conférer une invulnérabilité immédiate face à un pathogène (« The Cheshire Cat » dynamiques) [Frada *et al.*, 2008]). Dans cette étude, nous avons revisité ces deux stratégies du point de vue du pathogène. *Amoebophrya* spp. (Syndiniales) est un parasitoïde obligatoire de dinoflagellés marins, incluant certaines espèces toxiques (voir pour revue [Coats, 1999, Park *et al.*, 2004]. Nous avons observé que la sexualité de l'hôte, *Scrippsiella trochoidea*, était induite en présence du pathogène. En effet, cette espèce produit des kystes calcifiés diploïdes au contact direct de ce pathogène. Grâce à la fluorescence naturelle du parasite, nous avons été capables de détecter sa présence à l'intérieur même des kystes de résistance de son hôte (3 % de prévalence). Après une période de dormance, la germination du kyste libère le pathogène et de nouveaux cycles infectieux sont alors observés. Cette stratégie efficace lie durablement le parasitoïde à son hôte dans le temps et dans l'espace, ce qui permet d'expliquer la spécificité des associations observées années après années dans le milieu naturel. Dans ce cas, la sexualité de l'hôte, considérée généralement comme un mécanisme de défense efficace de l'hôte, est détournée, et pourrait être induite par le pathogène lui-même.

PARASITISM AND SEX : WHEN NATURE OVERRULES THEORY

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Sexual reproduction is widely considered as an advantage during antagonist associations, since innovative genetic recombinations generated during meiosis may bring new resistances (the Red Queen Hypothesis [Van Valen, 1973,Jaenicke, 1978]. In the microbial world, a change of ploidy, link with deep morphological and physiological metamorphoses, may confer immediate invulnerability towards pathogens (the Cheshire Cat dynamics [Frada *et al.*, 2008]. Here, we revisit both strategies from a pathogen perspective. *Amoebophrya* spp. (Syndiniales) are obligatory parasitoids of marine dinoflagellates, including several toxic species (for review [Coats, 1999, Park *et al.*, 2004]. Consistent with theory, we observed that sexuality of the host, *Scrippsiella trochoidea*, is promoted in the presence of its pathogen ; this species produces diploid calcified cysts, resistant to the pathogen. However, the pathogen can be detected inside 3% of these cysts. After a dormant period, cyst germination releases the pathogen, and new infective cycles are observed. This strategy efficiently binds the parasitoid to its host in time and space, thereby helping to explain the apparent specificity of these associations and their recurrence in the field over years [Chambouvet *et al.*, 2008]. Thus, in this case, the benefit

of host sexuality as an efficient defence against pathogens is overruled, with the pathogen possibly promoting host sexuality to ensure its own survival.

The Syndiniales (Alveolata) are marine parasitoids that infect a wide range of host species, ranging from unicellular protists to commercially important crustaceans, such as crabs and lobsters [Chatton, 1920]. Among them, the Amoebophryidae (Syndiniales Group II) preferentially infect protists, with many reports for dinoflagellates, a key component of the marine phytoplankton, with some toxic species also known to produce harmful algal blooms (HAB). Both *in situ* observations [Chambouvet *et al.*, 2008] and *in silico* modelisation [Montagnes *et al.*, 2008] suggest that these pathogens efficiently control their dinoflagellate populations, even toxic species. In an estuarine ecosystem, these interactions were found to be host specific ; one dinoflagellate species being infected by a genetically distinct pathogen [Chambouvet *et al.*, 2008]. Additionally, the same genetic parasitic type returns to infect the same host species year after year [Chambouvet *et al.*, 2008]. Because of the regulating capacity and specificity of these parasitoids, toxic dinoflagellate blooms were suggested to arise from the disruption of such biological controls.

However, at least one step is missing from this comprehensive overview. Most dinoflagellates have a marked seasonality and are virtually absent from the water column most of the year. Their survival during winter is widely ensured by the formation of diploid resistant cysts, produced throughout sexual reproduction [Perez *et al.*, 1998, Pfiester, 1977]. On the other hand, the free-living stage of the parasitoid only survives a few days outside its host, as evident from cultures [Coats and Park, 2002] and field observations [Chambouvet *et al.*, 2008]. Yet unresolved is how the parasitoid survives during the period of host dormancy, emerging the following year to infect the same dinoflagellate species.

We partially resolved this enigma using a culture of the dinoflagellate *Scrippsiella trochoidea*, a widespread species, typically blooming in eutrophic coastal waters during summer [Smayda and Reynolds, 2001]. In the Penzé estuary (Western Brittany, France), this species is chronically infected by a specific parasitoid belonging to the Amoebophryidae, clade 2 [Chambouvet *et al.*, 2008]. Last year, we successfully isolated this parasitoid. While uninfected host cultures typical showed growth rate of 0.6 div.d^{-1}, cultures mixed with the parasitoid rapidly crashed after two days (Fig2.9). Calcified cysts, produced by sexual reproduction, are oval and covered by numerous calcareous spines [Sgrosso *et al.*, 2001, Olli and Anderson, 2002, Meier *et al.*, 2007](Fig2.11). In all experimental and control treatments, monoclonal *S. trochoidea* cultures continuously produced these cysts. However, in only two days, total number of cysts was 8 times higher in presence of the parasitoid (Fig2.9). Thus, cyst production, resulting from the host sexuality, is clearly stimulated by the presence of the pathogen. After a few weeks, the vegetative haploid cells of the dinoflagellate and the free-living stage of the parasitoid completely disappeared from the culture, leaving only diploid resistant cysts of *S. trochoidea*. Cyst number remained constant for two months indicating that they were resistant to the pathogen.

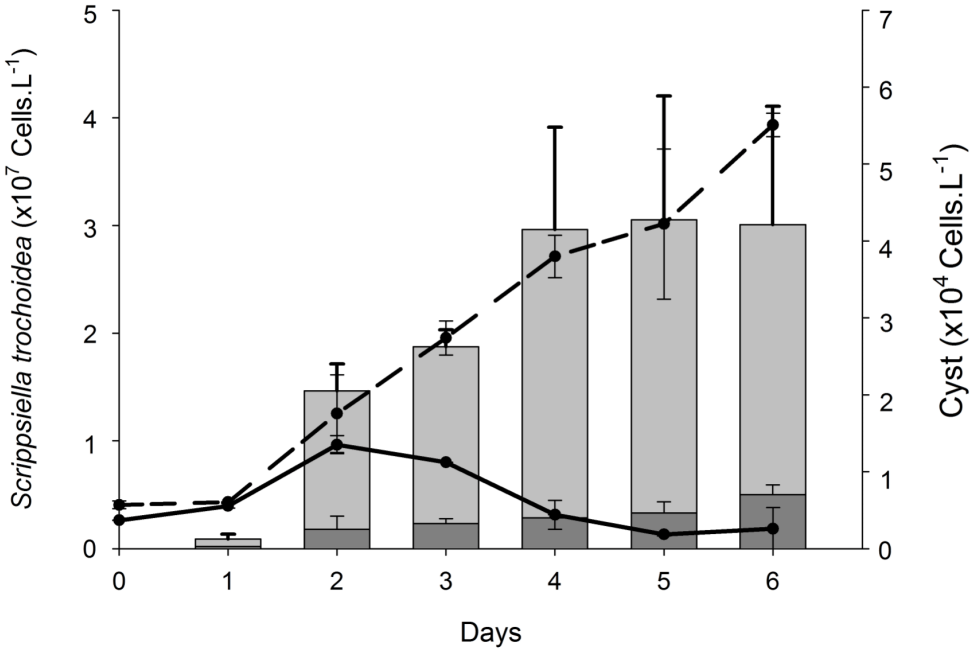

FIG. 2.9 – Host dynamics in cultures and simultaneous production of resistant cysts during infection of *Scrippsiella trochoidea* by its parasitoid (Syndiniales, Amoebophryidae Group II, clade 2). Dash line for non-infect, and continuous line for infect culture of *S. trochoidea*, left scale. Histograms in light grey for resistant cyst production over time in infected cultures and in black grey for uninfected cultures, right scale. Error bars indicate standard deviation between quadruplicates.

We tried to induce the production of cysts by inoculating fresh exponentially growing dinoflagellate culture with filtrate from a 2-days old parasitoid culture passed through a 0.22 μm pore-size filter (pathogen-free, incubations vol/vol). No significant difference in cyst production was detected compared to the negative controls (Fig2.12). Thus, activation of host sexuality appears to require physical contact with the parasitoid, rather than an allelopathic signal.
Sexuality is believed to be an efficient defence against parasitoids, because new host resistance may be acquired by random genetic recombinations. This hypothesis, also known as the Red Queen Hypothesis (RQH) [Van Valen, 1973], is currently considered as the main driving force for the evolution and the universality of sex in nature [Jaenicke, 1978]. More recently, diachronic ploidy variations were implicated in the physical escape from pathogens on short time scales. Thus, the widespread *Emiliania huxleyi* (Haptophyta) may escape its virus by a simple mitotic reduction, because the haploid stage is resistant (the Cheshire cat strategy) [Frada *et al.*, 2008]. Both strategies fit well with the massive production of resistant cysts by *S. trochoidea* in the presence of its parasitoid ; the change of ploidy driving both the capacity for the host to acquire innovative recombinations and a rapid physical resistance.
However, the parasitoid is able to overrule these theories. We carefully observed the cultures

by epifluorescence microscopy. The parasitoid has the particularity to naturally emit a bright green autofluorescence under a blue light excitation during all phases of its life cycle [Coats and Bockstahler, 1994]. Intriguingly, the parasitoid was detected inside its host cysts (Fig2.10), with about 3% of fresh host cysts produced in the presence of the pathogen exhibiting this typical bright green autofluorescence in part of their cellular content.

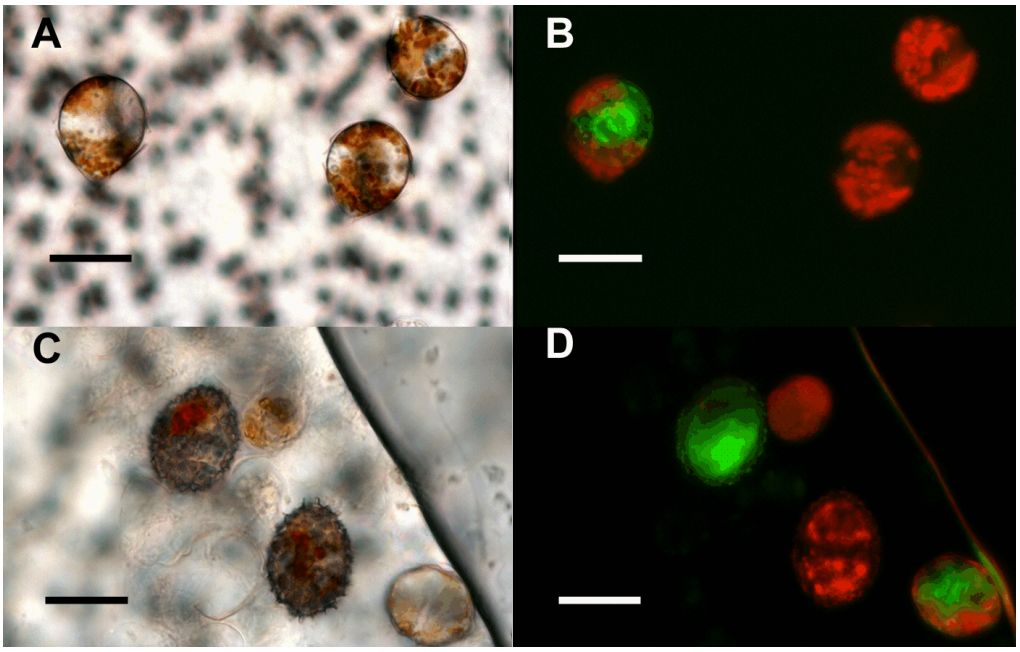

FIG. 2.10 – Microscopic observations of cultures infected by the parasitoid.
Visualization of cells by phase contrast under white light (left part) and blue light excitation (right part). A-B : three distinct vegetative cells of *Scrippsiella trochoidea*, the left one is infected. C-D : resistant cysts produced by *S. trochoidea*. Two are calcified (in the left part), one of them is infected. The right cyst is not calcified and infected. Scale bars = 20 μm.

Cysts germination can be experimentally induced after a dormant period of a few weeks by placing them in fresh medium at optimal growth conditions. After two months, we meticulously washed old plate cultures (12 in total) using fresh medium, helped by the fact that resistant cysts naturally glued on the bottom of the culture. In few days, germination of the first host cells was observed. After only 7 days, both the free-living stage of the parasitoid and newly infected host cells were detected in all replicates. We concluded that the parasitoid was surviving inside its host and still virulent after months. Thus, a very novel relationship for protists has been demonstrated, one in which parasite and host simultaneously enter dormancy, emerging months later to propagate both species. This strategy nicely explains the recurrence of parasitoids that chronically infect the same host species year after year [Chambouvet *et al.*, 2008].

Additionally, that most host cells (97%) encyst without being infected promotes survival of both species. The low frequency of infected cysts prevents extermination of the host as required for survival of the parasitoid and provides over-wintering seed populations of host and parasitoid to drive bloom formation of the host and propagation of the highly virulent parasitoid the following summer. These two considerations undoubtedly have more immediate advantages for the parasitoid on short time scales, than the random possibility of acquiring resistance via sexuality, as suggested by the RQH hypothesis, has for the host. Considering that cyst production is induced by physical contact with the pathogen, host sexuality could be also interpreted as being induced by the parasitoid as a means to ensure survival.

Supplementary information

Supplementary information-Life cycle of *S. trochoidea*

S. trochoidea is a cosmopolitan species from coastal environment [Wang *et al.*, 2007]. Like most dinoflagellates, this species reproduces vegetatively and sexually. Most *S. trochoidea* strains are considered to be homotallic (meaning that gametes from the same parent cell can fused) [Montresor *et al.*, 2003]. Vegetative cells of *S. trochoidea* are haploid and divide by binary fission after mitosis. Thus, vegetative reproduction allows rapid exponential growth. During sexual reproduction, two motile cells, which act as functional gametes, fuse to produce a mobile zygote (planozygote). This diploid cell, possessing two flagella, can swim for few days before becoming a sexual resistant cyst. In *S. trochoidea*, the resistant cyst is characterized by the formation of calcareous spines covering the cyst. After a mandatory dormancy period of 15 to 60 days [Wang *et al.*, 2007, Binder and Anderson, 1987, Kim and Han, 2000], depending on the strains, *S. trochoidea* cysts germination to liberate a planomeiocyte. Meiosis occurs at this stage, restoring the haploid state and resulting in a new inoculation of the water column. Indeed, the cyst bank provides a huge reservoir for new generations (Fig2.11).

Supplementary information-Incubations with different size fractions of infected cultures

Alga are not defenceless against biotic aggressions. Allelopathic molecules can be produced as chemical signals and liberated into the water [Pohnert, 2000]. These molecules have been described to induce temporary cyst formation or production of fungicide at very low concentrations [Toth *et al.*, 2004, Pohnert, 2000]. In this experiment, we were interested in the production of such allelopathic signals during infection of *Scrippsiella trochoidea* by *Amoebophrya* sp. A previous experiment demonstrated that cyst production is maximal at two days, although host concentration remains at the same order of magnitude as in the controls (Fig2.9). Thus, a mix-

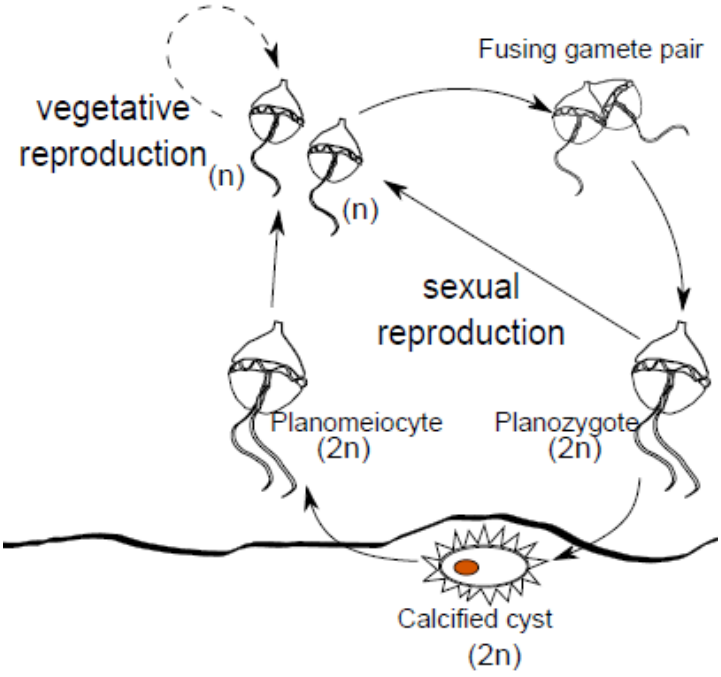

FIG. 2.11 – Life cycle of *S. trochoidea*. Black lines represented sexual reproduction and the dash line, vegetative reproduction.

ture of host contaminated by the parasitoid was prepared two days before the experiment in the same conditions as for the experiment in Figure 1. From this initial culture, we extracted the medium containing the potential allelopathic molecules by passage through a 0.22 μm filter to insure removal of both the host and the parasite. We inoculated untreated culture plates (Nalgene, 2 ml wells) using 1 ml exponentially growing *S. trochoidea* stock cultures at 5.4 $\times$ 10^6 cells per litre and 1 ml of this filtrate (Vol/Vol, corresponding to 1 ml each and a dilution of 50%). In parallel, the free-living stage of the parasitoid was recovered by serial filtrations from 1 ml of the initial infected culture ($<$ 10 μm to remove host, and $>$ 0.22 μm to collected and concentrated the free-living stage of the parasitoid) and placed in fresh medium. We used these parasites to inoculate *S. trochoidea* culture (Vol/Vol, corresponding to 1 ml each and a 2 :1 dinospores :host ratio). Finally, we inoculated *S. trochoidea* with fresh medium as a control (Vol/Vol, corresponding to 1 ml each). All these experimental conditions were tested in 4 replicates. Cysts production was significantly higher when the parasitoid was physically present (Fig2.12).

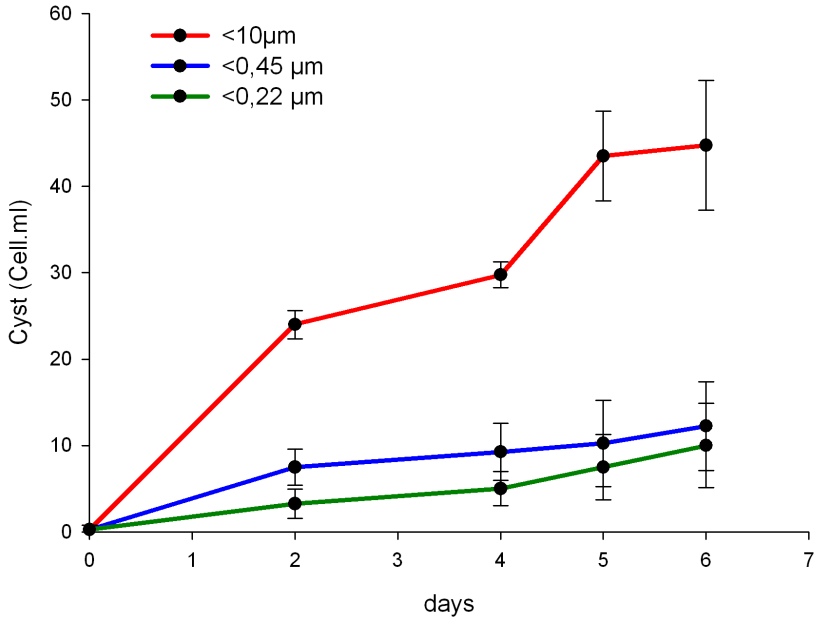

FIG. 2.12 – Cyst production using different inoculums. In green : Host culture alone. In purple : Host culture incubated with < 0.22 μm filtrate of a two-days-old culture of *Scrippsiella trochoidea* infected with *Amoebophrya* sp. In red : Host culture incubated in the presence of the free-living stage of the parasitoid.

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Methods

Cultures

Clonal cultures of *Scrippsiella trochoidea* (Roscoff Culture Collection, RCC1627) were obtained from one vegetative cell obtained after the germination of resting cysts collected in 2005 form the Penzé estuary sediments (North Brittany, France). Cultures were maintained in autoclaved natural seawater from Penzé estuary used as the basis for classical F/2 medium, adjusted to 27 salinity with distilled water, and complement with 5% (v/v) soil extract [Starr and Zeikus, 1993]. Cultures of the parasitoid infecting *Scrippsiella trochoidea* (RCC1626) were established from the Penzé estuary during summer 2007, as described by [Coats *et al.*, 1996]. All cultures were maintained at 18°C and on a light regime of 12L :12D, at 80 μEinstein/m^2/s^{-1}. The green fluorescence of the parasite was detected under epifluorescence microscope (cube U-MWB2, 450 to 480 nm excitation, 500 nm emission).

Experimental infections

Fresh free-living infective cells of the parasitoid were obtained by gravity filtration throughout 10-μm pore-size filter (Whatman). Half of the exponentially growing *Scrippsiella trochoidea* (5,400 cells) were inoculated with the free-living stage of the parasitoid in a 2 :1 (Host :Parasite) ratio (final volume of 2 ml). A total of 32 incubations were initiated at the same time using untreated culture plates (Nalgene). Cysts in all replicates were counted daily by inverted microscope. Each day, 2 replicates of each condition were fixed with glutaraldehyde (0.25% final concentration) and examined by flow cytometry to further deduce the abundances of hosts and parasitoid free-living stages.

Conclusions et Perspectives

CHAPITRE 3

CONCLUSIONS

Sommaire

Au cours de cette thèse, nous avons donc étudié la phylogénie des Syndiniales et des Amoebophryidae. La dynamique et la spécificité entre ces Amoebophryidae et leurs hôtes primaires, des dinoflagellés, ont été suivies *in situ*, dans l'estuaire de la Penzé durant trois années successives en début d'été, ainsi que durant les efflorescences biannuelles d'*A. catenella* dans l'étang de Thau. Nous avons modélisé nos observations et confirmé le rôle de ces pathogènes dans la régulation des populations hôtes. Enfin, l'établissement d'une culture du couple *Scrippsiella/Ameobophrya* nous a permis de mieux comprendre le couplage qui existe dans le temps et dans l'espace entre ces pathogènes et leurs hôtes. Toutes ces approches nous ont permis de détailler l'impact des populations parasites sur leurs hôtes, les dinoflagellés. La conclusion principale de ce travail est l'établissement de l'hypothèse d'un contrôle parasitaire fort sur toutes les espèces de dinoflagellés hôtes observées, une efflorescence algale étant alors interprétée comme un dérèglement du couplage existant entre ces deux partenaires.

3.1 Phylogénie et spectre d'hôtes des Syndiniales

3.1.1 Phylogénie des Syndiniales

Le séquençage environnemental a permis l'acquisition d'un nombre important de séquences provenant d'une grande variété d'environnements. Il est apparu à travers une analyse phylogénétique basée sur l'ARNr 18S que l'ordre des Syndiniales formait un groupe monophylétique. Ce premier résultat confirme les conclusions basées sur des études cytologiques de Cachon [Cachon, 1964], puis de Loeblich et Loeblich, ces derniers auteurs regroupant même cet ordre au sein d'une nouvelle classe distincte des dinoflagellés [Loeblich III, 1976]. Cette première étude clarifie la phylogénie de ce groupe grâce à un nombre important de séquences. Cette dernière était jusqu'alors controversée. En effet, les phylogénies basées sur l'ARNr 28S situaient le groupe II comme proche des Perkinzosoa, alors que les études basées sur l'ARNr 18S ne montraient pas de monophylie entre les groupes I et II [Massana *et al.*, 2008, Skovgaard and Daugbjerg, 2008]. Pour confirmer cette première étude, il pourrait donc être intéressant de refaire des analyses phylogénétiques à partir d'autres gènes tels que des gènes mitochondriaux ou des gènes nucléaires codants (comme celui de la β-tubuline).
Il est apparu que l'ordre des Syndiniales était composé de 5 groupes considérés comme constitués de parasites exclusivement marins (Table 3.1). Parmi ces groupes, seulement trois sont affiliés à des organismes de référence, le groupe I qui inclue les genres *Dubosquella* et *Ichtyodinium*, le groupe II qui inclue le genre *Amoebophrya* et le groupe IV incluant les genres *Hematodinium* et *Syndinium*. Parmi les espèces décrites par Cachon [Cachon, 1964], beaucoup ne sont pas encore caractérisées génétiquement. Ainsi leur séquençage permettrait de mieux décrire l'ensemble des Syndiniales [Cachon, 1964]. Enfin, l'utilisation de sondes oligonucléotidiques générales des groupes III et V permettrait la description de nouveaux organismes de

référence, de confirmer leur fonction de parasite, et peut-être d'étendre le spectre des hôtes de ce groupe.

Syndiniales	Organisme de référence	Hôtes	Habitats
Groupe I	*Duboscquella* sp. *Ichthyodinium* sp.	Ciliés Œuf de poissons	Marin
Groupe II	*Amoebophrya ceratii* *Amoebophrya* sp.	Dinoflagellés, Radiolaires, Ciliés et Chaetognates	Marin
Groupe III	ND	ND	Marin
Groupe IV	*Hematodinium* sp. *Syndinium* sp.	Décapodes	Marin
Groupe V	ND	ND	Marin

TABLE 3.1 – Détail de la nouvelle nomenclature des Syndiniales avec les organismes de référence [Guillou *et al.*, 2008] (Section 2.1)

3.1.2 Les Amoebophryidae

Les séquences appartenant à l'ordre des Syndiniales ont été retrouvées dans tous les écosystèmes marins étudiés [Moon-van der Staay *et al.*, 2001, Lopez-Garcia *et al.*, 2001]. Ainsi, l'importance écologique de ces parasites est probablement encore à l'heure actuelle sous-estimée. Cette étude s'est révélée fondamentale pour mettre au point la classification de ces parasites. En effet, il était nécessaire de faire un lien entre la classification de Cachon [Cachon, 1964] et l'actuelle dénomination « alvéolés du Groupe I ou II ». Ainsi il est apparu que les Alvéolés du Groupe II sont probablement synonymes de la famille des Amoebophryidae appartenant à l'ordre des Syndiniales. La diversité génétique intragroupe s'est aussi révélée très importante, composée d'au moins 44 clades. Ce nombre n'est pas exhaustif, le séquençage de nouveaux écosystèmes permettra sans doute de rajouter des clades supplémentaires. Cependant, au niveau des Amoebophryidae, à l'heure actuelle, seul *Amoebophrya ceratii* est relativement bien décrit. Ainsi l'utilisation de sondes clades-spécifiques ainsi que le séquençage des autres espèces d'*Amoebophrya* décrites (par exemple, *Amoebophrya grassei* ou *A. rosei*), permettrait de caractériser plus précisément ce groupe. Les organismes décrits par Cachon [Cachon, 1964] ont la capacité d'infecter un large choix d'hôtes, dinoflagellés, radiolaires jusqu'aux métazoaires. Cependant, en baie de Penzé (Section 2.2) les clades décrits (1, 2 et 14) ne semblent infecter que

les dinoflagellés. Ainsi des études complémentaires sur les hôtes infectés sont donc nécessaires pour mieux comprendre la complexité de ce groupe. Une étude de coévolution hôte-parasite pourrait par la même occasion retracer son histoire évolutive, si les changements d'hôtes n'ont pas été trop nombreux au cours du temps.
Entre chaque clade génétique, il existe au moins 44 points de mutations. D'après les réseaux d'haplotypes (Section 2.2), il semblerait qu'il n'y ait pas de recombinaison intraclade. Il est donc vraisemblable que nous ayons à faire à des espèces cryptiques, qu'il reste à décrire selon des critères physiologiques. Les critères d'identification d'espèces au sein des Syndiniales sont relativement flous. Jusqu'à présent, les parasites infectant des espèces différentes ont été généralement considérés et décrits sous des noms d'espèces différentes, sauf pour l'espèce *A. ceratii*, capable d'infecter un très grand nombre d'hôtes différents, mais qui masque effectivement une très grande diversité génétique.
Actuellement, il n'existe aucune preuve de l'éxistence de sexualité chez les Amoebophryidae. Elle pourrait avoir lieu lorsque deux parasites infectent le même hôte, ou au cours de la phase libre du parasite. Néanmoins, la caractérisation du nombre de chromosomes et la taille du génome de ces parasites par PFGE (Pulsed Field Gel Electrophoresis) associées à une analyse du niveau de ploïdie par cytométrie des différentes étapes du cycle infectieux pourraient apporter des preuves de la présence ou non de sexualité.

3.2 Dynamique et spécificité des Amoebophryidae en milieu naturel-Considérations méthodologiques

L'application de la technique du FISH-TSA, déjà utilisée avec succès sur le picoplancton [Not *et al.*, 2002], nous a permis d'identifier tous les stades du cycle de vie végétatif du parasite. Cette méthode est plus sensible que les techniques habituellement utilisées (coloration nucléaire ou fluorescence naturelle) [Cachon, 1964, Coats and Bockstahler, 1994], qui sous-estiment le taux d'infection car seuls les stades matures (trophontes) sont clairement visibles, et surtout elles ne permettent pas de quantifier la partie libre du parasite (les dinospores).
Grâce à cette méthode, l'impact du parasite a donc pu être finement évalué *in situ* dans l'estuaire de la Penzé. Cet écosystème est caractérisé par des efflorescences toxiques récurrentes de plusieurs millions de cellules par litre causées par le dinoflagellé *Alexandrium minutum* depuis 1988. Toutefois, depuis 2001, *A. minutum* paraît limité à de faibles concentrations cellulaires (de l'ordre de 10^5 cellules par litre), ce qui est corrélé à l'absence de toxicité des bivalves. L'utilisation d'une sonde oligonucleotidique ciblant la plupart des séquences environnementales appartenant à la famille des Amoebophryidae (la sonde Alv01) a permis d'identifier clairement ce groupe comme parasitoïdes de dinoflagellés photosynthétiques, toutes les espèces observées dans cet écosystème étaient infectées. De plus, le marquage des dinospores a clairement permis

de mettre en évidence cette dynamique hôte-parasite, et de relier l'augmentation de la concentration de la forme libre du parasite aux déclins des différentes espèces de dinoflagellés comme *Alexandrium minutum*. La prévalence moyenne s'élève à 20 % de la population totale. On aurait pu s'attendre à des valeurs bien supérieures, surtout lors de la disparition de l'hôte. La technique du FISH pourrait en être responsable. En effet, elle nécessite sur filtre le comptage d'un minimum de 50 cellules pour l'établissement d'une prévalence fiable, et tout dépend du volume filtré lors du déclin de l'hôte. Or, ce volume est limité sous peine de saturer le filtre en question. Durant le déclin de l'hôte, il devient donc techniquement impossible d'obtenir des prévalences fiables. Enfin, les prélèvements ont été effectués en surface. Or, Park *et al.* ont décrit une séparation physique entre les cellules infectées et saines le long de la colonne d'eau [Park *et al.*, 2002]. Il est probable que le comportement des cellules infectées soit profondément modifié par l'infection. Par exemple, de nombreuses espèces de dinoflagellés effectuent des migrations nycthémérales, qui peuvent être altérées. Enfin, les cellules augmentent considérablement de volume. Plus lourdes, elles pourraient avoir tendance à sédimenter plus rapidement, et ainsi disparaître des eaux de surface.
L'utilisation de sondes oligonucléotidiques spécifiques de chaque clade, nous a révélé que chacun était spécifique d'une espèce hôte donnée (Section 2.2). Cependant, nous avons été incapables de déterminer le clade infectant spécifiquement *A. minutum*. A cela, nous pouvons formuler plusieurs hypothèses :

1. Le clade génétique du parasitoïde spécifique d'*Alexandrium minutum* est sous-représenté dans nos librairies génétiques ou non couvert par la spécificité des sondes utilisées. Par exemple, ce parasite pourrait correspondre aux clades 32 ou 42 (minoritairement détectés dans nos librairies génétiques). Par ailleurs, la sonde spécifique du clade 2 ne cible que 95 % des séquences récupérées en rivière de Penzé (Section 2.2). Ainsi, un éventuel sous clade pourrait peut-être infecter *A. minutum* et représenter une potentielle nouvelle divergence sur le réseau d'haplotypes. L'utilisation du séquençage à partir d'une seule cellule infectée d'*A. minutum* ou bien la création de nouvelles sondes oligonucléotidiques pourraient permettre de répondre à ces questions.

2. Ce clade pourrait être absent des librairies génétiques. Un biais de l'utilisation du couple d'amorces Alv01-329r pourrait être incriminé, bien que le parasite soit détecté efficacement par FISH avec la sonde oligonucleotidique Alv01, et préférentiellement amplifié par le couple d'amorces 328f-329r (Section 2.1). Le séquençage avec un autre couple d'amorces spécifiques des Amoebophryidae pourrait diminuer les biais de PCR. Cependant, le primer Alv01 a été créé sur la seule région conservée de tous les Amoebophryidae le long de la séquence 18S. L'utilisation d'une autre région, comme celle correspondant au ARNr 28S, pourrait alors résoudre le problème.

3.3 Comparaison entre observations *in situ* et simulation par la modélisation

Certaines disparités apparaissent entre les résultats de la simulation par le modèle construit et nos observations *in situ*. Par exemple, le nombre de dinospores libérées lors des cycles infectieux s'avère dix fois plus élevé *in silico* qu'*in situ*, suggérant qu'un facteur de régulation non inclus dans le modèle est capable de limiter la production (ou d'augmenter les pertes) de dinospores.

Certaines explications sont d'ores et déjà implicites. Par exemple, il est apparu dans la section 2.5, que la présence du parasite pouvait induire la sexualité de *S. trochoidea*, et la formation rapide de kystes de résistance. Ce mécanisme de défense pourrait constituer une explication supplémentaire dans la disparition des cellules hôtes (et donc une production moindre de dinospores) et nécessiterait son intégration dans le modèle. Avant toute généralisation, ce mécanisme doit également être vérifié sur les autres espèces de dinoflagellés, en particulier chez *Alexandrium minutum*.

D'autres facteurs biotiques peuvent avoir été sous-estimés *in situ*, où la situation est souvent bien plus compliquée que ce que nous imaginons. En effet, le couple hôte-parasite est intégré dans un réseau d'interactions complexes à travers le réseau trophique. Dans le cas d'*Alexandrium minutum*, la pression de broutage considérée comme relativement constante car non basée sur des liens de spécificité élevées était visiblement insuffisante pour contrer le développement de la microalgue dans les années 1990-2000. Cette situation apparaît clairement également dans nos simulations. Il n'y a pas de raison de penser que cette pression de broutage puisse avoir augmenté considérablement ces dernières années. Par contre, l'introduction d'un pathogène spécifique même en faible quantité dans le modèle (pour un rapport 1×10^{-2} dinospores par hôte, Section 2.3) provoque le déclin rapide de la population hôte. Cependant, il existe d'autres pathogènes pouvant assurer une pression sur les populations hôtes. Par exemple, le parasitoïde *Parvilucifera infectans* a été observé dans les eaux de la Penzé comme infectant *Alexandrium minutum* [Erard-Le Denn *et al.*, 2000]. Ce parasite n'est pas reconnu par les sondes utilisées dans ce travail (il appartient aux Perkinsozoa). Actuellement aucune information sur la compétition entre ces deux parasites n'existe, néanmoins ils sont susceptibles d'assurer conjointement une pression de sélection sur les populations d'*Alexandrium minutum*. On peut également imaginer des attaques virales comme facteur de régulation, capables d'infecter aussi bien les hôtes que les dinospores, ce qui expliquerait en partie les différences observées entre modèle et milieu naturel (Figure 3.1).

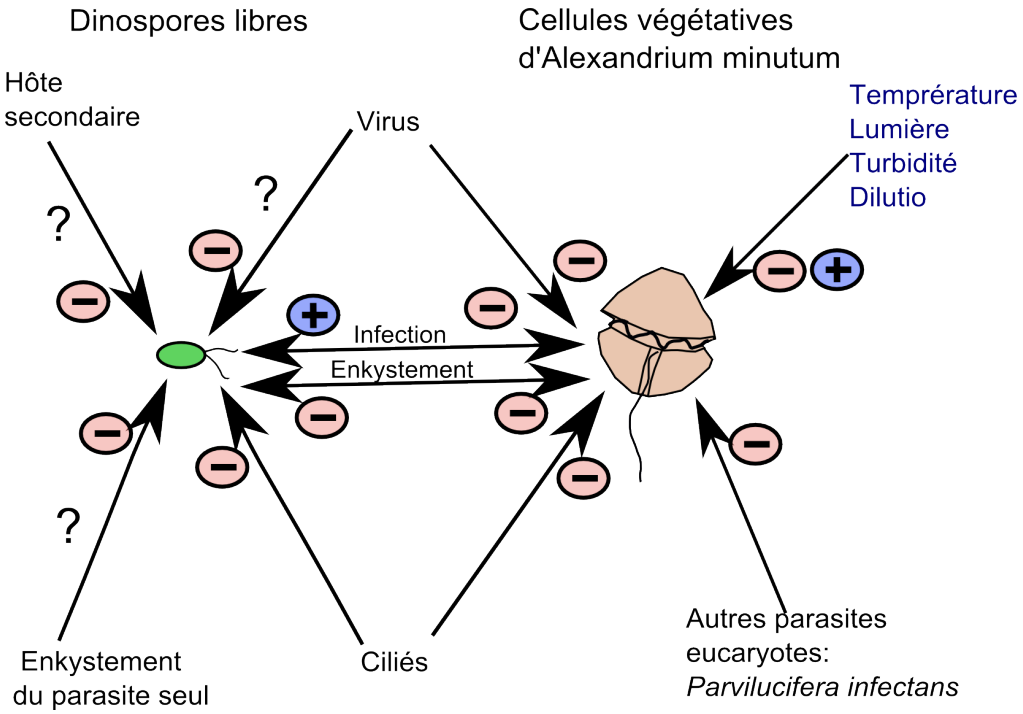

FIG. 3.1 – Schéma des impacts positifs et négatifs sur les populations végétatives de dinospores (parasites) et d'*Alexandrium minutum* (hôtes) dans la colonne d'eau. Les facteurs influençant positivement la croissance des populations de parasite ou d'*Alexandrium minutum* sont caractérisés par un $\oplus$ alors que les facteurs négatifs sont caractérisés par un $\ominus$.

3.4 Impact des Amoebophryidae sur le réseau trophique

Nous avons montré qu'*in situ* et *in silico* ces parasites avaient des capacités de régulation très fortes sur leurs populations hôtes. Or, les dinoflagellés sont des producteurs primaires importants, en particulier dans les écosystèmes côtiers en été. Les Amoebophryidae contrôlent donc un compartiment clef au sein du réseau trophique. Leur impact se répercute forcément sur les échelons trophiques supérieurs. Cependant leur rôle dans le transfert du carbone est différent du rôle des virus. En effet, ces derniers induisent un « Shunt » viral, c'est-à-dire que le carbone assimilé par les hôtes est libéré sous la forme de matière organique dissoute. Le rôle des parasites eucaryotes du type Amoebophryidae est différent car certes une partie de ce carbone retourne à l'état de matière organique dissoute mais une grande partie peut être transmise aux échelons trophiques supérieurs via entre autres le broutage. Par contre, la stratégie de « kill the winner », initialement appliquée aux bactériophages marins pourrait être également appliquée aux pathogènes eucaryotes [Thingstad and Lignell, 1997]. Cette stratégie s'illustre par le fait que les batériophages provoquent préférentiellement la lyse des bactéries dominantes, permettant la croissance d'espèces moins compétitives, et une plus grande richesse spécifique [Thingstad and Lignell, 1997]. En effet, les Amoebophryidae semblent induire spécifiquement

la mortalité des espèces dominantes permettant des successions rapides des hôtes. Tous ces résultats pourraient de plus expliquer en partie le paradoxe du plancton pour les espèces de dinoflagellés [Hutchinson, 1961], qui peut se résumer par cette interrogation : Comment, dans un environnent apparemment homogène, de si nombreuses espèces peuvent-elles coexister ? Dans l'estuaire de la Penzé, chaque clade est spécifique d'une espèce hôte donnée, ce qui induit la succession des espèces, très rapide (l'espèce dominante change toute les semaines), et le maintien d'espèces peut-être moins compétitives, même si ce dernier point reste encore à démontrer.

3.5 « The enemy release hypothesis »

Les efflorescences d'*Alexandrium minutum* en Baie de Penzé ont été un grave problème de santé publique durant de nombreuses années. Des moyens relativement importants ont été dédiés à l'analyse des facteurs environnementaux favorisant le développement de cette microalgue, permettant de modéliser et de prédire les concentrations cellulaires de la microalgue toxique. Ainsi, un modèle hydrodynamique a été développé par l'IFREMER. Ce dernier permet entre autre la simulation de la localisation et de l'intensité des efflorescences d'*A. minutum* dans l'estuaire de la Penzé (`http://www.ifremer.fr/delec/faits_marquants/algues_toxiques_fichiers/algues_toxiques.htm`).
Des analyses statistiques de régression ont permis de définir certains facteurs clefs dans le déterminisme des efflorescences d'*Alexandrium minutum*, en particulier, la température, la pluviométrie (reliée au débit de la rivière), et les coefficients de marées (résultats en cours de publication par IFREMER, et publiés sur le site internet précédemment cité). Ces facteurs permettent d'expliquer parfaitement l'apparition des efflorescences en Baie de Penzé jusqu'en 2001, date à partir de laquelle les conditions environnementales semblent optimales mais où aucune efflorescence n'a été observée. Ces résultats vont dans le sens de nos conclusions générales. Dans ce travail, nous avons émis l'hypothèse que les efflorescences d'*Alexandrium minutum* avaient été récemment limitées par l'action de pathogènes du type Amoebophryidae. Autrement dit, les efflorescences de dinoflagellés (ou marées rouges) traduiraient l'incapacité des pathogènes à infecter efficacement la microalgue localement. Cette hypothèse est à rapprocher de celle plus générale qui consiste à expliquer les capacités d'une espèce à être invasive localement par l'absence d'ennemis adaptés ou « Enemy Release Hypothesis » [Keane and Crawley, 2002]. Le cas certainement le plus médiatisé en milieu marin est celui de la macro-algue *Caulerpa taxifolia* introduite en 1984 accidentellement sur les côtes méditerranéennes françaises. Depuis, en absence de tout prédateur, elle est devenue invasive, avec des conséquences désastreuses pour l'environnement [Meinesz *et al.*, 1995].
Cette hypothèse semble se vérifier dans l'Etang de Thau, où *A. catenella* vient d'être récemment introduit et continue à produire des efflorescences toxiques. Les résultats de ce travail de

thèse suggèrent que la souche d'*A. catenella* est résistante à tous les clades d'Amoebophryidae présents dans l'étang de Thau (10 au total). Il serait intéressant d'étendre cette étude le long de côtes méditerranéennes, où cette algue s'est également dispersée [Vila *et al.*, 2001], et de suivre dans le temps l'évolution de cette résistance le long des côtes méditerranéennes. Dans le cas de l'estuaire de la Penzé, et si l'on adhère à cette hypothèse, il aura fallu une dizaine d'années pour qu'un tel contrôle ait pu s'établir. Dans le port d'Alexandrie, il aura fallu près de 30 ans ! En baie de Penzé, et pour définitivement tester cette hypothèse, l'étude de la stratification des kystes et des parasites dans le sédiment peut être une alternative. En effet, la datation des sédiments pourrait permettre d'évaluer le moment où la rencontre entre le pathogène et la microalgue s'est établie. Malheureusement, il semble encore techniquement très difficile non seulement d'appliquer la technique de FISH sur des kystes très résistants et trop imperméables, mais aussi de dater des sédiments récents, et sur une aussi faible échelle temporelle.
Bien sûr, cette théorie ne peut concerner que les microalgues relativement opportunistes, et capables de se multiplier fortement dans des écosystèmes non limitants, c'est-à-dire des dinoflagellés présentant une stratégie de types C ou R [Smayda and Reynolds, 2001].
Une autre hypothèse permettant d'expliquer la diminution dans le temps de la concentration d'espèces de dinoflagellés potentiellement invasive a également été proposée dans la littérature. D'après Ismael [Ismael, 2003], ce phénomène observé dans le port d'Alexandrie pour *A. minutum* après 30 années d'efflorescences toxiques régulières, pourrait être directement relié à une diminution du nombre de kystes dans le sédiment, ne permettant plus de fournir un inoculum suffisant pour produire des efflorescences. Dans le cas de la baie de Penzé, chaque année depuis 2001, les efflorescences diminuent de façon drastique (Section 2.2). Cette diminution du nombre de cellules végétatives diminue par la même occasion la capacité de la population à réapprovisionner d'une année sur l'autre cette banque de kystes. Le parasite induit la formation de kystes, mais l'inoculum pourrait être de moins en moins important, et naturellement limiter les efflorescences. La régulation des populations hôtes n'est pas seulement une régulation des cellules végétatives, mais aussi indirectement celles des banques de kystes. Pour conclure, il est fort probable que ces deux hypothèses soient liées.

3.6 Déconnection entre un hôte et son pathogène

On peut également se demander comment l'hôte peut échapper pour un temps à son pathogène, considérant le couplage très fort qui existe entre les deux partenaires. En effet, nous avons montré dans la section 2.4 que le parasite pouvait entrer dans un état de dormance à l'intérieur même du kyste de son hôte. Ce phénomène expliquerait la régularité année après année de l'apparition du parasite parfaitement calquée sur celle de son hôte. Cette étude permet de quantifier ce phénomène sur culture. On peut alors se demander si une prévalence de 3 % est

suffisante pour assurer la survie du pathogène, surtout si l'on considère que le nombre total de kystes produit diminue lui aussi de façon importante. Ce mécanisme pourrait permettre pour un temps à la microalgue d'échapper à son pathogène. Cependant, d'autres stratégies de dissémination peuvent être développées par le pathogène, telles que l'existence d'un hôte secondaire plus permanent dans l'écosystème ou la formation de kystes de résistance, très fréquente chez les Alveolata.
Cette déconnection peut aussi être liée à l'activité humaine. En effet, les introductions d'organismes dans un écosystème « sain » sont un phénomène récent et bien connu qui peut avoir plusieurs origines, via les eaux de ballast, les transports de coquillages, ainsi que le réchauffement climatique [Anderson, 1997].
Enfin, on ne peut exclure que certaines mutations puissent être positivement sélectionnées par l'hôte pour contrer les attaques du parasite, ce qui permettrait d'expliquer le succès soudain de certaines espèces jusqu'alors relativement stables à de plus faibles concentrations cellulaires. En effet, la présence du parasite induit la sexualité de l'hôte (Section 2.4). Ainsi de nouvelles recombinaisons génétiques sont générées à chaque génération pouvant conduire à l'apparition de phénomènes de résistance [Jaenicke, 1978]. Le parasite de son côté essaye d'augmenter son pouvoir infectieux et de contrer la résistance de son hôte. C'est ce qu'on appelle la course aux armements [Van Valen, 1973]. Une telle pression de sélection est clairement illustrée par l'importance de la micro diversité retrouvée intra clade chez les Amoebophryidae en baie de Penzé (Section 2.2). Ces ajustements permanents entre hôtes et parasites ont été bien étudiés sur un autre Alvéolé parasite tel que *Plasmodium falciparum*. En effet, ce dernier possède des gènes de résistance ou des gènes d'adaptation localisés préférentiellement dans les régions subtélomériques de ses chromosomes. Ces dernières subissent de fortes recombinaisons spontanées lui permettant d'augmenter ses capacités d'adaptations [Gardner *et al.*, 2002]. Les gènes de résistance, s'ils existent chez les dinoflagellés hôtes pourraient être à rechercher au niveau de ces régions chromosomiques.

3.7 Utilisation des Amoebophryidae comme arme biologique ?

En 1968, Taylor [Taylor, 1968] proposa l'utilisation de bio-contrôle comme *Amoebophrya* pour lutter contre les efflorescences toxiques algales. Cependant en 1985, cette idée a été rejetée par Nishitani du fait du manque apparent de spécificité de ces parasites [Nishitani *et al.*, 1985]. Dans le cas de cette étude, nous avons montré que la convergence morphologique des différentes souches d'*Amoebophrya* masque en réalité une diversité génétique importante, et une forte spécificité de chaque génotype pour une espèce locale. L'utilisation de la souche d'*Amoebophrya* isolée aux Etats-Unis et capable d'infecter la souche de *A. catenella* poussant dans l'étang de Thau comme bio contrôle afin d'éviter les efflorescences pourrait être tentant. Cependant,

même si les efflorescences toxiques sont en pleine augmentation, l'histoire nous rappelle qu'il est souvent dangereux d'introduire une espèce pour en contrôler une autre. En effet, l'utilisation de bio-contrôle a souvent aboutit à une cascades d'événements ayant parfois des conséquences de plus en plus désastreuses. De plus, dans le cas des parasites Amoebophryidae, la possible présence d'un hôte secondaire ainsi qu'une grande capacité d'adaptation pourraient dans le cadre d'une introduction dérégler tout le réseau trophique et apporter de nouveaux problèmes encore imprévisibles. Enfin, l'adaptation à un nouvel hôte chez les parasites est un phénomène connu. Par exemple, le parasite de saumon *Gyrodactylus* (Plathelminthes) a changé d'hôte au cours de l'évolution, et a été capable d'infecter *Gasterosteus aculeatus* (l'épinoche). Avec le temps, il s'est parfaitement adapté à son nouvel hôte [Zietaria and Lumme, 2002].

CHAPITRE 4

PERSPECTIVES

Au cours de cette thèse, un travail exploratoire a été réalisé, centré sur les contrôles des populations hôtes, la spécificité et le cycle de vie du parasite. L'approche de cette thèse a été essentiellement écologique. Il serait maintenant intéressant de détailler la spécificité hôte-parasite d'un point de vue plus comportemental ou moléculaire par une étude plus poussée sur culture. D'autre part la spécificité d'infection induit potentiellement la manipulation de son hôte. Dans le monde des parasites terrestres, de telles manipulations existent, par exemple un groupe de vers parasites, les nématomorphes, ne sont parasites du grillon qu'au stade larvaire. Une fois adultes, ils sont en revanche libres et aquatiques. Ces parasites manipulent le comportement des insectes hôtes, les grillons, obligeant ces derniers à se « suicider » en se jetant à l'eau. Une fois dans son milieu liquide, le vers sort de l'insecte et commence sa vie au stade libre [Hertel and Coustau, 2008]. Nous pouvons donc envisager différents axes pour caractériser ces « manipulations » de la part du parasite :

Des études plus comportementales pourraient être envisagées. Par exemple, le long de radiales de salinité dans l'estuaire de la Penzé en 2004, il est apparu que le maximum de concentration des dinospores était décalé par rapport à celui d'*Alexandrium minutum* vers des salinités plus importantes. Nous savons que les Syndiniales sont des espèces exclusivement marines. Ainsi les maxima de concentration atteint par *Alexandrium minutum* pour des salinités avoisinant les 27 ‰pourraient résulter de l'incapacité du parasite à contrôler son hôte à de faibles salinités. Ces faibles salinités pourraient servir de refuge aux populations d'*Alexandrium*. Des tests en culture en utilisant des salinités différentes pourrait révéler si (1) le parasite est capable de supporter des salinités très basses (2) si le maximum de croissance de la microalgue est bien à 27 ‰. D'autre part il serait intéressant de comprendre comment le parasite arrive à trouver puis à infecter un hôte dans la colonne d'eau. En effet, un signal chimiotactique entre le parasite et son hôte pourrait exister. L'utilisation de la technique de « microfluidic channels »

testée sur des bactéries face à des nutriments, pourrait être une solution. De façon générale, les parasites cibles pourraient être mis sur une lame à compartiment et les hôtes, ou différents substrats pourraient être injectés par pulse [Stocker *et al.*, 2008].

L'obtention des cultures de parasites infectant *Scrippsiella trochoidea* permet de nouvelles ouvertures au niveau génomique. En effet, par PFGE, en collaboration avec Evelyne Derelle (Observatoire océanologique de Banyuls), nous avons estimé la taille du génome du parasite à ∼5 Mpb. La petite taille de ce génome comparée au génome de dinoflagellés en fait un modèle de choix et ouvre des possibilités de séquençage du génome complet. Ce résultat pourrait nous apprendre beaucoup sur les mécanismes d'infection. En parallèle une analyse de l'expression (banque EST) (1) du parasite seul (dinospores) (2) de l'hôte seul ainsi que (3) pendant différents stades de maturation du parasite, pourrait nous révéler les cibles moléculaires induisant la spécificité du parasite pour son hôte, et les processus de prise de contrôle de l'hôte mis en place pour son développement intracellulaire.

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Abstract

Syndiniales are marine parasitoids infecting a wide range of planktonic hosts, from dinoflagellates, decapods, to fish larvae. One family, the Amoebophryidae mostly infects dinoflagellates. In the Penzé estuary (North of Brittany, France), all species of dinoflagellates observed were infected by these pathogens, including the toxic species *Alexandrium minutum*, which produces paralytic shellfish poisoning. A strong specificity has been detected : a dinoflagellate species being infected by a genetically distinct parasitoid clade, the same clade infecting the same host species year after year (from 2004 to 2006). These pathogens have the capacity to control their host populations, resulting in the rapid host successions from field. This host population control was studied by integrating an Anderson-May model with the predation pressure. The parasitoid infecting the non-toxic *Scrippsiella trochoidea* was obtained in pure culture. The presence of the pathogen induced the host sexuality, resulting in the production of diploid resistant cysts. However, a few of them were also infected by the parasitoid. After a dormancy of two months, both the host and the parasitoid were able to germinate, and new infectious cycles were observed. This behaviour strongly links in time and space both partners, and explains the recurrence of infections. We hypothesized that blooms can only occur when parasitic controls are absent, explaining why the toxic blooms of *A. minutum* in the Penzé estuary that began in the 80' suddenly ended in 2001. This hypothesis was also tested in the Thau lagoon (Mediterranean Sea), where the blooming *Alexandrium catenella* species arise from the 90'. This dinoflagellate species was the only one to not be infected by Amoebophryidae during toxic blooms that occurred in spring and autumn 2007.

Keywords : Parasitoid ; *Amoebophrya* ; dinoflagellate ; *Alexandrium* ; *Scrippsiella* ; plankton ; toxic blooms ; invasive species ; population controls.

Résumé

Les Syndiniales sont des parasitoïdes marins capables d'infecter un très grand nombre d'organismes planctoniques, des dinoflagellés jusqu'aux larves de poissons. Parmi ces pathogènes, les Amoebophryidae infectent tout particulièrement les dinoflagellés. En rivière de Penzé (Bretagne Nord, France), ces parasitoïdes infectent toutes les espèces présentes, y compris l'espèce toxique *Alexandrium minutum* qui produit des toxines paralysantes. La spécificité de ces interactions est grande : une espèce hôte est infectée par un parasitoïde génétiquement distinct, le même pathogène infectant la même espèce d'une année sur l'autre (entre 2004 et 2006). Ces pathogènes semblent capables de réguler les populations hôtes, la spécificité d'interaction entraînant une succession rapide des espèces de dinoflagellés. Dans cette étude, ce contrôle a été simulé en reprenant le modèle d'Anderson-May et en l'incluant dans un réseau trophique microbien intégrant la prédation. Une souche d'un parasitoïde infectant le dinoflagellé non toxique *Scrippsiella trochoidea* a été isolée. La présence du pathogène induit la production de kystes de résistance de l'hôte, issus de la reproduction sexuée. Cependant, un faible pourcentage de ces kystes est infecté. Après deux mois de dormance, la germination a pu être observée, aussi bien pour l'hôte que pour le parasitoïde, et de nouveaux cycles d'infection ont également été observés. Cette interaction physique entre l'hôte et son pathogène induit donc un couplage très fort entre les deux partenaires, ce qui permet d'expliquer la récurrence des infections d'une année sur l'autre en baie de Penzé. Nous avons émis l'hypothèse que le nombre croissant de marées rouges (ou efflorescences de dinoflagellés) pouvait être une conséquence du découplage entre le dinoflagellé hôte et son pathogène naturel. Cette hypothèse permet de mieux comprendre l'historique de certaines efflorescences toxiques récurrentes, comme celle d'*A. minutum* en Baie de Penzé, qui avait produit des efflorescences toxiques entre la fin des années 1980 et 2001, mais qui aujourd'hui apparaît parfaitement régulé par ces pathogènes naturels. Cette hypothèse a été également confirmée dans l'étang de Thau (mer méditerranée), où *Alexandrium catenella*, invasives depuis la fin des années 1990, était la seule espèce de dinoflagellés à ne pas être infectée par des Amoebophryidae durant les efflorescences toxiques printanières et automnales de 2007.

Mots Clés : Parasitoïde ; *Amoebophrya* ; dinoflagellé ; *Alexandrium* ; *Scrippsiella* ; plancton ; efflorescence toxique ; espèce invasive ; contrôle des populations.