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Amel Dhib

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Amel Dhib. Contribution à l'étude des successions écologiques du phytoplancton dans la lagune de Ghar El Melh. Biodiversité et Ecologie. Université de Franche-Comté, 2015. Français. NNT : 2015BESA2033 . tel-01693267

HAL Id: tel-01693267

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Université El MANAR II
Faculté des sciences de Tunis



Institut National des Sciences et
Technologies de la Mer



Université de
Franche-Comté

THESE DE DOCTORAT

Présentée par
Mme Amel DHIB

Pour obtenir le titre de
Docteur de l'université de Tunis El-Manar
Docteur de l'université de Franche-Comté

Discipline
Sciences Biologiques (Tunisie)
Sciences de la Vie et de l'Environnement (France)

Contribution à l'étude des successions écologiques du phytoplancton dans la lagune de Ghar El Melh

Soutenue publiquement le 20 juillet 2015 devant le jury composé de :

- Pr. Rafika FEHRI-BEDOUI (Faculté des Sciences de Tunis) : Présidente de jury**
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- Pr. Lotfi ALEYA (Université de Franche-Comté) : Encadrant**
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- Pr. Michel DENIS (Université d'Aix Marseille) : Rapporteur**
- Dr. Estelle MASSERET (Université Montpellier/ IRD) : Examinatrice**

A mon ange NOOR

Pour la joie de la vie qu'elle m'offre

A mon père Mustapha & ma mère Najet

*Pour l'éducation qu'ils m'ont prodigué; avec tous les moyens et au prix de
toutes les sacrifices qu'ils ont consentis à mon égard*

A mes sœurs Hanen & Boutheina & mon frère Anis

A mon cher mari Ibrahim

A ma chère Tante Souad

Pour tout le soutien moral et matériel

Trouvez ici l'expression de ma profonde gratitude

Remerciements

Ce travail a été réalisé en cotutelle internationale entre l'Université de Tunis El- Manar et l'Université de Franche Comté au sein du Laboratoire Milieu Marin de l'Institut National des Sciences et Technologies de la Mer (La Goulette) et du laboratoire Chrono-Environnement de Besançon. Que ces honorables institutions soient remerciées de m'avoir permis de réaliser ce travail dans les meilleures conditions.

Je tiens à exprimer ma profonde gratitude et ma sincère reconnaissance à mes deux directeurs de thèse :

- ❖ A **Mme. Souad TURKI**, Maître de conférences à l'INSTM. Au cours de mon stage de mastère, son encadrement de qualité a motivé mon désir de poursuivre ma formation au travers d'une thèse, je le remercie chaleureusement pour la confiance qu'il m'accordée en soutenant ma candidature. Tout au long de ma thèse, j'ai pu bénéficier de sa grande disponibilité, de son aide, et de ses connaissances en matière d'écologie du phytoplancton. Je vous suis reconnaissante, Madame, pour votre patience, vos encouragements et vos conseils constructifs.*
- ❖ A **Mr. Lotfi ALEYA**, Professeur à l'Université de Franche Comté. Je tiens à rendre hommage à la pertinence de ses conseils, à sa capacité à élargir la réflexion ainsi qu'à ses qualités en matière de communication, et en particulier de rédaction scientifique. J'ai été particulièrement sensible à son respect des initiatives personnelles ainsi qu'à ses encouragements. A maintes reprises, il a su me faire profiter de ses compétences scientifiques, mais également de sa bonne humeur et de son humour ; j'ai été très heureuse de faire mes premiers pas dans le domaine de la recherche à ses côtés. Il a ainsi grandement contribué à ma formation mais aussi à me laisser un très bon souvenir de ces années écoulées, qu'il soit ici chaleureusement remercié.*

Je tiens ensuite à exprimer ma reconnaissance aux membres du jury :

- ❖ A **Mme. Rafika BEDOUI**, Professeur à la Faculté des sciences de Tunis, qui a accepté de juger ce travail en tant que présidente de jury. Qu'elle trouve ici l'expression de mon profond respect et ma sincère reconnaissance.
- ❖ A **Mme. Asma SAKKA**, Professeur à la Faculté des sciences de Bizerte, et à **Mr. Michel DENIS**, Professeur à l'Université d'Aix Marseille, de m'avoir fait l'honneur d'être les rapporteurs de cette thèse. Merci pour l'intérêt que vous avez porté à ce travail. Vos suggestions, vos remarques pertinentes et vos commentaires judicieux m'ont été très utiles. Merci infiniment.
- ❖ A **Mme. Estelle MASSERET**, maître de conférences à l'Université Montpellier, d'avoir accepté d'examiner ce travail. Sincèrement merci.

Je veux exprimer aussi ma profonde gratitude au Professeur **Cherif SAMMARI**, Directeur du Laboratoire du Milieu marin de l'Institut National des Sciences et Technologies de la Mer. Je le remercie pour m'avoir accueilli dans son laboratoire.

Je tiens également à remercier **Mr. Fourat AKROUT**, Ingénieur au laboratoire du Milieu Marin à L'INSTM, **Mme. Noura BALTI**, Technicienne au laboratoire du Milieu Marin à L'INSTM, **Mme. Mouna FERTOUNA-BELLAKHAL**, Chef laboratoire à l'Institut Supérieur de Pêche et d'Aquaculture de Bizerte, **Mme. Aude BARANI**, Technicienne à l'Université de Marseille, **Mr. Nicolas ROUGE**, Ingénieur à l'Université de Franche-Comté et **Mr. Victor FROSSARD**, docteur à l'Université de Franche-Comté, pour leur aide à réaliser ce travail.

L'aide de tous les pêcheurs de la lagune m'a été indispensable pour la réalisation de mes sorties de terrain. Je remercie particulièrement **Riadh OUELDI**.

Boutheina, Amine, Imen, Mouna, Afef, mes compagnons, merci pour votre amitié, votre soutien, votre gentillesse, votre disponibilité, votre amour, vos petits coups de pouce et vos gros coups de main...et tout le reste ! Ce fut un grand plaisir de vous avoir connue.

Voilà le temps de remercier mes parents qui m'ont toujours soutenu, et à qui je dois mon épanouissement tant personnel que professionnel. Sans leur soutien, ce travail n'aurait pas vu le jour. Je remercie également mes sœurs **Hanen** et **Boutheina** et ma cousine **Amna**, pour ses nombreux coups de pouce et encouragements au cours de ces années de thèse. Merci d'avoir pris ce temps-là pour moi.

A mon mari et mon meilleur ami **IBRAHIM**, à celui qui a partagé les joies et satisfactions mais aussi les moments difficiles de découragement ou de doutes de ces années, merci d'avoir toujours cru autant en moi et de m'avoir toujours soutenue.

A tous les membres de famille **LANDOULSI** (am **EL HEDI**, omi **JAMILA**, **Imen**, **Abdou**, **Mohamed ALI**, am **BOUJEMAA ...**) mille mercis pour votre présence et votre soutien.

J'en oublie certainement encore et je m'en excuse.

Je remercie finalement tous ceux qui de près ou de loin ont contribué à la réalisation de ce travail.

Amel

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LISTE DES ABREVIATIONS

ANOVA : Analyse de variance

ASP: Amnesic Shellfish Poisoning

BF: Bloom Forming

CCA : Analyse Canonique des Correspondances

DSP: Diarrheic Shellfish Poisoning

FAT : Fast Acting Toxin

GML : Ghar El Melh Lagoon

HAB: Harmful Algae Bloom

HNA: High Nucleic Acid

HP: Heterotrophic Prokaryotes

LNA: Low Nucleic Acid

Max : maximum

Min : minimum

OA: Okadaic Acid

PCA : Analyse en Composantes Principales

PERMANOVA : Permutational Multivariate Analysis of Variance

PSP : Paralytic Shellfish Poisoning

R : eaux rouges

RDA : Analyse de redondance

INTRODUCTION

Chaque lagune, lieu d'échange permanent entre les eaux marines et les eaux continentales, est un écosystème particulier dont le fonctionnement hydrobiologique doit être étudié (Crouzet, 1972). En Méditerranée, les lagunes sont nombreuses, jouant souvent un rôle important sur le plan écologique et socio-économique : (1) un rôle écologique de tampon en protégeant la côte de l'influence de la mer, ceci en filtrant les eaux de ruissellement et en abritant d'importantes communautés végétales et animales. Beaucoup d'espèces, rares ou menacées, sont inféodées aux lagunes, du fait des conditions écologiques spécifiques qui y règnent. De plus les lagunes sont des zones de nidification importantes pour les oiseaux migrateurs et à ce titre, de très nombreuses lagunes sont protégées par la convention RAMSAR, (2) un rôle économique : les lagunes sont des zones de production biologique intense et font l'objet d'une importante exploitation de leurs ressources.

Pourtant, au nord comme au sud de la Méditerranée, les lagunes font face à plusieurs types de pressions qu'elles soient naturelles et/ou anthropiques : (1) contraintes naturelles : en étant un réceptacle final des eaux du bassin versant, caractérisé par un faible renouvellement des eaux, les lagunes sont des milieux riches en éléments nutritifs et à forte productivité. Cette caractéristique, propre aux lagunes méditerranéennes, peut rapidement devenir une contrainte due au phénomène de l'eutrophisation, (2) contraintes anthropiques : par leur position au sein de zones d'urbanisation et d'industrialisation très dissemblables, et ayant fait l'objet d'activités diverses et intenses (pêche, aquaculture, aménagement hydraulique...).

Parmi les lagunes les plus représentatives du sud de la Méditerranée, on retrouve la lagune de Ghar El Melh (Ayache *et al.*, 2009). Située au nord-est de la Tunisie et bien entourée du Golfe de Tunis, elle se caractérise par plusieurs qualités distinctes (1) nommée « site RAMSAR » pour son importance internationale en tant qu'habitat pour les poissons et les oiseaux migrateurs (RAMSAR, 2007), (2) un milieu semi fermé avec une seule communication avec la mer méditerranéenne *via* la passe dite « El Boughaz » subissant un ensablement périodique (3) a une très faible profondeur de 2 m maximum et moins de 0.5 m dans certaines parties de la lagune (Chakroun, 2004), (4) la salinité des eaux atteignent des valeurs caractéristiques qui peuvent dépasser les 50 en été (Ktari-Chakroun et Romdhane, 1985; Beyrem *et al.*, 2002), (5) caractérisée par une large prolifération du macrophyte « *Ruppia cirrhosa* », permettant une

couverture de 80 à 100 % en été de la superficie (Shili *et al.*, 2002). C'est une espèce résistante aux conditions environnementales extrêmes, parmi les rares qui peuvent avoir une bonne croissance dans des eaux hyperhalines (Verhoeven, 1979; Pergent *et al.*, 2006). Elle est considérée comme une macrophyte bio-indicatrice, signe de la détérioration de la qualité des eaux (SCET-ERI, 2000 ; Moussa *et al.*, 2005), (6) a une grande sensibilité à l'eutrophisation du fait des conditions répertoriées au-dessus (faible profondeur, ensablement de la communication avec la mer) et à l'accumulation de sels nutritifs en provenance du bassin versant (agriculture, industries, eaux pluviales...) (Romdhane, 1999; Chakroun, 2004; Ayache *et al.*, 2009), et (7) caractérisée par l'incidence d'épisodes de mortalité de poissons (Romdhane *et al.*, 1998; Turki *et al.*, 2007) avec une production halieutique de plus en plus faible avec, par exemple, de 200 tonnes en 1993 à 26 tonnes en 1996 (Moussa *et al.*, 2005).

Toutes ces caractéristiques combinées, indiquent l'aptitude de la lagune de Ghar El Melh à être une zone sujette à la prolifération des microalgues (microphytoplancton) y compris les espèces nuisibles. En effet, le régime altéré de la lagune pourrait facilement affecter les communautés de microalgues en termes d'abondance et de composition. Le phytoplancton, en édifiant le premier maillon de la chaîne trophique, contrôle tous les écosystèmes aquatiques et peut être ainsi un outil pour caractériser l'état écologique de ces derniers.

Le plus souvent, les proliférations microalgales sont des phénomènes naturels qui se produisent régulièrement, parfois amplifiés par un enrichissement important du milieu en éléments nutritifs mais sans conséquence néfaste. Cependant, dans certaines situations ces proliférations peuvent avoir plusieurs répercussions sur l'écosystème. En effet, les microalgues peuvent causer des problèmes et former ce qu'on appelle les eaux colorées qui affectent les espèces marines, et/ou produire des toxines avec des risques sur la santé publique (ex. GEOHAB, 2001; Anderson *et al.*, 2002; Glibert *et al.*, 2005a). Les proliférations d'algues nuisibles sont en nombre croissant presque chaque année et, virtuellement, chaque région côtière est affectée (Hallegraeff, 1993). Cette augmentation peut être attribuée à l'inclusion récente des microalgues benthiques (Hallegraeff, 1993; James *et al.*, 2003; Landsberg, 2002). Ces dernières sont d'un intérêt croissant, étant donné que la plupart de leurs représentants sont des producteurs de toxines potentielles (Nakajima *et al.*, 1981; Foden *et al.*, 2005; Aligizaki *et al.*, 2009). En Tunisie et depuis 1988, le phénomène des eaux colorées est provoqué par des blooms monospécifiques à cyanophycées (*Trichodesmium erythreum*), à dinophycées, à prasinophycées et dernièrement à cryptophycées (ex. Turki et El Abed, 2002 ; Abdennadher *et al.*, 2012). Des proliférations phytoplanctoniques sont apparues, dans certaines lagunes

tunisiennes induisant généralement des phénomènes d'anoxie qui affectent la faune qu'elles abritent (Belkhir, 1981 ; Guelorget, 1991 ; Kéfi, 1993 ; Turki et El Abed, 2001 ; Sakka *et al.*, 2003, 2009 ; Armi *et al.*, 2009 ; Turki *et al.*, 2007, 2014). Une présence importante des espèces épiphytes a été signalée également dans les côtes tunisiennes (Turki, 2005; Mabrouk *et al.*, 2011; Ben Brahim *et al.*, 2013), et même une immense dispersion de ces dernières dans la colonne d'eau ce qui est le cas de *Coolia monotis* dans le lac de Tunis (Armi *et al.*, 2010). Concernant la lagune de Ghar El Melh, elle reste relativement inexplorée surtout pour les proliférations microalgales nuisibles. Les seules données occasionnelles sont celles associées à la signalisation de la mortalité des poissons en 1995-1996, principalement liée à la prolifération d'*Alexandrium* sp. (Romdhane *et al.*, 1998), et en 2007 suite à celle du dinoflagellé *Kryptoperidinium foliaceum* (Turki *et al.*, 2007).

Ainsi, une étude qui traite les successions écologiques du phytoplancton dans la lagune de Ghar El Melh serait indispensable. Le traitement, aussi, du maximum de facteurs abiotiques et biotiques en étroite relation avec le compartiment phytoplanctonique pourrait nous aider à mieux comprendre l'évolution spatio-temporelle de ces populations. En effet, la dynamique des efflorescences microalgales est complexe et pourrait être le résultat d'un mécanisme de couplage faisant intervenir des facteurs physiques, chimiques et biologiques (Karl *et al.*, 1997; Yin *et al.*, 1999; Fistarol *et al.*, 2004; Solé *et al.*, 2006; GEOHAB, 2006; Adolf *et al.*, 2007; Waggett *et al.*, 2008). On ne pourrait pas étudier et comprendre la dynamique du peuplement phytoplanctonique sans considérer de près les facteurs abiotiques et biotiques qui sont en relation étroite avec ce peuplement. Le défi d'étudier le phytoplancton est de plus en plus compliqué au niveau des écosystèmes lagunaires dont la structure et le fonctionnement des réseaux trophiques sont complexes et demeurent ainsi peu connus.

Dans la lagune de Ghar El Melh, les travaux qui traitent les interactions phytoplancton - facteurs abiotiques sont rares (Ramdani *et al.*, 2004) et aucune étude n'a exploré les relations existant entre le phytoplancton et les composants biologiques associées telles que les microalgues épiphytes, les kystes de dinoflagellés, l'ultraplancton et les ciliés. Certains travaux en Tunisie, ont reporté des corrélations importantes entre le phytoplancton et les ciliés suggérant une relation trophique potentielle (Daly Yahia *et al.*, 2005 ; Sakka *et al.*, 2008 ; Drira *et al.*, 2010 ; Hannachi *et al.*, 2011), entre les concentrations des microalgues épiphytes dans les feuilles des macrophytes et l'abondance du phytoplancton dans la colonne d'eau (Mabrouk *et al.*, 2011) et entre les kystes de dinoflagellés et les espèces phytoplanctoniques numériquement abondantes dans la colonne d'eau (Fertouna-Bellakhal *et al.*, 2014).

Dans cette optique, l'intérêt général du présent travail est l'étude des successions écologiques du microphytoplancton dans la lagune de Ghar El Melh. En effet, l'aspect systématique (composition spécifique) et la dynamique saisonnière (répartition spatio-temporelle) en relation avec les facteurs de l'environnement sont d'une importance capitale pour les investigations écologiques. D'autres intérêts particuliers s'ajoutent à savoir l'étude des microalgues épiphytes, des kystes de dinoflagellés, de l'ultraphytoplancton, de la population bactérienne et des ciliés, ceci dans le but d'aboutir à la compréhension des relations entre ces communautés et le développement des principales populations phytoplanctoniques dans la lagune de Ghar El Melh. En effet, l'étude de chaque compartiment est indispensable pour les raisons suivantes :

- ✓ Les microalgues épiphytes : tenant compte de la large répartition de *Ruppia cirrhosa* dans la lagune de Ghar El Melh dont les feuilles colonisent toute la colonne d'eau aux mois d'été, aussi, cette espèce fait partie des macrophytes les moins connues à l'échelle mondiale (Triest et Sierens, 2009). De plus, selon plusieurs études, des rapports importants entre l'abondance des microalgues toxiques sur les feuilles des macrophytes et celles colonisant la colonne d'eau ont été observés (Vila *et al.*, 2001; Aligizaki et Nikolaidis, 2006; Mabrouk *et al.*, 2011).
- ✓ Les kystes de dinoflagellés : compte tenu de l'importance primordiale des kystes des dinoflagellés dans le déclenchement des efflorescences microalgales dont les espèces sont dominantes, aucune étude sur les kystes de dinoflagellés n'a été réalisée au niveau de la lagune de Ghar El Melh et les travaux sur les kystes sont rares en Tunisie et restrictifs à la lagune de Bizerte (Fertouna-Bellakhal *et al.*, 2014 ; Zmerli Triki *et al.*, 2014).
- ✓ L'ultraphytoplancton et la population bactérienne : au sein du réseau trophique aquatique, ces deux compartiments se situent juste en amont de la population microphytoplanctonique. La composition, l'abondance et les relations existantes entre ces communautés demeurent primordiales afin de mieux comprendre le réseau trophique dans un système lagunaire. Aucune étude n'a été réalisée dans la lagune de Ghar El Melh sur ces deux populations au moyen des techniques émergentes telle que la cytométrie en flux. Dans cette étude, et en utilisant cette technique, l'ultraphytoplancton peut renfermer le pico et le nanophytoplancton inférieur à 5 µm.
- ✓ Les ciliés : ces microzooplanctons se situent, au sein du réseau trophique aquatique, juste à la suite du peuplement microphytoplanctonique constituant ainsi un lien majeur

de transfert de l'énergie du niveau trophique inférieur au niveau trophique supérieur (Azam *et al.*, 1983; Sherr *et al.*, 1986). Aussi, les ciliés sont connus par leur potentielle alimentation en phytoplancton (Gismervik *et al.*, 1996; Loder *et al.*, 2011), atteignant même des taux de croissance optimale lorsqu'ils s'alimentent par certaines microalgues nuisibles (Kamiyama, 1997; Jeong *et al.*, 1999), et constituant ainsi un facteur de contrôle des efflorescences microalgales (Riegman *et al.*, 1993; Fonda Umani *et al.*, 2005). C'est la première étude des ciliés dans la lagune de Ghar El Melh.

Dans le présent travail, les différents chapitres développés sont les suivants :

- ✓ Le premier chapitre est consacré à la synthèse bibliographique relative aux traits biologiques du phytoplancton (diversité, cycle de vie des principaux groupes, migration), sur l'expansion des efflorescences planctoniques nuisibles (espèces responsables, historique de leurs apparitions dans le monde et en Tunisie, les facteurs environnementaux contrôlant leur prolifération et leur déclin).
- ✓ Le deuxième chapitre est consacré à la présentation du site d'étude (la lagune de Ghar el Melh) et de ses caractéristiques hydrobiologiques.

Les 3 chapitres suivants sont consacrés à la présentation des différents articles :

- ✓ Le troisième chapitre a été l'objet du développement des herbiers à *R. cirrhosa* et les microalgues épiphytes associées durant la période estivale. Il s'agit des résultats publiés dans « l'article 1 ».
- ✓ Le quatrième chapitre a été consacré à :
 - ✓ L'étude du cycle annuel du phytoplancton et l'importance des microalgues épiphytes dans la colonisation de la colonne d'eau (Article publié 2).
 - ✓ L'étude de la distribution des kystes des dinoflagellés (Projet d'article) a été effectuée afin d'associer leur présence à l'initiation des proliférations des populations phytoplanctoniques de certaines espèces dominantes du milieu.
 - ✓ L'étude de l'ultraphytoplancton et les hétérotrophes procaryotes (Article soumis), a été élaborée afin de mettre en évidence l'importance de cette classe de taille du plancton d'une part et sa relation avec le microplancton de la lagune de Ghar el Melh d'autre part.
- ✓ Le cinquième chapitre est consacré à des interactions biotiques entre le microzooplancton caractérisé par la présence des ciliés et les principales populations du phytoplancton de la lagune de Ghar el Melh afin d'analyser leurs capacités de prédation dans le contrôle des efflorescences phytoplanctoniques nuisibles. Il s'agit des résultats publiés dans « l'article 3 ».

Chapitre I. Le monde phytoplanctonique

1. Définition et classification

Le monde phytoplanctonique regroupe les micro-organismes unicellulaires dont la taille est comprise entre 1 μm et 1 mm et que l'on retrouve au niveau de tous les écosystèmes aquatiques (Sournia, 1986). Le peuplement phytoplanctonique regroupe des espèces autotrophes, mixotrophes ou hétérotrophes, des formes mobiles, fixées, planctoniques, benthiques, libres, symbiotes, ou parasites (Sournia, 1986). Selon le critère de taille, les communautés phytoplanctoniques sont formées essentiellement du microphytoplancton et d'ultraphytoplancton (nanophytoplancton et picophytoplancton). La majorité du nano- et du microphytoplancton est représentée par les eucaryotes. Toutefois, les procaryotes sont représentés essentiellement dans le picoplancton (Tableau 1). Le phytoplancton, représenté par une importante variété d'espèces, constitue la base des écosystèmes marins et sert de nourriture aux organismes benthiques filtreurs, au zooplancton et aux poissons planctonophages. Le phytoplancton représente un compartiment remarquable par le rôle qu'il joue, non seulement dans le milieu aquatique mais aussi dans tous les domaines de notre vie : qualité d'environnement (oxygène), alimentation (poissons et fruits de mer), bien-être (santé, beauté), ressource industrielle.

Tableau 1. Classification simplifiée des organismes phytoplanctoniques (Margullis et Schwartz, 1998 ; Van den Hoek *et al.*, 1995)

Super-règne	Règne	Division	Classe
Prokarya (Procaryotes)	Bactéries	Cyanobactéries	Cyanobactéries (ou cyanophycées)
Eukarya (Eucaryotes)	Protistes	Dinophyta	Dinophyceae (dinoflagellés)
		Heterokontophyta (silicoflagellés)	Chrysophyceae (chrysophycées) Bacillariophyceae = Diatomophyceae (diatomées) Dictyochophyceae Raphidophyceae (chloromonadines)
		Prymnesiophyta = Haptophyta	Prymnesiophyceae = Haptophyceae (prymnésiofycées ou haptophycées)
		Cryptophyta	Cryptophyceae (cryptophycées)
		Chlorophyta	Chlorophyceae (chlorophycées) Prasinophyceae (prasinophycées)

1.1. Microphytoplancton

Le microphytoplancton (20-200 μm) regroupe deux grandes classes (Bougis, 1974): les Bacillariophycées (ou diatomées) et les Dinophycées (ou dinoflagellés). D'autres groupes sont rassemblés dans le règne du phytoplancton à savoir: les Cryptophycées, les Prymnésiophycées, les Chrysophycées, les Dictyochophycées, les Raphidophycées, les Euglénophycées, les Prasinophycées et les Chlorophycées. Le microphytoplancton constitue le principal producteur primaire au niveau des écosystèmes marins pélagiques et qui reflète les changements écologiques du biotope où il vit (Polat et Isik, 2002).

1.1.1. Bacillariophycées (ou diatomées)

Les diatomées (Bacillariophyceae) (Phylum des Chromophycophytes, Embranchement Chrysophycophytes) ont été reconnues comme le principal groupe du phytoplancton marin. Elles représentent l'une des composantes cruciales de la production primaire du milieu pélagique. Elles contribuent, seules, à presque 43 % de la production primaire marine à l'échelle annuelle (Nelson *et al.*, 1995). Les diatomées sont caractérisées par une grande diversité : Il existe de 10×10^3 à 12×10^3 espèces de diatomées dans le phytoplancton marin.

- Structure

Les diatomées sont caractérisées par la présence d'une paroi siliceuse (la frustule) autour de la cellule. Cette paroi montre une différenciation morphologique importante. En effet, la morphologie, la structure et l'ornementation du frustule sont les critères de détermination systématique chez la plupart des diatomées. La frustule est doublée extérieurement par une couche organique composée des polysaccharides et des polypeptides. L'ensemble (frustule et revêtement organique) est percé de pores permettant le passage des éléments nutritifs à l'intérieur du protoplasme. Les diatomées produisent une substance dite: mucilage, un phénomène qui se présente essentiellement pour attacher les cellules entre elles au sein des colonies ou pour permettre l'adhésion au substrat des espèces benthiques. Toutefois, ces espèces microalgales, sont tributaires du silicium pour leur développement et la duplication de l'ADN, qui précède la division cellulaire.

- Classification des diatomées

Selon la morphologie, les diatomées sont divisées en deux ordres : les Pennatophycideae (ou Pennées) et les Centrophycideae (ou Centriques) (Figure 1).

- Les Pennales : les valves possèdent deux plans de symétrie perpendiculaires entre eux et perpendiculaires au plan valvaire. Cette classe caractérise essentiellement le microphytobenthos. Chez les Pennales, on distingue généralement un ou deux plastes de grande taille.

- Les Centrales : les valves possèdent un axe de symétrie radiale. Cette classe constitue le groupe majoritaire du phytoplancton. Chez les centriques, les plastes contiennent toujours un pyrénéoïde dont la forme est variable. Les plastes sont de petites tailles, nombreuses et en forme de disques aplatis localisés à la périphérie de la cellule.

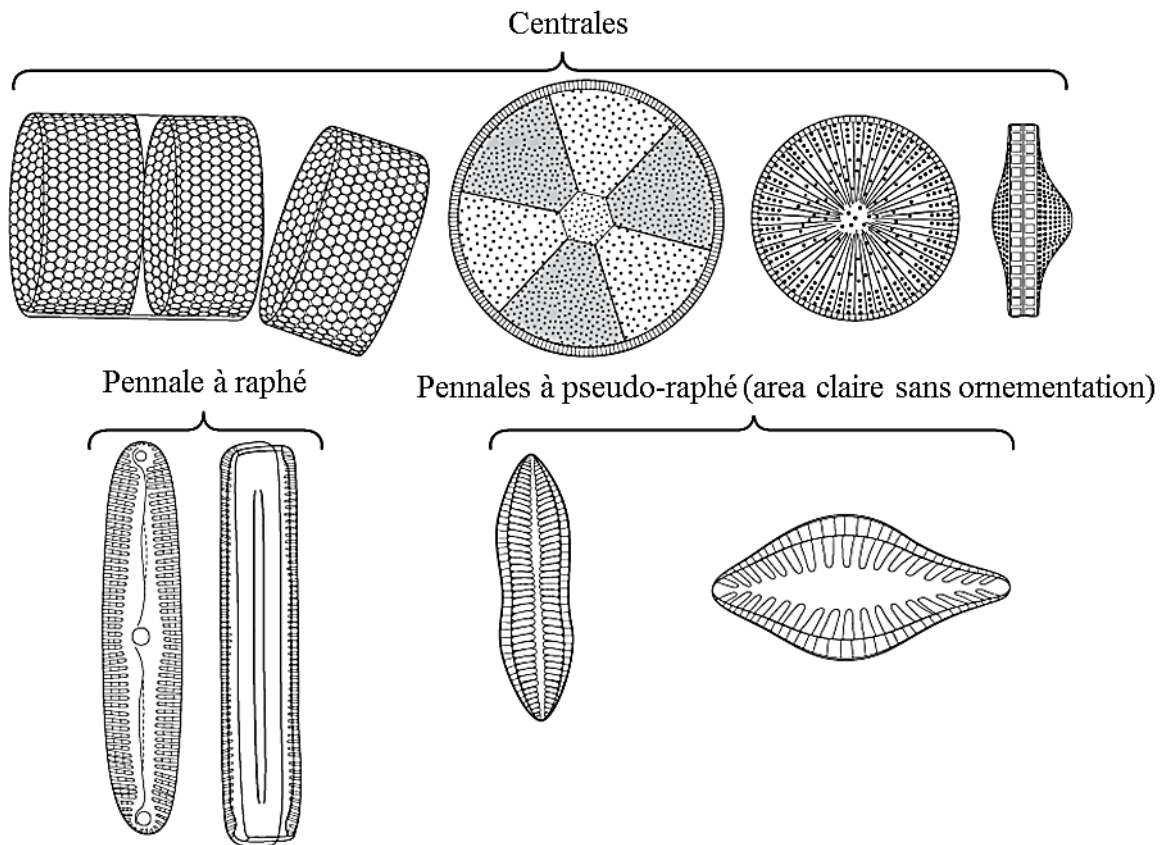


Figure 1. Variété des frustules de diatomées (Mathieu *et al.*, 2011)

1.1.2. Dinophycées (dinoflagellés)

Les dinoflagellés appelés encore Péridiniens (Phylum des Chromophycophytes, Embranchement des Pyrrophytophytes) sont après les diatomées le groupe le plus représenté dans le phytoplancton marin. Il comporte 20 ordres et plus de 1300 espèces. A la différence des diatomées, les individus vivent libres et rarement regroupés en chaînes. Ils peuvent être trouvés dans les eaux douces, saumâtres ou marines. Les espèces peuvent être planctoniques, benthiques ou épiphytiques, certaines sont symbiotiques, comme par exemple les zooxanthelles associées aux coraux ou à d'autres invertébrés, d'autres sont strictement parasites (Hansen *et al.*, 2001). Ils sont généralement adaptés à la vie pélagique, cependant plusieurs genres présentent dans leur cycle biologique une phase benthique ; phase de repos

enkystée déposée sur le fond (Trégouboff et Rose, 1978). Environ 50 % des 2500 espèces décrites sont hétérotrophes en se nourrissant d'autres organismes, principalement d'autres protistes (Gaines et Elbrachter, 1987). Bien que les espèces restantes soient phototrophes, beaucoup d'entre elles sont mixotrophes (Skovgaard, 1996 a,b ; Stoecker *et al.*, 1997).

- Structure

Les cellules des dinoflagellés peuvent être nues ou couvertes de plaques cellulosiques formant la thèque (Seguin *et al.*, 1997) (Figure 2). Elles se distinguent par des caractères propres au groupe (i) une partie antérieure appelée épïcône ou épisôme ou encore épithèque, (ii) une partie postérieure appelée hypocône ou hyposôme ou hypothèque. (iii) deux flagelles logés dans deux sillons orthogonaux : le sulcus et le cingulum (appelé aussi ceinture), dont la jonction définit la face ventrale de ces organismes (Sournia, 1986 ; Lassus, 1988). Chez quelques groupes comme les Prorocentrales, le cingulum et le sulcus sont absents et les deux flagelles sont insérés antérieurement (Taylor, 1987) et (iv) un dinocaryon constituant le noyau des dinoflagellés. Il se caractérise par des chromosomes toujours condensés quel que soit le cycle cellulaire et au microscope photonique, ces chromosomes sont habituellement visibles et forment des petites tâches ou de courtes bandes (Hansen *et al.*, 2001).

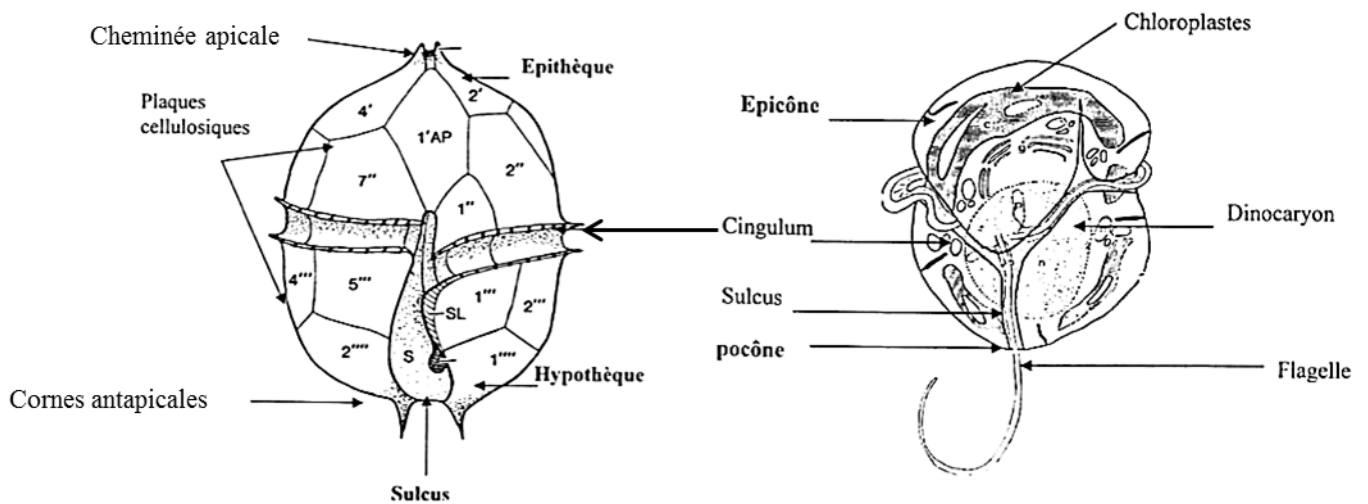


Figure 2. Organisation générale d'un dinoflagellé cuirassé (AP : plaque apicale, S : sulcus, SL : suture sulcal) (d'après Dodge, 1985) et d'un dinoflagellé nu (Sournia, 1986)

- **Reproduction**

Le cycle biologique des dinoflagellés présente deux phases de reproduction : asexuée et sexuée. Seuls les dinoflagellés formant des kystes permanents semblent présenter un mode de reproduction sexuée. Le cycle biologique des dinoflagellés explique leurs aptitudes à former des eaux colorées. Ce phénomène de bloom est souvent lié à une remise en suspension des kystes et au développement des cellules végétatives.

1.1.3. Organismes procaryotes : les cyanobactéries

Les cyanobactéries sont des organismes caractérisant généralement les eaux douces. Ils présentent une écologie des bactéries et une physiologie des algues. Conformément aux Procaryotes, les organites cellulaires sont dépourvus de membrane. Les cyanobactéries peuvent être unicellulaires libres ou formant des colonies filamenteuses. Une caractéristique de ce groupe c'est que les thylakoïdes et le matériel génétique sont libres dans le cytoplasme. Qu'ils soient unicellulaires solitaires ou formant des colonies filamenteuses, les Cyanobactéries se divisent en deux groupes (Sournia, 1986) : les Chroococcales et les Hormogonales, respectivement. Ces procaryotes, surtout sous forme coloniale, sont susceptibles de fixer l'azote atmosphérique par le biais de cellules spécialisées dites : hétérocystes. La reproduction sexuée est inconnue.

1.2. Nanophytoplankton

Le nanophytoplankton renferme un ensemble d'organismes eucaryotes autotrophes de taille comprise entre 2 et 20 μm et qui peuvent parfois être mobiles (cas des flagellés). Ce sont les principaux responsables du transfert de l'énergie et de la matière, des bactéries vers les niveaux trophiques supérieurs, notamment le microzooplankton (Azam *et al.*, 1983; Porter *et al.*, 1985; Sherr *et al.*, 1986; Verity *et al.*, 2002). Plusieurs travaux ont montré l'importance quantitative de la fraction nanophytoplanktonique dans le milieu marin (Watson et Klaff, 1981 ; Christaki *et al.*, 2001; Sanders *et al.*, 2000; Lovejoy *et al.*, 2002).

1.3. Picophytoplankton

Il est composé de picocyanobactéries et de picoeucaryotes (Stockner et Antia, 1986; Riemann et Christoffersen, 1993), caractérisé par une taille de l'ordre du micron (0.2 – 2 μm) (Sieburth *et al.*, 1978). Parmi les cyanobactéries, le genre *Synechococcus* (0.6 – 0.9 μm) qui a été détecté à l'aide de la microscopie à épifluorescence par leur forte intensité de fluorescence jaune-orange d'un de leurs pigments photosynthétiques : la phycoérythrine (Waterbury *et al.*, 1979). Le genre *Prochlorococcus* (0.6 – 0.8 μm) a été découvert par cytométrie en flux (Chisholm *et al.*, 1988) et il est reconnu comme l'organisme photosynthétique le plus abondant de la biosphère (Partensky *et al.*, 1999). Les *Synechococcus* sont généralement

moins abondantes (Morel *et al.*, 1993) et plus ubiquistes que les *Prochlorococcus*. Les picoeucaryotes regroupent des espèces très variées et renferment un grand nombre de classes d'algues (Chlorophyceae, Cryptophyceae, Pelagophyceae, Prasinophyceae...) (Simon *et al.*, 1994). En effet, ces dernières années de nombreux classes, genres et espèces ont été décrits (Courties *et al.*, 1994). Un des rôles primordiaux des picoeucaryotes est leur contribution à la production en milieu marin qui est de 16 % et en milieu lacustre de 70 % (Magazzù et Decembrini, 1995; Metzler *et al.*, 2000). Plusieurs études montrent que les picoeucaryotes jouent un rôle très important dans le flux de la matière et de l'énergie et dans la boucle microbienne (ex. Weisse et Stockner, 1992; Bertrand et Vincent, 1994; Šimek *et al.*, 1995; Stockner *et al.*, 2000). En effet, cette communauté a fait l'objet de nombreux travaux dans les lacs et les rivières (ex. Ruggiu *et al.*, 1998; Vörös *et al.*, 1998; Stockner *et al.*, 2000; Callieri et Stockner, 2002), et en milieu marin (ex. Moon-van *et al.*, 2001; Massana *et al.*, 2004; Romari et Vaultot, 2004; Jiao *et al.*, 2005).

2. Efflorescences microalgales nuisibles

Les microalgues sont des organismes magnifiques qui contribuent à la plus grande diversité de formes dans les milieux aquatiques et, sans ces producteurs primaires, il n'y aurait pas de vie dans les mers et les océans. Cependant, dans certaines situations, les proliférations microalgales peuvent avoir plusieurs répercussions sur l'écosystème. Outre les problèmes d'anoxie connus depuis très longtemps, diverses études signalent actuellement des espèces provoquant des nuisances diverses telles que des intoxications parfois fatales chez l'Homme et la faune marine. En effet, parmi les 4000 espèces microalgales planctoniques marines recensées à travers le monde 5.5 à 6.7 % (184-267 espèces) ont été identifiées comme responsables d'efflorescences algales massives (Sournia, 1995). Parmi celles-ci, environ la moitié, sont des dinoflagellés, les diatomées arrivant en seconde position, neuf autres classes étant également impliquées. Les espèces productrices de toxines sont moins nombreuses, 60 à 78 espèces ayant été recensées (soit 1.8 à 1.9 % de la «microflore pélagique mondiale»). Parmi ces espèces, 90 % sont des flagellés, et plus particulièrement des dinoflagellés (Smayda, 1997).

Les intoxications humaines associées à la consommation de coquillages sont connues depuis plusieurs siècles et en divers endroits du globe. La première mention la mieux documentée étant l'intoxication par des toxines paralysantes (PSP) (Kao, 1993). En Méditerranée, de tels problèmes ont été enregistrés depuis les années 1975, mais les réelles manifestations toxiques

ont été enregistrées pour la première fois en 1989 sur les côtes espagnoles concernant les toxines PSP, et en Italie pour les toxines diarrhéiques DSP (PNUE, 1995).

L'ensemble de ces phénomènes est aujourd'hui rassemblé sous l'appellation internationale : « Harmful Algal Bloom » ou « HAB ». Au cours de ces efflorescences, les concentrations cellulaires observées peuvent dépasser plusieurs millions de cellules par litre d'eau. Les efflorescences de micro-algues sont le plus souvent mono- ou oligospécifiques et elles induisent une diminution de la biodiversité du phytoplancton local (Crossetti *et al.*, 2008). Néanmoins, les nuisances engendrées ne sont pas nécessairement liées à une augmentation significative de la biomasse : de nombreuses espèces responsables de HAB sont «nuisibles» à faible concentration (quelques centaines de cellules par litre). Ainsi deux groupes d'organismes impliqués dans des phénomènes HAB peuvent être distingués : les organismes producteurs de toxines qui peuvent être nocifs même à faible concentration d'une part, et les organismes qui ne produisent pas de toxines, mais qui ont un effet néfaste sur l'environnement ou sur les organismes marins d'autre part.

Au cours des dernières années, l'observation accrue d'efflorescences «toxiques ou nuisibles», liées à l'extension des usages du domaine côtier, a vu l'augmentation de la liste des espèces suspectes (Zingone et Enevoldsen, 2000). Une partie de cette augmentation est due à l'intégration récente des microalgues benthiques (Hallegraeff, 1993; James *et al.*, 2003; Landsberg, 2002) qui, au cours de cette dernière décennie, ont étendu leur distribution des mers tropicales-subtropicales jusqu'aux mers tempérées telles que la Méditerranée (Tognetto *et al.*, 1995; Vila *et al.*, 2001a,b; Ismael et Halim, 2006; Monti *et al.*, 2007; Aligizaki *et al.*, 2008; Mangialajo *et al.*, 2008). La majorité des microalgues benthiques est potentiellement toxique (Nakajima *et al.*, 1981; Foden *et al.*, 2005; Aligizaki *et al.*, 2009) et certaines d'entre elles ont été associées à l'intoxication de type ciguatera (Fraga *et al.*, 2008). Contrairement à la plupart des autres espèces benthiques, le dinoflagellé *Prorocentrum lima* est capable de se développer dans la colonne d'eau à des concentrations qui pourraient justifier des événements de DSP (Quilliam *et al.*, 1993 ; Nascimento *et al.*, 2005).

3. Successions écologiques du phytoplancton

L'analyse de l'évolution des communautés phytoplanctoniques a montré l'existence de successions de populations orientées (Margalef, 1958). En effet, la composition spécifique des communautés phytoplanctoniques, l'abondance relative des différentes espèces, ainsi que la dominance d'une population par rapport à une autre sont autant de traits et de

phénomènes en constante évolution qui caractérisent les successions phytoplanctoniques (Smayda, 1980).

Selon Margalef (1958), la succession écologique phytoplanctonique s'articule en trois stades : le premier stade est caractérisé par une croissance élevée des petites diatomées, puis des diatomées de plus grande taille et à faible taux de croissance deviennent dominantes, le troisième stade étant caractérisé par la présence de dinoflagellés, de grande taille également, et à taux de croissance encore plus faible. Le passage progressif du premier état au troisième s'accompagne d'une diminution de la concentration en nutriments dans le milieu.

Cependant, cette description n'intègre pas les relations interspécifiques ni les exigences écologiques de chaque espèce, ce qui est nécessaire de prendre en compte pour comprendre la variation des perturbations environnementales et de la dominance d'une espèce.

En effet, la dynamique des populations phytoplanctoniques est examinée à travers la réponse globale de la communauté aux variations environnementales : les efflorescences phytoplanctoniques sont des événements de production rapide qui sont des réponses aux changements provenant de la masse d'eau, de l'atmosphère ou de la terre (précipitations et apports fluviaux) (Maestrini et Granéli, 1991 ; Franks, 1992 ; Rhodes *et al.*, 1993; Cloern, 1996). Il en résulte que les efflorescences peuvent être des événements épisodiques de courte durée et d'amplitude variable, des phénomènes saisonniers récurrents ou des événements rares associés à des conditions hydrologiques ou météorologiques ponctuelles récurrentes ou exceptionnelles. Ce phénomène provoque d'importants changements dans la structure des communautés.

Au laboratoire, l'étude de la dynamique phytoplanctonique est principalement effectuée à des échelles de temps courtes (Fouillaron *et al.*, 2007), tandis que, *in situ*, l'étude de la dynamique phytoplanctonique est généralement effectuée en réalisant des prélèvements mensuels ou bi-mensuels (Jouenne *et al.*, 2007 ; Spatharis *et al.*, 2007).

Comprendre et caractériser la variabilité de la structure des communautés phytoplanctoniques, en particulier la succession des groupes, dans des écosystèmes particuliers tels que les lagunes est relativement important. En effet, au sein des milieux semi-fermés, tout changement de communautés peut être amplifié et avoir des effets majeurs sur l'écosystème, en particulier si ces populations sont toxiques. A titre d'exemples, les échanges entre les eaux estuaires et marines pourraient influencer la composition des espèces directement à travers l'introduction de nouvelles espèces et indirectement par l'augmentation des éléments nutritifs. La concentration de nutriments peut donc être modifiée en quelques jours et pourrait induire des conséquences transitoires sur la croissance, la biomasse, et la concurrence entre les groupes et

les espèces. Aussi, la disponibilité de la lumière peut changer en quelques jours (par exemple suite à l'entrée de la matière en suspension), ce qui peut affecter la structure des communautés phytoplanctoniques en particulier dans les systèmes turbides et riches en nutriments (Alpine et Cloern, 1988).

Selon Breton *et al.* (2006), les facteurs environnementaux peuvent être utilisés pour prédire l'apparition des principaux groupes de phytoplancton.

Toutefois, dans tous les cas, il est impossible de prédire quel groupe phytoplanctonique va dominer ou bien quelles espèces vont se développer. La dynamique des espèces côtières de phytoplancton est encore mal comprise, et le fait de trouver le lien entre les changements des facteurs environnementaux et leur influence sur la croissance et la concurrence entre les espèces représente une étape critique dans l'amélioration de la compréhension des systèmes côtiers.

4. Microphytoplancton en relation avec les facteurs abiotiques et biotiques

4.1. Relation avec les facteurs abiotiques

Les facteurs abiotiques jouent un rôle plus au moins décisif, suivant les périodes de l'année, dans les fluctuations de la composition et de l'abondance du phytoplancton. Parmi ces paramètres nous relaterons les plus importants :

- La lumière qui dirige non seulement la photosynthèse mais aussi, selon sa quantité et de sa qualité, régit aussi une partie de sa croissance et, par conséquent, la structure de la communauté algale. Ce facteur varie en fonction de l'heure du jour, les saisons et la couverture nuageuse. Selon Robert et Catesson (2000), dans un système aquatique dont les caractères ne changent pas, la production phytoplanctonique est directement influencée par le degré d'ensoleillement durant les 48 heures précédentes. La qualité spectrale de la lumière conditionne fortement la production primaire en intervenant dans la stratification des organismes dans la colonne d'eau. En effet, le phytoplancton se développe en abondance dans la couche euphotique et se raréfie en profondeur.

Dans un milieu aquatique, les variations rapides de l'angle d'incidence de la lumière sous l'effet des vagues provoquent des fluctuations dans la pénétration des rayons lumineux (Robert et Catesson, 2000).

- La température influence plusieurs paramètres physicochimiques de l'eau tels que les concentrations en oxygène dissous et en nutriments. En effet, elle est un stimulus des phénomènes d'accroissement, de la reproduction et du développement de la majorité des espèces végétales macro et microscopiques (Amniot et Chaussepied, 1983). Chaque espèce

possède son propre gradient de température optimale. Les variations saisonnières de la température influencent ainsi de façon importante la structure de la communauté algale. Selon Eppley (1972), la croissance phytoplanctonique se trouvera ralentie dans les eaux froides (écosystèmes des hautes latitudes) par rapport à des eaux plus chaudes (régions tempérées). L'élévation de la température de l'eau agit, positivement, sur la production primaire du phytoplancton en influençant le métabolisme des organismes par action sur la vitesse des réactions enzymatiques (Doney, 2006; Chavez *et al.*, 2011). La température pourrait aussi provoquer le déclin des proliférations microalgales dans le cas du réchauffement climatique (Boyce *et al.*, 2010; Moran *et al.*, 2010).

- La salinité : ce facteur peut être considéré comme paramètre définissant l'habitat des espèces et délimitant leur répartition (Kirst, 1995). Les fortes densités de salinité stimulent le développement du phytoplancton (Yin *et al.*, 2000) et augmentent la biomasse chlorophyllienne (Revelante et Gilmartin, 1976). Plusieurs travaux ont reporté l'influence de la salinité sur la distribution du phytoplancton (ex. Lobban and Harrison, 1994 ; Kies, 1997, Fu and Bell, 2003; Kutlu *et al.*, 2012).

-Le pH doit être compris entre 5 et 8.5 dépassant cette fourchette, les espèces peuvent réguler leur croissance ou transformer même leur physiologie en développant ainsi un pouvoir de toxicité (Arrignon, 1998).

Dans les milieux très eutrophes, l'augmentation du pH entraîne une diminution de la solubilité des bicarbonates dans l'eau pouvant créer une limitation de croissance du phytoplancton (Sevrin-Reyssac *et al.*, 1996).

- Les nutriments : plusieurs études écologiques ont mis l'accent sur l'effet des nutriments sur le phytoplancton (Granéli *et al.*, 1999). La disponibilité des nutriments constitue le principal facteur qui contrôle le phytoplancton lorsque les conditions de lumière et de température sont adéquates (Hecky et Kilham, 1988). En effet, elle peut affecter son taux de croissance (Sakka *et al.*, 2006), sa biomasse et sa composition spécifique (Caron *et al.*, 2000).

De plus, l'abondance des microalgues nuisibles est maintenant bien connue comme une expression de l'eutrophisation (Anderson *et al.*, 2002 ; Glibert *et al.*, 2005; Glibert et Burkholder, 2006 ; Heisler *et al.*, 2008). En effet, la perturbation du ratio azote/phosphore se traduit par la sélection de certaines microalgues notamment favorisant les espèces nuisibles (Smayda, 1990; Smayda et Reynolds, 2003; Heisler *et al.*, 2008).

Cependant, dans tous les cas, un lien évident entre une augmentation des HAB (la fréquence, l'ampleur ou la durée) et un changement d'azote ou phosphore est encore difficile à établir. Davidson *et al.* (2012) ont apporté l'hypothèse selon laquelle la valeur du rapport des

nutriments n'a pas d'importance dans la détermination de la compétition entre les espèces si la concentration de chaque nutriment n'est pas assez faible pour limiter la croissance.

4.2. Relation avec les facteurs biotiques

4.2.1. Microalgues épiphytes

Les macrophytes possèdent une place très importante dans plusieurs lagunes du monde (Menéndez, 2002 ; Sfriso *et al.*, 2002). Elles constituent un habitat favorable pour plusieurs organismes (Agostini *et al.*, 2003; Heck *et al.*, 2008) et elles ont un rôle primordial du point de vue économique et écologique (Skinner et Zalewski, 1995; Giovanetti *et al.*, 2010; Cullen-Unsworth *et al.*, 2013). Aussi, grâce à leur grande distribution et à leur grande sensibilité aux changements des zones côtières, les herbiers sont considérés comme des bioindicateurs de la qualité des eaux (Bhattacharya *et al.*, 2003; Pergent-Martini *et al.*, 2005) et ils ont été récemment inclus parmi les composants biologiques utilisés dans les programmes de suivi de la qualité des eaux (EC, 2000; Foden et Brazier, 2007). Les épiphytes qui colonisent ces herbiers, ne participent pas seulement à la production primaire (Nelson et Waaland, 1997; Duarte *et al.*, 2004) mais ils sont plus sensibles que l'herbier hôte aux changements environnementaux (Delgado *et al.*, 1999; Nesti *et al.*, 2008; Giovannetti *et al.*, 2010) et ils ont des réactions différentes aux interférences anthropiques qui peuvent toucher leur structure et leurs fonctions (Sfriso *et al.*, 2007, 2009; Orlando- Bonaca *et al.*, 2008). Par exemple, plusieurs études ont montré le surcroît de la biomasse épiphytique comme réponse à l'eutrophisation (Balata *et al.*, 2008; Frankovich *et al.*, 2009; Ben Brahim *et al.*, 2013).

Les herbiers constituent un habitat favorable pour les microalgues notamment les diatomées (Penhale, 1977; Cummins *et al.*, 2004). Plusieurs études ont trouvé des corrélations importantes entre l'abondance des microalgues toxiques sur les feuilles des macrophytes et les microalgues au sein de la colonne d'eau (Vila *et al.*, 2001b; Aligizaki et Nikolaidis, 2006; Mabrouk *et al.*, 2011). En effet, les microalgues épiphytes pourraient être à l'origine de la colonisation des masses d'eau, constituant une part importante du phytoplancton.

4.2.2. Kystes de dinoflagellés

Les dinoflagellés ont la particularité de produire, durant leur cycle biologique, des kystes qui constituent un stade de dormance suite à la reproduction sexuée (ex. Wall et Dale, 1968; Taylor, 1987). Les kystes, d'environ 15 à 20 % des dinoflagellés, sont constitués d'une membrane organique (sporo-pollénine) extrêmement résistante et fossilisable et présentent un intérêt particulier en micropaléontologie puisqu'ils ne sont pas affectés par la dissolution comme les microfossiles à test minéral.

Seulement 60 espèces (35 Peridiniacées, 18 Gonyaulacacées, 9 Gymnodinales, 2 Prorocentrales) sont connues actuellement comme productrices de kystes. Toutes les formes décrites sont reconnaissables par les critères suivants (Hallegraeff *et al.*, 1995) :

- la forme qui peut varier énormément même au sein d'un seul genre.
- la taille qui varie de 20 à 150 µm.
- la couleur qui varie du transparent au brun (le brunissement est souvent un indicateur de pré-germination de kyste).
- la présence d'une thèque ou de plusieurs couches (généralement, la première couche est constituée de sporo-pollénine qui permet la fossilisation). Des kystes calcaires et siliceux ont été retrouvés surtout parmi les organismes fossiles.
- la surface qui peut être lisse ou réticulée, sans ou avec des ornements dénommés « process » qui elles aussi ont des formes et des tailles très variables.
- le contenu cellulaire constitué de produits de réserve formés de grains d'amidon et de gouttelettes lipidiques.
- la forme de l'archéopyle qui est une ouverture par où s'échappe le protoplasme au moment de la germination et qui est couverte d'un fermoir dit operculum. Généralement, il y a 3 formes dépendant de la suture de l'operculum : une forme saphopylique où l'operculum est complètement détaché, une théropylique avec un operculum partiellement attaché et une cryptopylique qui est en forme de trou ou de fente (Figure 3).

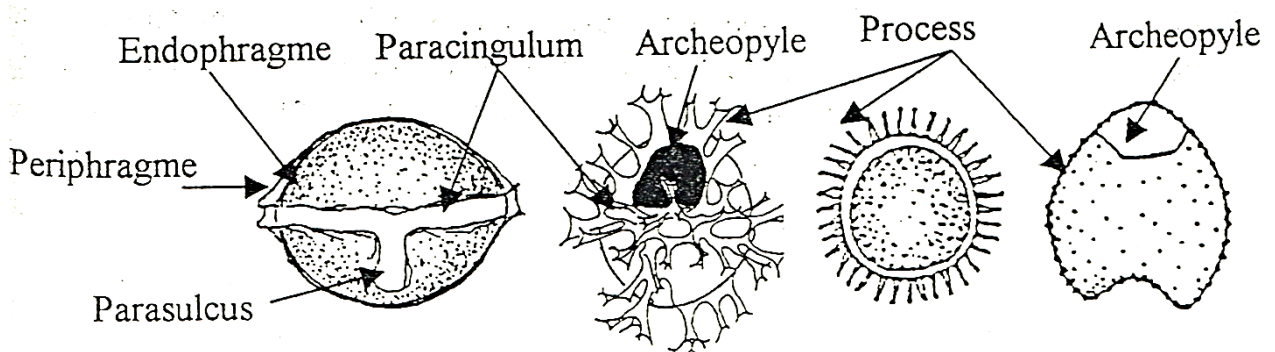


Figure 3. Différentes structures et ornements de la surface de la surface des kystes (d'après Matsuoka et Fukuyo, 1995)

Dans les domaines estuariens et côtiers, les assemblages de dinokystes peuvent refléter l'état d'eutrophisation du milieu (Matsuoka, 1999 ; Dale, 2001 ; Pospelova *et al.*, 2002, 2005). La germination de cette forme de résistance est contrôlée par les conditions environnementales

(température, lumière et O₂) ou par une horloge biologique interne (Anderson et Keafer, 1987; Yamaguchi *et al.*, 1996 ; Itakura et Yamaguchi, 2001). Les efflorescences sont fortement dépendantes de l'abondance et de la distribution des kystes benthiques dans le sédiment: rôle prépondérant dans la dispersion de l'espèce, l'initiation, et la terminaison des blooms, et dans la survie de l'espèce (Yamaguchi *et al.*, 1995, 1996 ; Persson *et al.*, 2000 ; Dale, 2001). Le dénombrement et l'étude de la germination des kystes paraissent intéressants pour une meilleure connaissance du cycle de vie des espèces côtières. Selon Dale (1977), il existe une assez bonne corrélation entre l'abondance des kystes et des cellules mobiles. D'autre part, les kystes représentant des populations relativement stationnaires par rapport aux stades pélagiques, il est plus facile de les étudier pour la prévision de l'apparition et la persistance des efflorescences phytoplanctoniques. Leur présence dans les sédiments côtiers et lagunaires constitue un risque potentiel pour la santé et l'environnement, au même titre que de nombreuses autres substances toxiques (Yamaguchi *et al.*, 1995). Les kystes toxiques représentent une source potentielle de toxification des coquillages (Yamaguchi *et al.*, 1995), car leur contenu toxique peut être plus fort que celui des cellules végétatives.

4.2.3. Ultraplankton

- **Hétérotrophes procaryotes (bactérioplancton)**

Avant les années 70, le seul composant microbien des réseaux trophiques pélagiques pris principalement en considération était le « net phytoplankton » (Steele, 1974). Les bactéries hétérotrophes ne jouaient alors qu'un rôle de « décomposeur » de matière, constituant simplement une source de nutrition pour les organismes benthiques (Steele, 1974). De plus, les mesures d'abondance réalisées à cette période par culture sur milieu solide (ex. gélose nutritive ou agar-agar, Zobell, 1937) ne donnaient pas d'estimation quantitativement fiable (Billen *et al.*, 1990 et références citées), dès lors qu'elles ne prenaient pas en compte les cellules viables (et plus précisément actives) non « cultivables » (Roszak *et al.*, 1984 ; Barer et Harwood, 1999). Suite aux avancées techniques (ex. cytométrie en flux ; Veal *et al.*, 2000 ; Vives-Rego *et al.*, 2000 ; Gruden *et al.*, 2004) en écologie microbienne, l'importance quantitative des bactéries hétérotrophes dans les flux de matière et d'énergie a largement été réévaluée (Legendre et Rivkin, 2002) et leur rôle dans le réseau trophique progressivement mis en évidence.

Statut trophique

Le bactérioplancton peut être considéré comme un « puits » de carbone organique ainsi qu'un « relais » trophique indispensable à l'alimentation de l'écosystème planctonique côtier en matière organique et donc à son fonctionnement (Rassoulzadegan *et al.*, 1993).

Au sein du réseau planctonique, les bactéries hétérotrophes peuvent être consommées à la fois par des protistes bactérivores (nanoflagellés et petits ciliés) (Sherr et Sherr, 1994), et par du phytoplancton mixotrophe (Neuer et Cowles, 1995 ; Strom et Morello, 1998 ; Caron, 2000).

- **Ultraphytoplancton (nanophytoplancton + picophytoplancton)**

En milieux oligotrophes marin et lacustre, le picoplancton autotrophe peut représenter une partie plus importante de la production primaire que les classes de taille nano et microphytoplanctoniques (Agawin *et al.*, 2000). Le picoplancton est très abondant dans les régions océaniques oligotrophes dont la colonne d'eau est caractérisée par une stratification thermique (Chisholm, 1992). En effet, ces petits organismes phototrophes peuvent être considérés comme des marqueurs d'oligotrophie. Ainsi, selon Fogg (1986), ce type de phytoplancton ne serait pas adapté à l'eutrophie de fait que leur production diminue avec l'augmentation de l'eutrophisation. En effet, ces petits organismes laisseraient leur place aux organismes les plus grands. En 1985, Murphy a montré que le phytoplancton de petite taille et mobiles (picoflagellés et picocyanobactéries) prédominent dans les eaux oligotrophes (Bell et Kalff, 2001; Schallenberg et Burns, 2001; Callieri et Stockner, 2002) tandis que les grandes cellules non-mobiles tel que le microphytoplancton dominant au niveau des écosystèmes eutrophes. Par conséquent, le picophytoplancton domine dans les eaux oligotrophes (Dorth et Packard, 1989) grâce à sa meilleure capacité d'adaptation.

Les petites cellules sont plus efficaces dans l'assimilation des nutriments limitants (Malone, 1980; Litchman et Klausmeier, 2008). En revanche, en cas de concentrations élevées en nutriments, le microphytoplancton avec une taille plus importante atteint généralement des taux de croissance plus élevés que les petites cellules (Laws, 1975). D'autre part, les grandes cellules phytoplanctoniques sont mieux adaptées à des fluctuations importantes de lumière que les petites cellules (Bode et Fernandez, 1992; Kiørboe, 1993; Gargett et Marra, 2002). En effet, la taille des populations phytoplanctoniques et l'abondance de ces cellules sont déterminées par des facteurs physiologiques, tels que l'efficacité de la capture de la lumière ou l'assimilation des éléments nutritifs, mais dépend aussi d'autres facteurs comme la concurrence et la prédation qui influence la taille de la cellule (Lewis, 1976; Reynolds, 1997).

4.2.4. Ciliés

Ce sont des microorganismes dont la taille variable est comprise entre 20 et 200 μm (Dussart, 1965, Karayanni *et al.*, 2003). Les protozoaires ciliés appartiennent au règne des protistes, phylum des Ciliophora. Ce dernier est considéré comme un taxon monophylétique, malgré la diversité des assemblages (Katz *et al.*, 2005). Le phylum des ciliés constitue un ensemble homogène, très différencié et nettement séparé des autres protistes. Les ciliés sont des

organismes très fragiles (Carrias, 1996 ; Johansson *et al.*, 2004) et possèdent une durée de vie limitée (Madoni, 2006).

- Classification

La première classification a été établie par Bütschli (1887-1889) et Petz (1999). Selon Lynn et Small (2002), les ciliés se divisent en 8 classes principales (Tableau 2). La classification est généralement basée sur la ciliature. En effet, comme leur nom l'indique, les protozoaires ciliés sont caractérisés par la présence de cils vibratiles à leur surface, arrangés radialement autour du cytopharynx (Lynn, 1996 ; Lynn et Small, 1997, 2002 ; Li et Song, 2006). Les cils possèdent un double rôle : la locomotion et l'alimentation.

Tableau 2. Classification des protozoaires ciliés (Lynn et Small, 2002)

Classe	Ordre	Famille	Genre
Spirotrichea	Oligotrichia	Strombidiida	<i>Strombidium</i>
	Choreotrichia	Choreotrichida	<i>Strombidinopsis</i> , <i>Strobilidium</i> , <i>Lohmanniella</i> , <i>Leegaardiella</i>
		Tintinnida	<i>Tintinnopsis</i> , <i>Tintinnidium</i> ,
	Hypotrichia	Euplodida	<i>Euplote</i>
Prostomatea		Prostomatida	<i>Urotricha</i> , <i>Balanion</i>
Heterotrichea		Heterotrichida	<i>Blepharisma</i>
Litostomatea	Haptoria	Haptorida	<i>Mesodinium</i>
Oligohymenophorea	Peniculia	Peniculida	<i>Urocentrum</i>
	Scuticociliatia	Philasterida	<i>Uronema</i>
	Peritrichia	Sessilida	<i>Vorticella</i>
Colpodea		Colpodida	<i>Colpoda</i>
Karyorelictea		Loxodida	<i>Loxodes</i> , <i>Aspidisca</i>
Armophorea		Odontostomatida	<i>Saprodinium</i>

Certaines espèces de ciliés sont capables de former des logettes (lorica) de nature variée et spécifiques dont la cellule ne constitue que 30 % à 50 % de cette loge : ce sont les tintinnides.

En effet, du point de vue fonctionnel, les ciliés peuvent être divisés en :

- ciliés aloriqués : qui sont des bactérivores/microbivores et renferment plusieurs espèces de *Strombidium*.

- ciliés avec loriques (les tintinnides) : qui sont généralement herbivores.

- Ecologie

Les ciliés sont des organismes cosmopolites (Gonzalez *et al.*, 2005). En Méditerranée, ils ont été largement étudiés dans les milieux marins et côtiers (Admiraal et Venekamp, 1986; Cariou *et al.*, 1999; Dolan, 2000; Dolan *et al.*, 2002; Kršenic' et Grbec, 2006) alors que les lagunes ont été négligées. En Tunisie, la plupart des études ont été signalés dans les écosystèmes côtiers et marins (Daly Yahia *et al.*, 2005, Hannachi *et al.*, 2009, 2011; Kchaou *et al.*, 2009 ;

Drira *et al.*, 2009; Rekik *et al.*, 2012; Ben Brahim *et al.*, 2013) ou les salines solaires (Elloumi *et al.*, 2006, 2009). Une seule étude a été réalisée au niveau d'une lagune tunisienne (lagune de Bizerte, Sakka *et al.*, 2008).

Des proliférations printanières majeures des ciliés ont été rapportées dans plusieurs études (Montagnes *et al.*, 1988; Brussaard *et al.*, 1995; Johansson *et al.*, 2004; Loder *et al.*, 2011).

- Rôle trophique

Les ciliés peuvent être autotrophes, mixotrophes, ou hétérotrophes :

- ciliés hétérotrophes : Ils ne possèdent pas des chloroplastes. Ils se nourrissent par phagocytose (De Puytorac, 1994).

- ciliés mixotrophes : Ils sont capables d'exploiter les plastes des algues qu'ils consomment et deviennent ainsi mixotrophes (Dolan, 1992) tel que *Strobilidium gyrans* (Setälä, 2004).

- ciliés autotrophes : ce groupe est représenté par une seule espèce : *Mesodinium rubrum* (Lindholm, 1985 ; Leppanen et Bruun, 1986 ; Johansson *et al.*, 2004). Cette espèce est capable de faire la photosynthèse contribuant ainsi à une partie significative de la production primaire (Stoecker *et al.*, 1989; Ota et Taniguchi, 2003).

Les ciliés sont considérés parmi les groupes les plus fonctionnels dans les réseaux trophiques aquatiques (Landry et Calbet, 2004; Pomeroy *et al.*, 2007; Fenchel, 2008; Sherr et Sherr, 2008). En effet, ils se nourrissent du bactérioplancton (Simek *et al.*, 1998; Karayanni *et al.*, 2008) et du phytoplancton (Gismervik *et al.*, 1996; Loder *et al.*, 2011) constituant ainsi un lien majeur de transfert de l'énergie du niveau trophique inférieur au niveau trophique supérieur (Azam *et al.*, 1983; Sherr *et al.*, 1986).

- Les ciliés comme indicateurs de pollution

A cause de leur reproductivité élevée, leur sensibilité et variabilité au niveau de la niche trophique, les ciliés peuvent répondre plus vite aux contaminations environnementales que les autres organismes (Aleya *et al.*, 1992; Foissner et Berger, 1996; Jiang, 2006). Le changement au niveau de la diversité des espèces est généralement utilisé pour voir l'effet biologique de la pollution. En effet, au niveau des milieux non pollués on trouve surtout les ciliés algivores mais au niveau des milieux pollués on constate que les ciliés bactérivores dominent (Madoni, 2005). Aussi, les communautés ciliaires sont sensibles à tout changement affectant la qualité des eaux tel que le changement de concentration des nutriments (Hannachi *et al.*, 2009) et les poisons organiques comme le HgCl₂ (Weitere et Arndt, 2003, Jiang *et al.*, 2006). Ils sont donc utilisés pour la détermination des effets toxiques non détectables par les moyens chimiques (Jiang *et al.*, 2006).

5. Aperçu sur les travaux réalisés sur le phytoplancton en Tunisie

Hamza et El Abed (1994) et Turki et El Abed (2001) ont reporté pour la première fois en Tunisie, en 1988, des blooms mono-spécifiques à cyanophycées qui ont été à l'origine de la coloration des eaux et la mortalité des poissons au niveau du littoral sud de la Tunisie dans le golfe de Gabès. Des mortalités de l'ordre de 1 à 2 tonnes par jour des différentes espèces des poissons (*Labridae*, *Gobidae*, *Mugulidae*, *Sparidae*...) ont été enregistrées dans le même milieu en automne 1994, suite à des blooms des dinoflagellés (Hamza et El Abed, 1994). Aussi, des proliférations massives du dinoflagellé *Karenia selliformis* ont provoqué des phénomènes d'anoxie dans le golfe de Gabès (Arzul *et al.*, 1997). En octobre 2000, les épiphytes *Prorocentrum lima* et *Ostreopsis siamensis* ont représenté 86.4 % des dinoflagellés du golfe (Turki *et al.*, 2006). Les travaux de Mabrouk *et al.* (2011) dans les côtes d'El Mahdia ont signalé des rapports importants entre l'abondance des microalgues toxiques sur les feuilles de *Posidonia oceanica* et celles colonisant la colonne d'eau.

Un suivi de dix ans dans le golfe de Gabès (1995-2007) a permis de repérer des efflorescences de dinoflagellés pendant une longue durée en été et pendant une courte durée en hiver ainsi qu'une faible abondance relative de diatomées pendant toutes les saisons. La salinité et le pH ont été révélés comme les facteurs principaux pour le développement des dinoflagellés notamment les espèces toxiques (Hamza *et al.*, 2009). Drira *et al.* (2010) ont couplé la dynamique du phytoplancton sur les côtes du golfe de Gabès avec les concentrations élevées du nutriment. Ils ont signalé aussi le rôle des ciliés comme prédateurs de phytoplancton.

Une campagne estivale (juillet 2006), conduite par Hannachi *et al.* (2011) dans le golfe de Hammamet, a révélé la dominance des dinoflagellés (55 %) dans les eaux côtières représentés essentiellement par les genres de *Protoperdinium* et *Gymnodinium*, et l'espèce *Scrippsiella trochoidea*, tandis que, les eaux du large a été dominé par les diatomées (68 %). Selon Hannachi *et al.* (2011), la stabilité de la colonne d'eau au cours de la campagne et la disponibilité du phosphate inorganique peuvent être parmi les facteurs les plus importants qui régissent la dynamique du phytoplancton dans le golfe de Hammamet. Des corrélations importantes entre le phytoplancton et les ciliés ont été révélées aussi dans le milieu ce qui suggère une relation trophique potentielle.

Au niveau de la baie de Tunis, une analyse systématique, biogéographique, quantitative et qualitative du phytoplancton a été réalisée durant la période allant de décembre 1993 à novembre 1995 par Daly Yahia (1998). Les résultats de cette étude ont permis d'identifier 130 espèces qui appartiennent à la classe des diatomées et 158 à celle de dinoflagellés. Parmi ces Dinoflagellés, la répartition biogéographique des 10 espèces du genre *Alexandrium* a été

étudiée par Daly Yahia *et al.* (2001). Les marées rouges dont l'espèce *Gymnodinium* sp. est responsable, semblent être associées à l'excès des phosphates (Daly Yahia *et al.*, 2005). Des associations spécifiques entre les espèces de tintinnides dominantes et les dinoflagellés susceptibles d'être nuisibles ont été considérées comme des relations prédateurs - proies (Daly Yahia *et al.*, 2005).

Concernant les lagunes tunisiennes, des efflorescences à *Karenia selliformis* ont été enregistrées pour la première fois en août 1991, au niveau de la lagune de Boughrara, ayant provoqué des mortalités des poissons mis en élevage dans les cages (Guelorget, 1991). Ce dinoflagellé a été responsable de plus de 70 % des cas de nuisances dans les différents sites aquacoles (Dammak *et al.*, 2009; Drira, 2009; Medhioub, 2011; Feki *et al.*, 2013). Un net accroissement du rapport dinoflagellés / diatomées a été signalé dans la lagune de Boughrara avec des proliférations de plus en plus accentuées (de l'ordre de $6 \cdot 10^6$ cellules par litre) dans les zones confinées (Benrejeb et Romdhane, 2002).

Dans la lagune de Bizerte, ce sont les proliférations des diatomées du genre *Pseudonitzschia* qui ont été le plus étudiées. Entre 2004 et 2006, un suivi de ces efflorescences a montré que les conditions physico-chimiques de cette lagune sont favorables au développement des espèces du genre *Pseudonitzschia* (Sahraoui *et al.*, 2007, 2009). Sahraoui *et al.* (2012) ont mentionné pour la première fois dans les eaux tunisiennes la présence de 4 espèces du genre *Pseudonitzschia* : *P. brasiliensis*, *P. calliantha*, *P. multistriata* et *P. cf. seriata*. Concernant les dinoflagellés, *Dinophysis* spp. et *Prorocentrum lima* ont été les espèces les plus dominantes dans la lagune de Bizerte (Turki *et al.*, 2004).

Selon Sakka *et al.* (2006), la composition phytoplanctonique de la lagune de Bizerte change de manière significative entre les saisons: les petits flagellés dominent en hiver et les diatomées deviennent plus abondantes en été. Sakka *et al.* (2008) ont signalé (i) l'influence de l'apport anthropique des nutriments sur la dynamique du phytoplancton notamment sur le développement des diatomées toxiques et (ii) le rôle des ciliés comme prédateurs de phytoplancton notamment le pico et le nanoplancton.

Un suivi de 5 ans dans la lagune de Bizerte (2007-2011) a révélé la présence des blooms hivernaux d'*Alexandrium pacificum* (Turki *et al.*, 2014). Des fortes concentrations des kystes d'*Alexandrium pacificum* ont été découvertes dans le sédiment de la lagune et pouvant être une source permanente et non négligeable pour la dispersion et l'initiation de blooms de cette espèce dans la colonne d'eau (Fertouna-Bellakhal *et al.*, 2014).

Dans la lagune Nord de Tunis, la dynamique des efflorescences des espèces des dinoflagellés potentiellement toxiques (*Dinophysis* spp., *Prorocentrum lima*, *Coolia monotis*) en relation

avec les conditions physicochimiques ont été établies (Turki, 2004; Armi *et al.*, 2008; Armi *et al.*, 2010). Selon Armi *et al.* (2010), la diminution hivernale de la température d'eau est la cause principale de l'absence des chlorophytes et des cyanophycées dans la lagune, tandis que les conditions printanières sont les plus favorables à la présence de tous les groupes de phytoplancton.

Chapitre II. Site d'étude

1. Situation géographique

La lagune de Ghar El Melh, anciennement appelée lac de Porto Farina, est située au Nord Est de la Tunisie et au Nord-Ouest du golfe de Tunis entre $10^{\circ}08'$ et $10^{\circ}15'$ de longitude $37^{\circ}06'$ et $37^{\circ}10'$ de latitude (Figure 4). Elle est localisée au Nord-Est de la Tunisie à 44 km de Bizerte et à 57 km de Tunis. La lagune est située au niveau de Ras Tarf : Cap Farina. Elle est abritée, du côté Nord, par l'extrémité de la dorsale tunisienne qui culmine à 334 m du Jebel Nadhour. Cette chaîne montagneuse forme le Cap Farina au niveau de la mer. Du côté Ouest et du côté Sud, cette lagune est limitée par la plaine alluviale et marécageuse de la basse vallée de la Mejerda. A l'Est, elle est séparée de la mer par un cordon littoral de largeur variable « El Boughaz », découpé de passes qui font communiquer la lagune avec la mer (Romdhane, 1985 ; Ayache *et al.*, 2009).



Figure 4. Le complexe lagunaire de Ghar El Melh

La lagune de Ghar El Melh est en fait un complexe lagunaire de 3135 ha constitué par 3 bassins:

- La lagune principale appelée communément « Bhira », de forme elliptique, d'environ 7 km de longueur, de direction Est-Ouest et de 4.5 km de largeur en direction Sud Nord. La longueur des côtes est de 22 km et sa superficie est de 26.7 km^2 .

- La lagune de Sidi Ali Il Meki, située du côté Est et presque isolée de la première par la route menant au nouveau port. Sa superficie qui couvre 3.7 km², tend à se rétrécir suite à des remblaiements artificiels.
- La lagune de Koukou ou Sebkhath El Ouafi est située au Sud. Elle couvre 5.2 km² et communique largement avec la lagune principale (Romdhane, 1985 ; Ayache *et al.*, 2009).

2. Cadre géomorphologique

2.1. Formation de la lagune

La formation du complexe lagunaire de Ghar El Melh résulte de l'évolution du littoral, dans l'ancien golfe d'Utique, qui n'a cessé d'avancer dans la mer depuis les temps les plus reculés. Ce mouvement est dû aux apports solides charriés par l'Oued Mejerda (Rodier *et al.*, 1980 ; Romdhane, 1985). C'est au cours du cycle pliocène que ce fleuve a construit son delta au niveau de la mer d'Utique (Pimienta, 1959).

2.2. Evolution de la communication mer-lagune

Le cordon littoral qui sépare le complexe lagunaire de Ghar El Melh de la mer est en perpétuelle évolution. En effet, la communication mer lagune était assurée par une triple passe de largeur et de tirant d'eau variable (Figure 5). La plus importante (A) appelée localement El Boughaz à 70 m de largeur et 0.4 à 3 m de tirant d'eau. Les deux autres (B) et (C) se situent plus au Nord. La 3^{ème} passe (C) est apparue à partir de 1981 près du nouveau port. Elle est large mais à faible tirant d'eau. Deux ans après la construction du port, le problème d'ensablement et de transit littoral est posé, une opération de dragage de ce port effectué fin 1983 a engendré le colmatage du grau (C) (Romdhane, 1985). Ce même cordon séparant la lagune de la mer a subi des changements importants par rapport à la situation relevée en 1985 :

- Un ensablement de l'ancienne communication
- L'ouverture artificielle d'une nouvelle communication de 80 m de large proche du nouveau port
- Un dégraissement important de la partie Sud du cordon vers l'extrémité Sud de Sebkhath El Ouafi, avec même l'établissement d'une ouverture temporaire d'une dizaine de mètres.

L'origine de ces modifications est la construction du nouveau port de pêche de Ghar El Melh en 1975 à l'extérieur de la lagune (côté Nord-Est) et les épis de protection au Nord du port. Ces ouvrages enregistrent un ensablement annuel important et contribuent à la modification de la trajectoire du transit littoral (SCET-ERI, 2000 ; Oueslati, 2004 ; Thompson *et al.*, 2009).

Oueslati (2004) et MEDD et UNDP (2006) ont suggéré qu'une élévation de 50 cm du niveau de la mer d'ici l'année 2100 pourrait entraîner l'expansion de la lagune de Ghar El Melh d'environ 2000 ha. Une grande partie de cette expansion aurait lieu à l'Ouest sur la partie la plus basse de la plaine d'Utique. Aussi, les zones au sud, y compris les restes de l'Oued Merjerda, seraient également inondées.

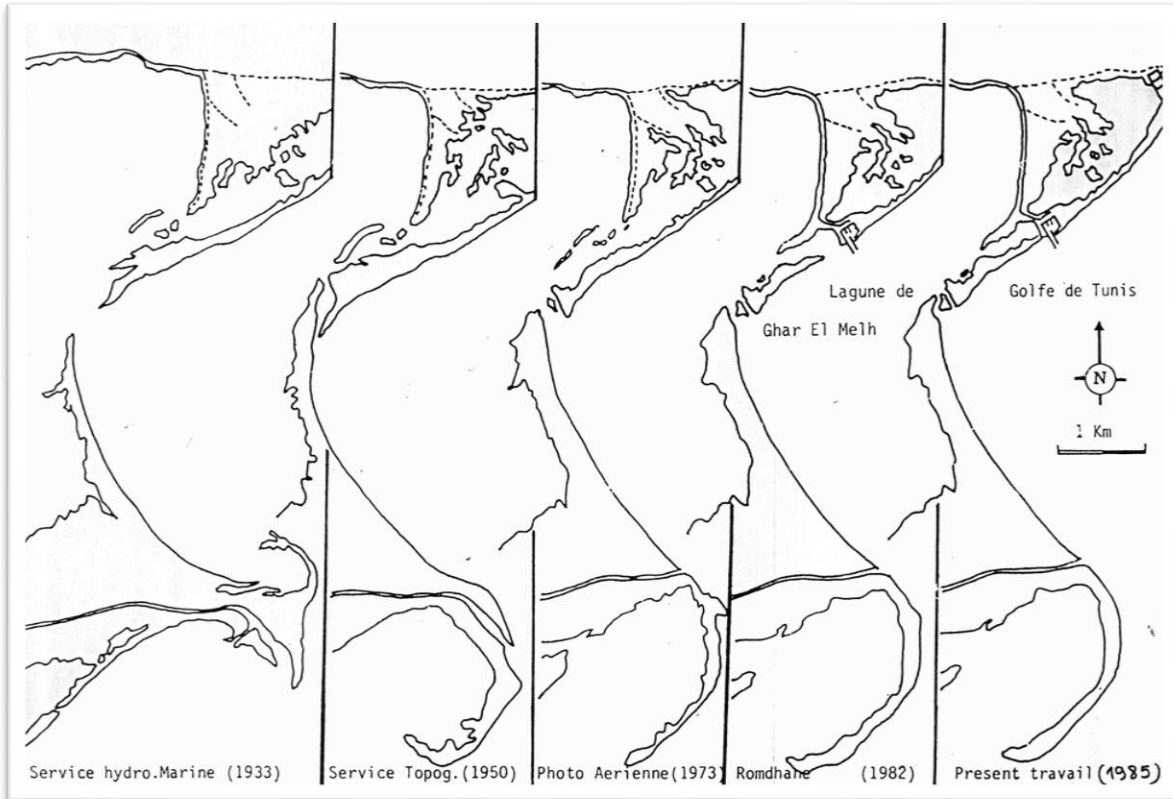


Figure 5. Evolution du cordon littoral et des passes de la lagune (Romdhane, 1985)

3. Bassin versant de la lagune

Le bassin versant de la lagune de Ghar El Melh se situe entre les coordonnées :

10°02'30'' et 10°17'00'' de longitude Est

37°06'30'' et 37°11'40'' de latitude Nord.

Il a une superficie totale de 131.25 km² et un périmètre de 57.5 km. Il peut être subdivisé en trois parties :

* Une première partie formée par le versant du Sud-Ouest d'Utique drainée par un certain nombre d'oueds qui se déversent dans la lagune et dont les principaux sont indiqués dans le tableau 3.

Tableau 3. Caractéristiques hydrologiques du bassin versant de Ghar El Melh (SCET-ERI, 2000).

Paramètres	Oued Tarfa	Oued Kherba	Oued Nechma	Oued Bourezem
Superficie (km ²)	17	10	12.5	6
Longueur de talweg principal (km)	6	9.5	5	3
Dénivelé H (cm)	210	185	155	85
Pente moyenne (%)	3.5	1.3	3	2.8
Vitesse d'écoulement (m s ⁻¹)	1.75	1.64	1.6	1.39

* Une deuxième partie formée par la lagune elle-même avec ses trois bassins (SCET-ERI, 2000).

* Une troisième partie formée par les eaux souterraines de la seule source « El Ayoun » située aux pieds de Jebel Nadhour (Ennabli, 1966; Beyrem, 2002). Elle contribue à une entrée modeste d'eau douce de l'ordre de 0.5 l s⁻¹ (Ennabli, 1966; Beyrem, 2002).

4. Cadre climatique

4.1. Pluviométrie

La région de Ghar El Melh est caractérisée par une pluviométrie moyenne de l'ordre de 520 mm an⁻¹ pour la période allant de 1961 à 1990. Les précipitations sont souvent de caractère bref et torrentiel. Les moyennes mensuelles indiquent aussi une variation importante. On remarque une sécheresse estivale pendant les mois de juin, juillet et août où les moyennes ne dépassent pas 10 mm mois⁻¹. La période pluvieuse commence à partir du mois de septembre et s'échelonne jusqu'au mois de mars. Les plus fortes pluies moyennes sont enregistrées pendant les mois d'octobre, novembre et décembre avec des quantités de l'ordre de 85 mm mois⁻¹ (SCET-ERI, 2000). Durant la période allant de 2011 à 2013, les précipitations mensuelles enregistrées dans la région de Ghar El Melh ont oscillé entre 0 et 216 mm mois⁻¹ avec les mois les plus pluvieux sont les mois de novembre, décembre et février (INM, 2015) (Figure 6).

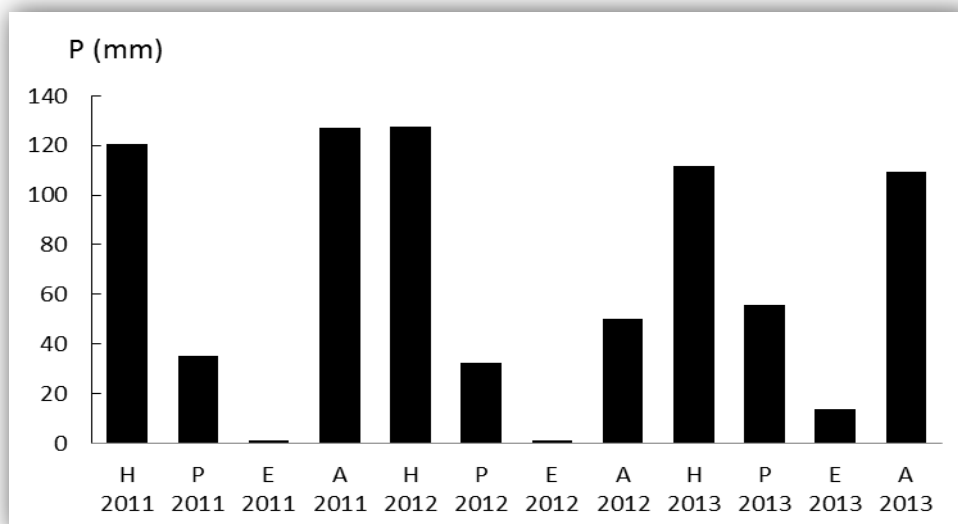


Figure 6. Précipitations enregistrées au niveau de la région de Ghar El Melh pendant les années 2011, 2012 et 2013 (H : hiver, P : printemps, E : été, A : automne) (INM, 2015).

4.2. Température de l'air

La région de Ghar El Melh se caractérise par un climat de type tempéré intermédiaire. La moyenne annuelle des températures, pour la période allant de 1961-1990, est de l'ordre de 18.5 °C (Labane, 1999 in SCET-ERI, 2000). L'écart de température moyenne entre le mois le plus chaud et le mois le plus froid ne dépasse pas 20 °C (SCET-ERI, 2000).

Les moyennes mensuelles des températures enregistrées durant la période allant de janvier 2011 à décembre 2013 oscillent entre 9.2 °C et 28.2 °C. Le mois le plus froid est le mois de février 2012 et le mois le plus chaud est le mois d'août 2012 (INM, 2015) (Figure 7).

Comme toute zone côtière, cette région est sous l'influence marine qui a tendance à réguler le climat (SCET-ERI, 2000).

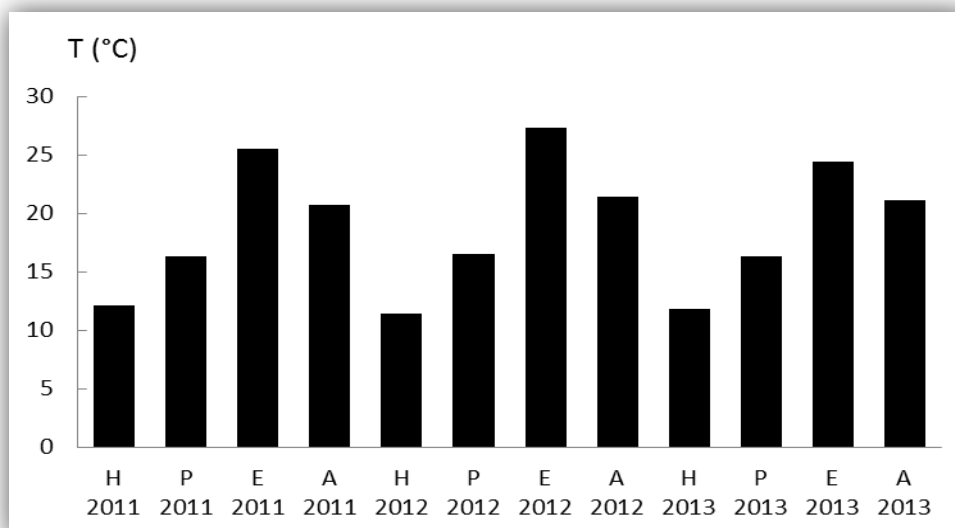


Figure 7. Températures de l'air enregistrées au niveau de la région de Ghar El Melh pendant les années 2011, 2012 et 2013 (H : hiver, P : printemps, E : été, A : automne) (INM, 2015).

4.3. Evaporation

L'évaporation est favorisée par l'insolation, la chaleur élevée et les vents. Elle est par contre contrariée par l'humidité relative. L'évaporation dans la région de Ghar El Melh est considérable, en effet, la moyenne annuelle de l'évaporation entre 1970 et 1990 est de l'ordre de 1450 mm an⁻¹. Les moyennes mensuelles montrent que les fortes évaporations se font pendant la saison estivale (les mois de juin, juillet et août) où elles atteignent 200 mm mois⁻¹ (SCET-ERI, 2000).

4.4. Humidité relative

La région de Ghar El Melh, est caractérisée par une forte humidité relative. La valeur moyenne entre 1951 et 1970, est de l'ordre de 71 %. L'humidité est moins accentuée à midi qu'au lever ou au coucher de soleil où elle tend à se rapprocher de la saturation (Mansouri, 1979).

Au cours de la période 1993 - 1999, l'humidité relative moyenne est de l'ordre de 63 %. La variation mensuelle du pourcentage d'humidité relative est proportionnelle à la pluviométrie. En effet, les mois à plus forts pourcentages de saturation correspondent aux mois les plus pluvieux et inversement (SCET-ERI, 2000).

Durant la période allant de 2011 à 2013, l'humidité relative moyenne enregistrée dans la région de Ghar El Melh est de l'ordre de 68. 89 %. L'humidité relative la plus élevée (83 %) a

été enregistrée en décembre 2013. Les valeurs de l'humidité les moins élevées ont été enregistrées surtout durant les mois d'été (INM, 2015) (Figure 8).

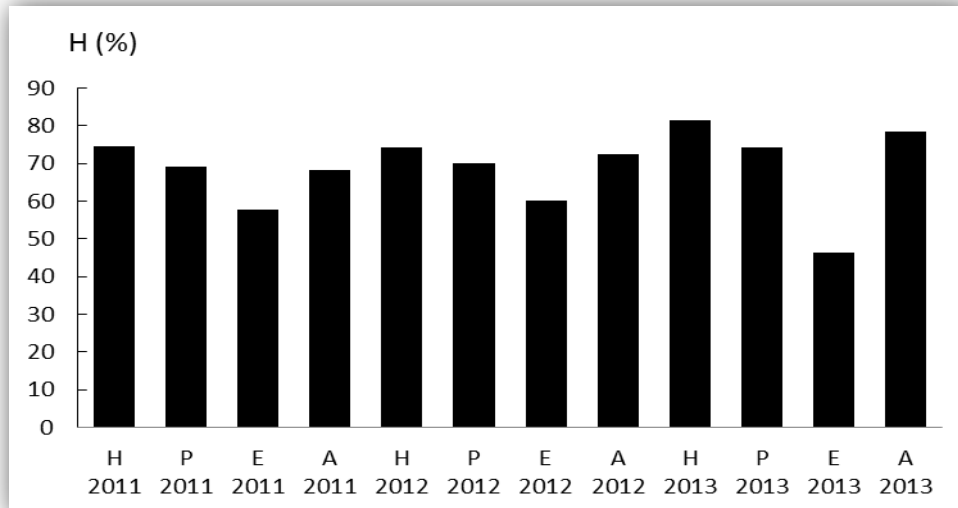


Figure 8. Les valeurs de l'humidité relative enregistrées au niveau de la région de Ghar El Melh pendant les années 2011, 2012 et 2013 (H : hiver, P : printemps, E : été, A : automne) (INM, 2015).

4.5. Vents

Les vents sont souvent violents et les calmes sont rares. La rose des vents entre 1951 - 1970 à Tunis montre une prédominance générale des vents du secteur Nord-Ouest avec des différences sensibles entre l'Ouest et l'Est (Mansouri, 1979 in SCET-ERI, 2000).

En général, les falaises de Ghar El Melh protègent la lagune des vents des secteurs Ouest et Nord-Ouest. Ainsi, seuls les vents du Nord et du Nord-est sont efficaces et engendrent les houles qui s'engouffrent dans le golfe de Tunis. Leur intensité dépasse rarement les 10 m s^{-1} .

On distingue deux catégories de vents :

- Les vents marins, frais et humides qui soufflent des secteurs Nord-Ouest à Est. Ils apportent principalement l'humidité et la pluie en saison froide. En été, leur effet est de tempérer la rigueur des chaleurs.
- Les vents continentaux, secs et chauds, soufflent des secteurs Sud-Est à Est et Ouest à Sud-Ouest. Ils provoquent de fortes augmentations thermiques (SCET-ERI, 2000).

La vitesse du vent varie entre 2.5 m s^{-1} et 5.61 m s^{-1} durant la période allant de janvier 2011 à décembre 2013 (INM, 2015) (Figure 9).

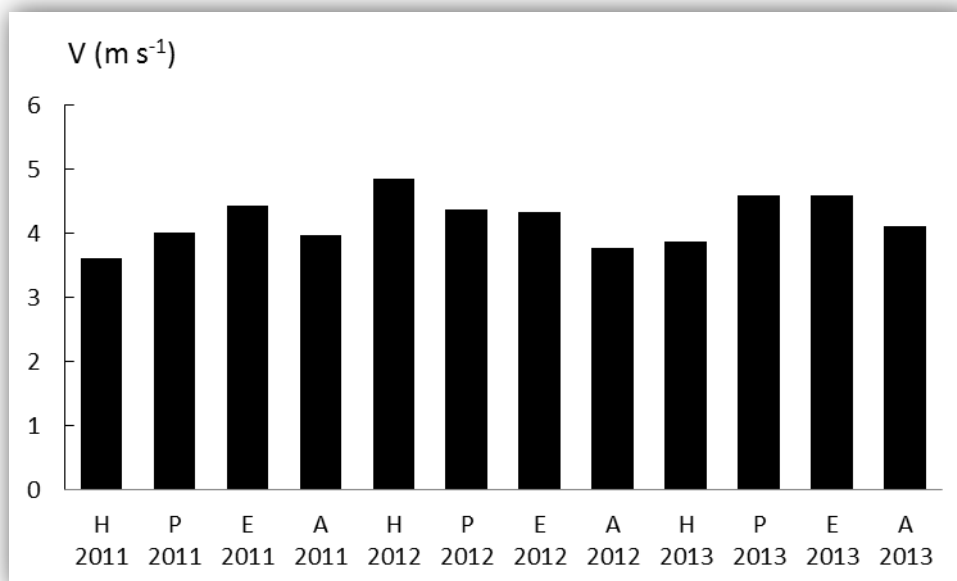


Figure 9. Vitesses du vent enregistrées au niveau de la région de Ghar El Melh pendant les années 2011, 2012 et 2013 (H : hiver, P : printemps, E : été, A : automne) (INM, 2015).

5. Hydrologie

5.1. Bathymétrie

Au total, la lagune de Ghar El Melh couvre une superficie d'environ 35 km² et sa profondeur moyenne est de 0.8 m par rapport au zéro NGT. La lagune principale incluant Sebket El Ouafi se décompose en quatre zones :

* Zone I : la hauteur d'eau est comprise entre 0 et 0.5 m. Cette zone borde toute la lagune et elle est plus importante au Sud et à l'Ouest.

* Zone II : la hauteur d'eau est comprise entre 0.5 et 1 m. Cette zone couvre la majeure partie de la lagune, surtout vers l'Ouest.

* Zone III : la hauteur d'eau est comprise entre 1 m et 1.5 m. Cette zone est emboîtée dans la zone II.

* Zone IV : la hauteur d'eau est supérieure à 1.5 m. Elle a une étendue très limitée à l'extrême Nord-Est ainsi qu'au niveau de la nouvelle communication (El Boughaz) où elle atteint 3.8 m de profondeur.

La profondeur moyenne de la lagune est d'environ 0.8 m par rapport au zéro NGT. Les récents résultats montrent, qu'en général, depuis les mesures de Mansouri (1979), la lagune n'a pas subi de modification perceptible quant à sa profondeur (SCET-ERI, 2000).

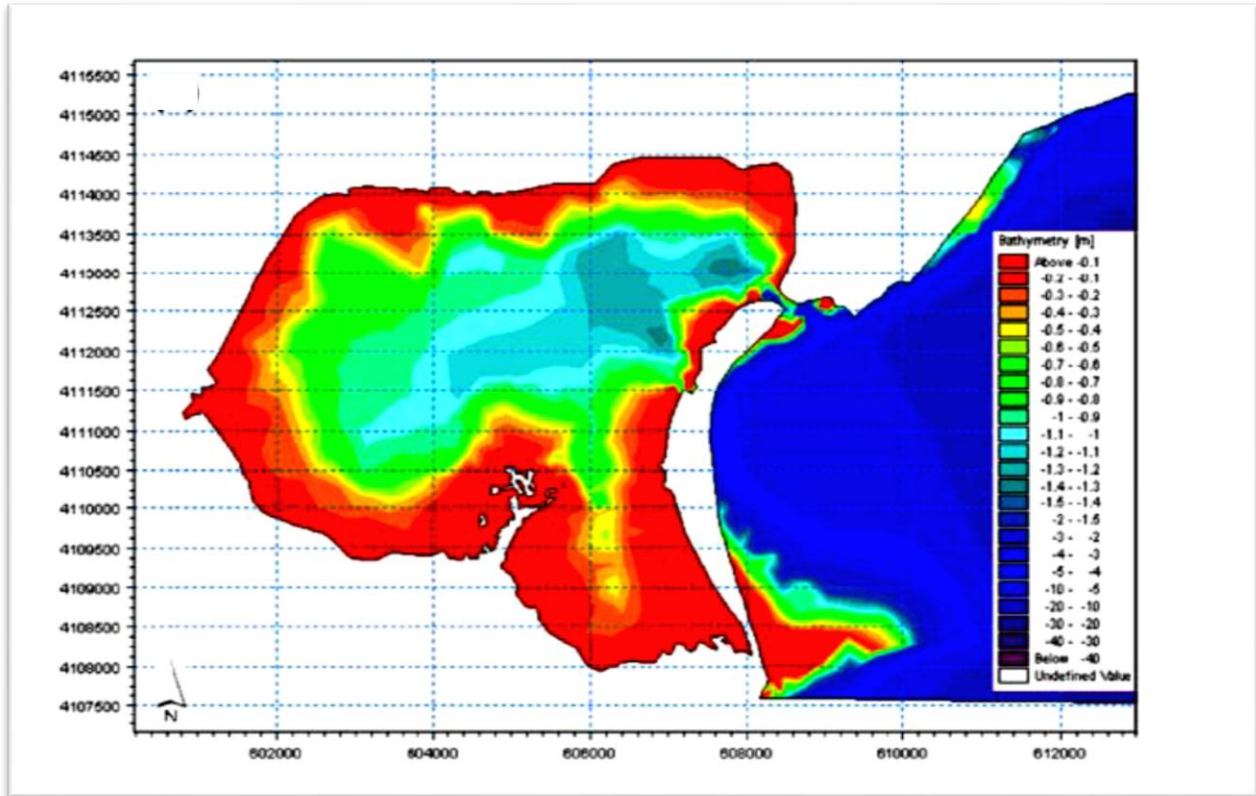


Figure 10. Relevé topographique de la lagune de Ghar El Melh et de la frange littorale (Thompson *et al.*, 2009)

5.2. Marée

La marée est une oscillation du niveau marin liée essentiellement à l'attraction de la lune et du soleil sur les molécules d'eau. Le marnage moyen est d'environ 15 cm, le marnage maximal est de l'ordre de 22 cm et le marnage minimal est estimé à 8 cm (Ben Khemis, 2003).

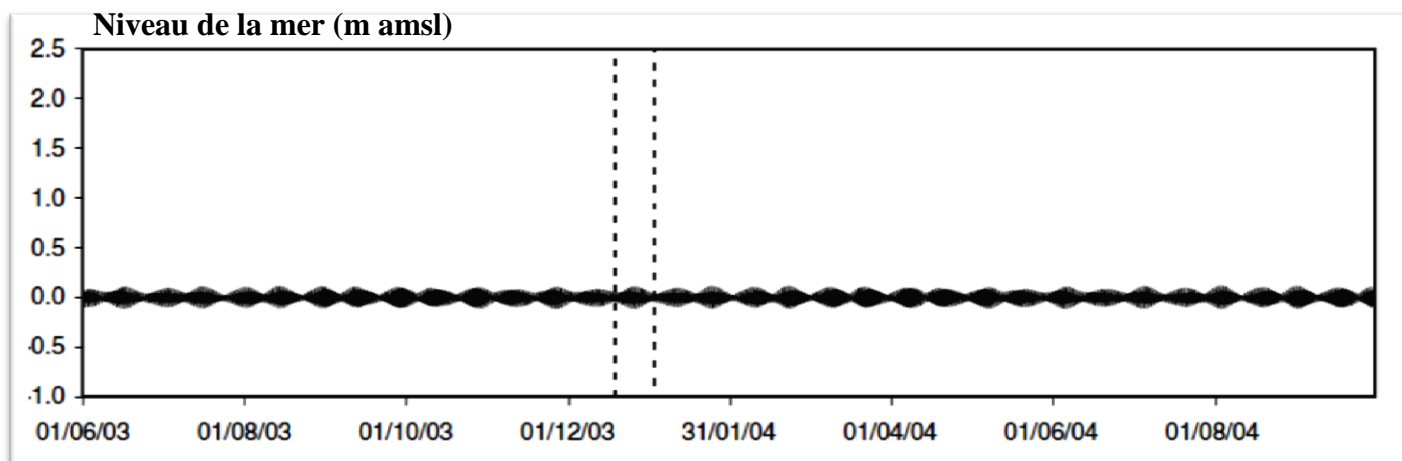


Figure 11. Courbes de variation de la marée dans la lagune de Ghar El Melh (Thompson *et al.*, 2009).

5.3. Courants de circulation

Le volume journalier maximal d'eau échangé entre la lagune et la mer est enregistré dans le cas de la marée moyenne avec évaporation et vent dominant à $3.22 \text{ mm}^3 \text{ s}^{-1}$. Il met ainsi en évidence l'influence positive du vent dominant venant du secteur Ouest en dépit de l'évaporation qui tend à minimiser l'échange mer-lagune. Pour le cas de marée moyenne avec évaporation et vent dominant, on enregistre le meilleur temps de séjour qui est évidemment le plus faible (estimé à 7.9 jours).

La stagnation des eaux dans une majeure partie de la lagune confère au temps de séjour moyen un aspect local. Dans le cas de la marée moyenne avec ou sans évaporation, les vitesses à l'intérieur de la lagune varient de 0.02 à 9 cm s^{-1} . Elles sont maximales à l'approche de la passe d'El Boughaz alors que partout ailleurs nous constatons un ralentissement remarquable de l'écoulement ce qui indique surtout, si la dispersion est négligeable, la présence des zones de stagnation. Ces zones sont principalement Sebket El Ouafi et toute la partie Ouest de la lagune. Par contre, lorsque nous sommes en présence du vent dominant, la circulation des eaux est meilleure. En effet, les vitesses sont beaucoup plus grandes à l'intérieur de la lagune. Les vitesses minimales sont de l'ordre de 1 cm s^{-1} , alors que les vitesses maximales atteignent 11.5 cm s^{-1} (SCET-ERI, 2000).

6. Caractéristiques biologiques de la lagune de Ghar El Melh

6.1. Phytoplancton

L'étude du phytoplancton dans la lagune de Ghar El Melh a été effectuée pour la première fois par Daly Yahia *et al.* (1997). 42 espèces de Diatomophycées, 33 espèces de Dinophycées,

plusieurs espèces de Chlorophycées, une Euglénophycée et une Prymnésiophycées ont été identifiées dont les concentrations enregistrées, durant cette période, ont oscillé entre 1140 et $1.4 \cdot 10^6$ cellules l^{-1} .

D'après l'étude portant sur l'amélioration de la qualité des eaux dans la lagune de Ghar El Melh (SCET-ERI, 2000), il y a une diminution quantitative et qualitative du phytoplancton qui peuple le milieu. 29 espèces de Diatomophycées, 23 espèces de Dinophycées, plusieurs espèces de Chlorophycées, une Euglénophycée et une cyannobactérie ont été identifiées et dont les concentrations ont oscillé entre 9115 et $2.4 \cdot 10^4$ cellules l^{-1} .

Ramdani *et al.* (2004) ont identifié 42 espèces de diatomophycées, 32 espèces de Dinophycées, 6 espèces de Chlorophycées, et une Cyannobactérie. Les concentrations de dinoflagellés ont oscillé entre 25 et $12 \cdot 10^3$ cellules l^{-1} . Selon ces auteurs, la dynamique planctonique est en étroite relation avec les concentrations élevées de nutriments et à la turbidité dans les eaux de la lagune.

Plusieurs espèces nuisibles ont été observées dans les eaux de la lagune (Tableau 4). Par ailleurs, des mortalités de poissons ont été enregistrées aussi bien en mai 1995 qu'en mai 1996 et ont coïncidé avec des concentrations élevées des espèces du genre *Alexandrium* (Romdhane *et al.*, 1998) et en 2007 suite aux proliférations du dinoflagellé *Kryptoperidinium foliaceum* (Turki *et al.*, 2007).

Tableau 4. Espèces nuisibles répertoriées dans la lagune de Ghar El Melh. **a, d:** compagnes d'été **b, c:** suivis annuels; **e:** compagnes d'automne et d'hiver.

Romdhane <i>et al.</i> (1998) ^a	SCET-ERI (2000) ^b	Ramdani <i>et al.</i> (2004) ^c	Turki <i>et al.</i> (2007) ^d	Dhib <i>et al.</i> (2013a) ^e
- <i>Alexandrium</i> sp. ($\approx 1.5 \cdot 10^6$ cellules l^{-1})	- <i>Akashiwo sanguinea</i> - <i>Gonyaulax polyedra</i> - <i>Prorocentrum micans</i> , <i>P. lima</i> , <i>P. triestinum</i> , <i>P. minimum</i> - <i>Scrippsiella trochoidea</i> - <i>Cylindrotheca closterium</i> - <i>Pseudo-nitzschia</i> spp.	- <i>Alexandrium</i> spp. - <i>Tripos furca</i> - <i>Coolia monotis</i> - <i>Dinophysis</i> spp. - <i>Gonyaulax spinifera</i> - <i>Karenia brevis</i> - <i>Prorocentrum</i> spp. (exp. <i>P. lima</i> , <i>P. micans</i>) - <i>Chaetoceros decipiens</i> - <i>Leptocylindrus danicus</i> - <i>Pseudo-nitzschia</i> spp. - <i>Thalassionema nitzschioides</i>	- <i>Kryptoperidinium foliaceum</i> ($1.83 \cdot 10^5$ cellules l^{-1}) - <i>Prorocentrum micans</i> - <i>Anabaena</i> sp.	- <i>Akashiwo sanguinea</i> - <i>Tripos furca</i> - <i>Tripos fusus</i> - <i>Dinophysis</i> spp. - <i>Gonyaulax polyedra</i> - <i>Gonyaulax spinifera</i> - <i>Prorocentrum micans</i> ($1.2 \cdot 10^5$ cellules l^{-1}), <i>P. lima</i> , <i>P. concavum</i> , <i>P. gracile</i> , <i>P. triestinum</i> , <i>P. minimum</i> - <i>Scrippsiella trochoidea</i>

6.2. Zooplancton (microzooplancton + mézozooplancton)

Les travaux réalisés dans ce domaine sont ceux de SCET-ERI (2000), de Ramdani *et al.* (2004), de Daly Yahia *et al.* (2005) et de Ziadi *et al.* (2015).

Selon l'étude de SCET-ERI (2000), le peuplement zooplanctonique est caractérisé par deux communautés :

- La première est typiquement lagunaire et bien adaptée aux fluctuations drastiques des conditions physico-chimiques et trophiques du milieu. Cette communauté est représentée par des espèces généralement côtières, ubiquistes, cosmopolites et très productives. Il s'agit par exemple des tintinnides du genre *Tintinnopsis* et *Stenosemella* et des copépodes tels que *Halicyclops neglectus*, *Harpacticus littoralis*...

- La seconde est typiquement néritique marine. Elle est représentée par des espèces cosmopolites parfois productives. Il s'agit d'espèces telles que les tintinnides : *Helicostomella subulata*, *Leprotintinnus bottnicu*, les copépodes : *Acartia clausi*, *Centropages typicus*, *Oithona nana*, les cladocères : *Evadne tergestina*, *Penilia avirostris*, les chaetognathes, les appendiculaires et les mysidacés.

D'après Ramdani *et al.* (2004), le zooplancton de la lagune de Ghar El Melh est dominé par les copépodes (55 espèces) surtout les espèces suivantes : *Acartia clausi*, *Calanus helgolandicus*, *Pseudocalanus elongatus*, *Oithona nana*, *Temora longicornis*, *Paracalanus parvus* et *Centropages typicus*.

L'étude de Daly Yahia *et al.* (2005) a été consacré seulement au groupe des tintinnides. Quinze espèces ont été identifiées, appartenant surtout aux genres *Tintinnopsis* et *Favella*, et avec des concentrations moyennes qui ne dépassent pas 62.3 cellules par litre.

Le travail récent de Ziadi *et al.* (2015) sur tout le groupe zooplanctonique a signalé la présence de 25 groupes comprenant 10 stades larvaires. L'assemblage zooplanctonique a été largement dominé par les copépodes (37.25 %) suivi respectivement par les ciliés (21.09 %), les larves de bivalves (14.88 %) et les gastéropodes véligères (12.53 %).

6.3. Macrophytes benthiques

Les travaux antérieurs relatifs à la flore benthique de la lagune ont été réalisés par Ben Alaya (1971), Prunus *et al.* (1978), Romdhane (1985), Romdhane et Ktari-Chakroun (1986) avec la présence de 15 espèces végétales benthiques. Cependant, l'inventaire établi par Shili *et al.* (2002) montre une diversité biologique relativement plus importante avec 26 espèces identifiées.

D'après Romdhane (1985), la majeure partie de la lagune était couverte dans sa partie centrale et à l'Ouest par des herbiers de *Zostera* (*Zostera marina* et *Zostera noltii*), ce peuplement

n'occupe plus d'après les dernières prospections faites par Shili *et al.* (2002), que des surfaces insignifiantes très limitées à l'Est de la lagune, a été remplacée par des peuplements à *Ruppia* supplantés par des touffes denses de *Cladophora*. Par ailleurs, et outre ce peuplement dominant, diverses modifications méritent d'être signalées:

- Les herbiers mixtes de *Zostera-Caulerpa* du Sud-Est ont laissé leur place à des herbiers à *Ruppia* et des touffes denses de *Cladophora* assez éparses.
- *Cymodocea nodosa* qui paraît abondant au début des années 70 et n'apparaissait que d'une façon sporadique à l'Est de la lagune au début des années 80, ne se trouve que dans des surfaces très réduites dans des zones à influence marine.
- *Caulerpa prolifera*, qui formait des pelouses mixtes avec les Zostères et qui était abondant au printemps et en été dans la partie Est et au centre de la lagune, n'occupe dernièrement que quelques petites surfaces.
- Les algues nitrophiles, comme *Ulva rigida*, *Enteromorpha intestinalis*, *Chaetomorpha lium* et *Cladophora* sp. qui ont été relativement moins abondantes et apparaissaient plus particulièrement sur les rivages Nord, se retrouvent de plus en plus abondantes dans la lagune.
- La zone située au niveau des graus qui était dépourvue de végétation à cause de l'instabilité des fonds, est occupée selon la dernière cartographie par *des Ruppia* et *des Cladophora* suite à l'atténuation de l'hydrodynamisme dans ce secteur (Shili *et al.*, 2002).

6.4. Faune ichtyque

La faune ichtyque de la lagune ayant une valeur commerciale est composée des espèces suivantes: *Mugil* spp., *Solea vulgaris*, *Sparus aurata* et *Anguilla Anguilla* (Kraiem *et al.*, 2009). Les captures annuelles durant la période allant de 1990 à 2004 sont de l'ordre de 80 tonnes dont l'espèce la plus commune est représentée par le mullet (40 %) (Ayache *et al.*, 2009). Sous l'influence de la pollution et des modifications des conditions environnementales, la production lagunaire de poissons a été diminuée (Figure 12) ainsi que la diversité ichtyque (Tableau 5). Dans le cadre de l'étude de l'amélioration de la qualité des eaux dans la lagune de Ghar El Melh (SCET-ERI, 2000), une liste des espèces (Tableau 5) a été dressée et comparée avec celle établie par Romdhane (1985).

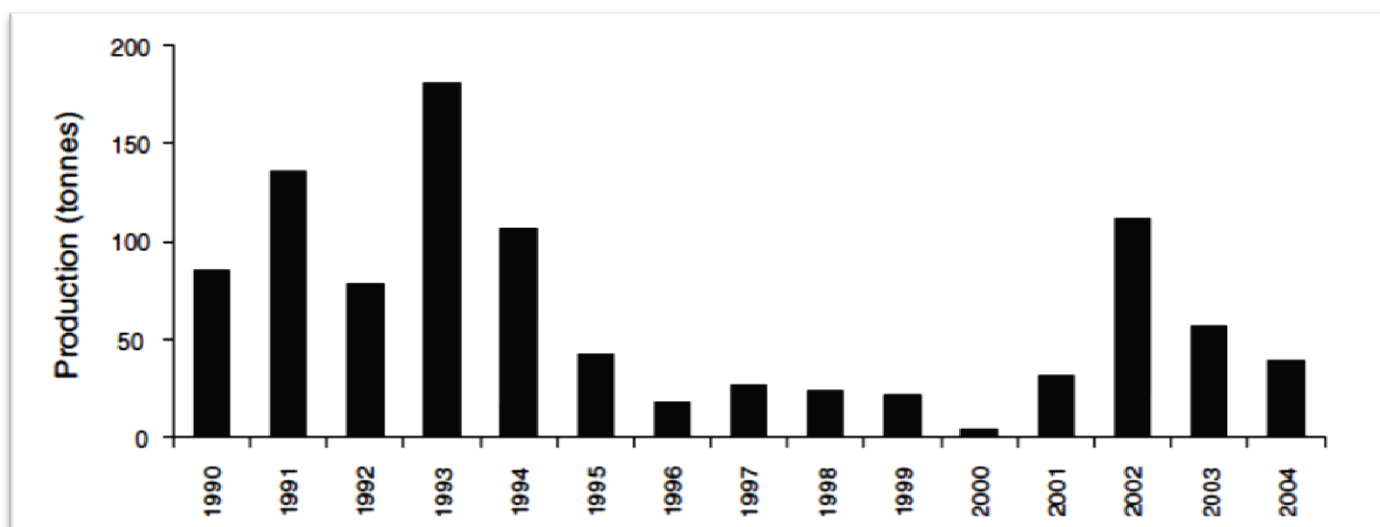


Figure 12. Production lagunaire de poissons durant la période allant de 1990 à 2004 (Kraiem *et al.*, 2009).

Tableau 5. Espèces de poissons de la lagune de Ghar El Melh (P : espèce présente, A : espèce absente).

Nom commun	Espèce	Rhomdhane (1985)	SCET-ERI (2000)
Mulet	<i>Mugil cephalus</i>	P	P
Bigeran	<i>Liza ramda</i>	P	P
Bigeran	<i>Liza aurata</i>	P	P
Bigeran / Mulet	<i>Chelon labrosus</i>	P	P
Bigeran	<i>Liza saliens</i>	P	P
Anguille	<i>Anguilla Anguilla</i>	P	P
Loup commun	<i>Dicentrarchus labrax</i>	P	P
Loup ponctué	<i>Dicentrarchus punctata</i>	P	P
Dorade	<i>Sparus aurata</i>	P	P
Spars	<i>Diplodus vulgaris</i>	P	P
Spars	<i>Diplodus annularis</i>	P	P
Sargue	<i>Diplodus sargus</i>	P	P
Oblade	<i>Oblada melanura</i>	P	P
Aiguille / Belone	<i>Belone belone</i>	P	P
Marbré	<i>Lithognathus mormyrus</i>	P	P
Pageau rouge	<i>Pagellus erythrius</i>	P	P
Rouget barbet	<i>Mullus barbatus</i>	P	P
Rouget rouge	<i>Mullus surmuletus</i>	A	P
Congre	<i>Congre conger</i>	P	P
Liche	<i>Lichia amia</i>	P	P
Ombrine	<i>Umbrina cirrosa</i>	P	P
Bogue	<i>Boops boops</i>	P	P
Saupe	<i>Sarpa salpa</i>	P	P
Ombrine	<i>Argyrosomus regius</i>	P	A
Corbeau	<i>Sciena umbra</i>	P	P
Limande	<i>Limanda limanda</i>	P	P

Turbot	<i>Scophthalmus maximus</i>	P	P
Saurel	<i>Trachurus trachurus</i>	P	P
Mendole	<i>Spicara maena</i>	P	P
Merlu	<i>Merliccius merliccius</i>	P	P
Maquereau	<i>Scomber scombrus</i>	P	P
Alose	<i>Allosa falax</i>	P	A
Sardine	<i>Sardina pilchardus</i>	P	A
Labre	<i>Symphodus rostratus</i>	P	A
Labre	<i>Symphodus melops</i>	P	A
Blennie	<i>Blennius pavo</i>	P	P
Blennie	<i>Blennius sanguinolentus</i>	P	A
Rousette	<i>Scyliorhinus canicula</i>	P	A
Chien de mer	<i>Mustelus mustelus</i>	P	P
Torpille	<i>Torpedo torpedo</i>	P	A
Trigla	<i>Trigla lyra</i>	A	P
Poulpe	<i>Octopus vulgaris</i>	P	P
Seiche	<i>Sepia officinalis</i>	P	P
Sole	<i>Solea vulgaris</i>	P	P

7. Pressions anthropiques

7.1. Activités agricoles

La région de Ghar El Melh est caractérisée par un système de culture très original à savoir le système horticole. Les systèmes horticoles sont les suivants : systèmes de jardin, H'mariet, N'jila et Gattaya.

- **Système de jardins** : l'aménagement en terrasse de ces parcelles continentales favorise l'infiltration de l'eau plutôt que son ruissellement qui aurait engendré une érosion des terres et une perte de l'eau de la lagune.

- **H'mariet** : ces terres sont cultivées presque exclusivement de pommes de terre, les espèces fruitières rencontrées sont le figuier, le poirier, le pommier et le grenadier. Les parcelles de pommes de terre sont travaillées manuellement on y apporte des engrais organiques sous forme de fumier avec un complément de fumure minérale sous forme d'ammonitre.

- **N'jila** : les cultures sont représentées surtout par la pomme de terre et par d'autres cultures maraîchères. Ce type de jardins n'est pas dominant par rapport aux autres, il est localisé au pied de djebel Sidi Ali El Mekki.

- **Gattaya** : les gattaya sont des jardins très spécifiques à Ghar El Melh. Le sol de ces parcelles fonctionne selon le principe d'une éponge en emmagasinant l'eau des plaines tout au long de l'année aidée en cela par l'eau de la lagune qui joue le rôle de fond de récipient pour l'eau douce, les deux eaux ne se mélangent pas.

L'apport de fumier à ces parcelles est systématique au début de chaque saison. D'importantes quantités de fumier sont incorporées manuellement au moment du travail de la terre. Cet apport est complété par un apport d'ammonitrite. Des produits insecticides sont utilisés entre autres pour traiter la pomme de terre à conserver et semblent être facilement contrôlables. Chaque agriculteur dispose au moins d'une gattaya sur la lagune de Ghar El Melh (SCET-ERI, 2000).

L'essentiel de l'activité agricole se concentre sur les rives de la lagune. La lagune va donc subir les conséquences de cette activité à savoir la pollution par les engrais.

7.2. Urbanisation

L'urbanisation des rivages de la lagune est de deux natures (i) l'existence et l'évolution des structures urbaines permanentes et (ii) l'occupation périodique par une population non résidente, estivante et abritée dans des structures légères.

L'action anthropique de l'urbanisation se manifeste comme suit :

- Le rejet des eaux usées urbaines : ces rejets atteignent la lagune par l'évacuation directe des eaux usées vers la lagune (par des émissaires de différentes tailles, mis en place par les services publics municipaux ou bien par des particuliers) et par l'alimentation indirecte par infiltration à partir d'un grand nombre de fosses septiques.

- L'évacuation des déchets solides : l'évacuation des déchets solides de tous genres s'effectue vers une décharge non contrôlée située sur les bords de la lagune. A cela s'ajoutent des quantités non négligeables de provenance extérieure, non identifiées mais indiquées par la population communale vigilante de Ghar El Melh. La tête de la décharge avance dans les eaux de la lagune et l'aspect de la surface des eaux aux alentours de la décharge ne cache plus les effets fortement compromettants et négatifs.

A cela s'ajoute aussi le tourisme balnéaire national qui constitue la principale activité estivale de la région. L'impact probable et majeur du tourisme, concerne l'action physique directe sur le(s) milieu(x) (piétinements...) et l'impact sur l'environnement suite aux résidus délaissés par les estivants (déchets solides, liquides..). Ces impacts affectent le site intégral : les plages et les eaux de baignade, les dunes et l'arrière dune ainsi que la zone humide à partir desquelles les pollutions seront périodiquement transférées dans la lagune à travers les communications (SCET-ERI, 2000).

7.3. Activités industrielles

Les activités industrielles sont des opérations qui induisent des déséquilibres écologiques de par la production de déchets solides gazeux et liquides. En effet, une zone industrielle est située dans le bassin versant de la lagune de Ghar El Melh : c'est la zone industrielle

d'Utique. De nombreuses industries se sont implantées en 1978 sur une parcelle de terrain agricole de 30 ha. Au total, cette zone comporte 34 unités industrielles réparties par secteur d'activité comme suit :

Tableau 6. Répartition des industries par secteur d'activité (SCET-ERI, 2000).

Secteur d'activité	Nombre d'unités industrielles
Industries agro-alimentaires (IAA)	5
Industries textiles d'habillement et du cuir (ITHC)	8
Industries chimiques (IC)	5
Industries mécaniques métalliques métallurgiques et électriques (IMMME)	5
Industries matériaux de construction céramique de verre (IMCCV)	2
Industries diverses (ID)	9
Total	34

Le réseau d'assainissement des eaux présente des problèmes de bouchage et de débordement qui sont dus essentiellement au sous-dimensionnement de la canalisation, aux eaux très chargées des unités industrielles et au manque total d'entretien. Le rejet hydrique industriel de l'ensemble des industries de la zone s'élève à $181\,250\text{ m}^3\text{ an}^{-1}$, acheminé par ce réseau vers l'Oued d'Utique (cours d'eau à sec), le rejet se détaille par secteur d'activité et par milieu récepteur comme suit :

Tableau 7. Répartition quantitative des rejets hydriques par secteur d'activité et par milieu récepteur (SCET-ERI, 2000)

Secteur d'activité	Oued d'Utique ($\text{m}^3\text{ an}^{-1}$)	Autres ($\text{m}^3\text{ an}^{-1}$)
Industries agro-alimentaires (IAA)	3500	-
Industries textiles d'habillement et du cuir (ITHC)	177000	-
Industries diverses (ID)	750	10 (stockage en bassins étanches)
TOTAL	183700	10

Parmi les 6 secteurs d'activité rencontrés dans la zone, le secteur des industries de textiles d'habillement et du cuir (ITHC) rejette à lui seul 98 % de l'ensemble du volume précité. Les industries de cuir de la zone sont uniquement des tanneries, elles rejettent environ $135000\text{ m}^3\text{ an}^{-1}$ d'eau usée industrielle, soit 75 % de la totalité des effluents hydriques industriels, leurs rejets se caractérisent par une charge organique importante et une concentration élevée des

produits chimiques (chrome et sulfure), outre la présence de matières en suspension et de matière organique.

Les industries de textiles de la zone, rejettent environ $42000 \text{ m}^3 \text{ an}^{-1}$ d'eau usée industrielle, soit 23 % de la totalité des effluents hydriques, leurs rejets se caractérisent par une charge importante en matières en suspension, par la présence de produits détergents et par une forte demande chimique d'oxygène (SECT-ERI, 2000).

Les industries agricoles et alimentaires rejettent annuellement environ 3500 m^3 , représentant 2 % de l'ensemble des effluents hydriques de la zone, leurs rejets se caractérisent essentiellement par une charge organique importante.

7.4. Activités halieutiques

L'activité halieutique dans la lagune de Ghar El Melh se base exclusivement sur la pêche. Une vingtaine de barques exploitent cette lagune où a sévi une crise dystrophique durant les périodes 1994 - 1995 et 1996. Les plus grandes difficultés ayant été connues en 1995, marquées par la fermeture de la communication mer-lagune et par voie de conséquence un arrêt du recrutement des jeunes poissons vers la lagune et une mortalité des poissons à l'intérieur de la lagune.

Actuellement, l'écosystème lagunaire se rétablit peu à peu et la faune ichthyque commence à conquérir toutes les zones de la lagune. Le rendement des barques côtières reste faible (5 à 10 Kg par unité et par sortie d'une nuit). Le rendement à l'hectare ($18 \text{ Kg ha}^{-1} \text{ an}^{-1}$) est loin de la moyenne des productions des lagunes méditerranéennes (50 à $100 \text{ Kg ha}^{-1} \text{ an}^{-1}$).

La distribution des fréquences des tailles des espèces capturées montrent que l'essentiel des captures s'effectue sur des classes d'âge jeunes 0.1 et 2 ans. Les poissons nouvellement recrutés n'ont pas eu le temps de grandir et sont en fait directement exposés à la mortalité par pêche. Il est nécessaire d'épargner ces jeunes individus pour qu'ils aient le temps de grandir dans la lagune.

Aussi, à cause de l'activité halieutique, le vieux port de pêche de Ghar El Melh, situé sur les berges Nord de la lagune principale, est à l'origine de plusieurs sources de pollution : une pollution chimique (les rejets des huiles usées, les eaux issues des opérations de carénage ou de peinture des coques), et une pollution par les macro-déchets flottants (SCET ERI, 2000).

8. Importance écologique de la lagune de Ghar El Melh

Ghar El Melh est récemment (Novembre 2007) désigné comme un site « RAMSAR ». Cette désignation couvre 10 168 ha et comprend la lagune et le delta d'Oued Medjerda (RAMSAR, 2007). Le site répond aux critères « RAMSAR » des zones humides importantes à l'échelle

internationale pour être représentatif des lagunes au sud de la Méditerranée. La lagune de Ghar El Melh constitue un habitat pour les poissons migrateurs, surtout en hiver, et un site d'hivernage et de reproduction pour de nombreux oiseaux, y compris les grèbes, les hérons, sternes, goélands et les flamants roses. Elle est également qualifiée d'importance internationale pour sa population de Glaréole à collier (*Glareola pratincola*) qui dépasse 1 % de la population totale paléarctique. L'importance de Ghar El Melh pour les oiseaux migrateurs est liée à sa position comme une zone de migration primaire vers et depuis l'Italie et sa position au sein d'un système de zones humides qui s'étendent de Raoued et Kalaat el Andalous à la lagune incluant la vallée de la Medjerda. Ces zones humides offrent une gamme d'habitats, y compris des zones étendues d'eau, des plaines inondables, des lacs de montagne, des dunes côtières, des canaux avec des roselières et des petites dépressions de zones humides... et donc étayent plusieurs avifaunes notamment les passereaux et les rapaces. A Ghar El Melh, la distribution des oiseaux reflète différents habitats. Selon le programme de surveillance de MELMARINA (Ayache *et al.*, 2009), les grèbes, les sternes et les cormorans se concentrent dans les régions centrales et les plus profondes de la lagune, tandis que les oiseaux de mer, particulièrement les goélands, se concentrent aux bords de la lagune.

Les plus grandes concentrations de ces oiseaux étaient dans le nord près des émissaires d'eaux usées de Ghar El Melh et d'Aousja où la prolifération d'algues est fréquemment observée. Les plus grandes concentrations de canards et flamants roses ont été trouvées sur les rives occidentales de Sebket El Ouafi qui sont soumises à moins de perturbation par rapport à la rive nord de la lagune principale (Hughes *et al.*, 1997).

Durant les années 1960 et 1970, le nombre de canards hivernant dans le sud de la lagune et autour de l'ancien delta Medjerda a régulièrement dépassé 20 000 (RAMSAR, 2007). Malheureusement, le manque de dénombrement systématique des oiseaux abritant la lagune de Ghar El Melh empêche l'évaluation de l'évolution du nombre des espèces et des individus au sein de la lagune en réponse aux pressions qui s'y exercent.

Chapitre III. Importance de *Ruppia cirrhosa* et les microalgues épiphytes dans la lagune de Ghar El Melh

Article 1. Contrasting key roles of *Ruppia cirrhosa* in a southern Mediterranean lagoon: Reservoir for both biodiversity and harmful species and indicator of lagoon health status

Article publié dans le journal 'Marine pollution bulletin' (IF = 2.99)

Marine Pollution Bulletin 76 (2013) 116–127



Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul



Contrasting key roles of *Ruppia cirrhosa* in a southern Mediterranean lagoon: Reservoir for both biodiversity and harmful species and indicator of lagoon health status



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ARTICLE INFO

Keywords:
Ruppia cirrhosa
Epiphytes
Environmental factors
Ghar El Melh lagoon

ABSTRACT

The distribution of *Ruppia cirrhosa* meadow density and its epiphytic organisms in relation with environmental factors were studied in summer 2011 at five stations in the Ghar El Melh lagoon (GML; southern Mediterranean Sea). Eleven epiphytic groups were recognised among which diatoms and dinoflagellates were the dominant groups and greatest contributors to temporal dissimilarity. An overwhelming concentrations of harmful microalgae was recorded, mainly represented by the toxic dinoflagellate *Prorocentrum lima* with maximal concentrations attaining 6×10^5 cells 100 g^{-1} of *Ruppia* fresh weight. The epifauna community accounted for only 1.4% of total epiphyte abundance and was comprised predominantly of nematodes (47.51%), ciliates (32.59%), fish eggs (7.2%) and larvae (4.95%). PERMANOVA analyses revealed a significant spatio-temporal variation of all epiphytic groups ($p < 0.01$). In this study, *R. cirrhosa* and its epiphytes were studied as potential early warning indicators of the health status of GML waters.

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Résumé

Dans ce chapitre, on dévoile l'importance du macrophyte « *Ruppia cirrhosa* » dans la lagune de Ghar El Melh. Ceci est à l'issue d'une stratégie d'échantillonnage ciblant ce macrophyte ainsi que les épiphytes abritant les feuilles, pendant les 3 mois d'été de l'année 2011 au niveau des 5 stations de prélèvement.

L'étude de la couverture de *R. cirrhosa* nous a permis d'estimer une densité maximale durant les 2 premiers mois de l'été (juin et juillet), dans plusieurs parties de la lagune, de l'ordre de 1500 pousses m⁻². Cette prolifération va en décroissant à partir du mois d'août, avec une abondance maximale de l'ordre de 350 pousses m⁻².

L'inventaire des espèces épiphytes de *R. cirrhosa* a révélé un rôle contradictoire de ce macrophyte faisant à la fois :

- (i) Une source immense de la biodiversité dans la lagune. En effet, 11 groupes épiphytes ont été identifiés parmi lesquels les diatomées et les dinoflagellés étaient les groupes dominants et les plus grands contributeurs à la dissemblance temporelle (Test de Simper). L'épifaune n'a représenté que 1.4 % de l'abondance totale des épiphytes et a été constituée principalement de nématodes (47.51 %), ciliés (32.59 %), oeufs de poissons (7.2 %) et des larves (4.95 %). Les analyses de PERMANOVA ont révélé une variation spatio-temporelle significative de tous les groupes épiphytes ($p < 0.01$).
- (ii) Une source préoccupante des microalgues potentiellement nuisibles. En effet, 10 espèces nocives ont été identifiées avec des concentrations alarmantes, parmi lesquelles le dinoflagellé toxique *Prorocentrum lima* avec des concentrations maximales atteignant $6 \cdot 10^5$ cellules par 100 gramme de matière fraîche de *R. cirrhosa*.

Dans cette étude, *R. cirrhosa* et ses épiphytes ont été analysés comme des indicateurs d'alerte potentiels de la qualité des eaux de la lagune de Ghar El Melh. Tenant compte de la faible profondeur du site d'étude, les microalgues épiphytes peuvent être d'une manière non négligeable à l'origine de la structure du phytoplancton de la lagune de Ghar El Melh.

Abstract

The distribution of *Ruppia cirrhosa* meadow density and its epiphytic organisms in relation with environmental factors were studied in summer 2011 at five stations in the Ghar El Melh lagoon (GML; southern Mediterranean Sea). Eleven epiphytic groups were recognised among which diatoms and dinoflagellates were the dominant groups and greatest contributors to temporal dissimilarity. An overwhelming concentrations of harmful microalgae was recorded, mainly represented by the toxic dinoflagellate *P. lima* with maximal concentrations attaining 6×10^5 cells 100 g^{-1} FW. The epifauna community accounted for only 1.4 % of total abundance and was comprised predominantly of nematodes (47.51 %), ciliates (32.59 %), fish eggs (7.2 %) and larvae (4.95 %). PERMANOVA analyses revealed a significant spatio-temporal variation of all epiphytic groups ($p < 0.01$). In this study, *R. cirrhosa* and its epiphytes were studied as potential early warning indicators of the health status of GML waters.

Keywords: *Ruppia cirrhosa*; epiphytes; environmental factors; Ghar El Melh lagoon

1. Introduction

Aquatic macrophytes, and especially aquatic Magnoliophyta, are key structural and functional components of numerous lagoon environments (Menéndez, 2002; Sfriso *et al.*, 2002). Generating highly favourable habitats (Agostini *et al.*, 2003; Heck *et al.*, 2008), they play essential roles of great ecological and economic importance (Skinner and Zalewski, 1995; Giovanetti *et al.*, 2010; Cullen-Unsworth *et al.*, 2013). Furthermore, due to their wide distribution and sensitivity to change in coastal zones, seagrasses are often used as biological indicators of water quality and health (Bhattacharya *et al.*, 2003; Pergent-Martini *et al.*, 2005) and have recently been included among components of biological quality in water quality monitoring programmes (EC, 2000; Foden and Brazier, 2007). Likewise, seagrass epiphytes not only contribute significantly to the primary production of meadows (Nelson and Waaland, 1997; Duarte *et al.*, 2004), but are also more sensitive to environmental changes than the plant host (Delgado *et al.*, 1999; Nesti *et al.*, 2008; Giovannetti *et al.*, 2010) and are expected to have different responses to human interference such as modifications in their structure and function (Sfriso *et al.*, 2007, 2009; Orlando-Bonaca *et al.*, 2008). For instance, various studies have reported increases in epiphyte biomass in parallel with eutrophication (Balata *et al.*, 2008; Frankovich *et al.*, 2009) and with respect to water quality (Meric *et al.*, 2005). We have thus hypothesised that, in an ecosystem exhibiting harmful algal blooms (HAB), seagrass leaf epiphytes should be examined to gain an overview of the potentially harmful species that are, in fact, protected by the leaves and that may go undetected in HAB monitoring in the water column. Epiphytic microalgae are of increasing interest as most of their representatives are potential toxin producers (Nakajima *et al.*, 1981; Foden *et al.*, 2005; Aligizaki *et al.*, 2009) that may cause mortality or disease at higher trophic levels by indirect biotic (predator-prey) and abiotic (hypoxia) factors (Doucette *et al.*, 2005; Hallegraeff, 2010).

In the Mediterranean Sea, most studies of seagrass meadows focus mainly on *Posidonia oceanica* (L.) Delile and *Cymodocea nodosa* (Ucria) Ascherson (Vizzini *et al.*, 2002; Prado *et al.*, 2007; Apostolaki *et al.*, 2011) which are considered as reliable bioindicators of meadow regression due to various causes (Ruiz and Romero, 2003; Oliva *et al.*, 2012). However, a few studies have dealt with *Ruppia cirrhosa* (Petagna) Grande (Calado and Duarte, 2000; Pergent *et al.*, 2006; Menéndez, 2009) which nevertheless remains one of the least studied seagrasses (Triest and Sierens, 2009). The Ghar El Melh lagoon (GML), located near the southern Mediterranean Sea, offers a good opportunity to study *R. cirrhosa* and its epiphytes for the following reasons: (i) the generally eutrophic conditions of GML waters due to agricultural

drainage from the Utica floodplain and the discharge of raw sewage from the town of Ghar El Melh (compounded by local tourism in summer months) (Romdhane, 1999; Chakroun-Glaoui, 2004; Ayache *et al.*, 2009), (ii) the high level and wide distribution of *R. cirrhosa* coverage during summer an area of 21.4 km², with dense, extensive beds covering 80-100 % of the lagoon's surface (Shili *et al.*, 2002), (iii) episodes of harmful microalgae detected in recent years (Turki and El Abed 2001; Turki *et al.*, 2007, Dhib *et al.*, 2013a) causing extensive fish mortality (Romdhane, 1998; Turki *et al.*, 2007), and (iv) the various papers that have been published linking the abundance of toxic species in the water column to their abundance among macrophytes (Vila *et al.*, 2001a; Aligizaki and Nikolaidis, 2006; Mabrouk *et al.*, 2011). As far as we know, studies of microepiphytes of *R. cirrhosa* are totally lacking, the only studies available being those dealing with the macroalgal epiphytes of *Ruppia* (Mannino and Sara, 2006) or with the epiphytic macrofauna found on these seagrass leaves (Verhoeven, 1980a).

Thus, and concordantly with the spectacular summer colonisation of the site by *R. cirrhosa*, we undertook a study, at five different sampling stations from early June to late August, of *R. cirrhosa* meadow density and its epiphytic organisms (microalgae + protozoans + metazoans) in relation to the physical and chemical variables. We hypothesised that 1) *Ruppia* leaves, which emerge and overwhelm the surface water in different parts of the lagoon, may provide a habitat for a wide range of epiphytic communities, including harmful algae which may find a refuge within these leaves and further contribute to HAB initiation if, due to water dynamics, they should leave the meadow, and 2) that the lagoon's eutrophication may influence both the meadow and epiphytic structure.

2. Materials and methods

Study area

Ghar El Melh lagoon is located in north-eastern Tunisia, 44 km from Bizerta (Figure 13). It is a complex of three wetlands comprising an area of about 35 km² (the main lagoon, Sabkhet El Ouafi and Sabkhet Sidi Ali El Mekki). The main lagoon is connected to the Mediterranean Sea by a narrow channel called "El Boughaz" whose average depth is 0.8 m, with a maximum of 2 m at the channel entrance (Chakroun, 2004). The bottom is relatively flat and muddy (Rasmussen *et al.*, 2009). GML is supplied with water volumes from the Utica floodplain, from several springs (north-east of the main lagoon) and from seasonal run-off from the Djebel Nadhour and Demina hills in the north (Ayache *et al.*, 2009). Thompson *et al.* (2009) have demonstrated that, in summer, the volumes of water provided to the lagoon are relatively

small and freshwater inflows are stagnant and essentially inexistent. Under these conditions, and along with high evaporation rates and small marine inflows through the narrow connection with the sea, the lagoon's salinity increases substantially (Ktari-Chakroun and Romdhane, 1985; Beyrem, 2002) with ultimately a wide spread of *R. cirrhosa* in accordance with its extremely high resistance to variations in environmental conditions and being among the rare macrophytes that survive and exhibit healthy growth in salinities above 20 psu (Verhoeven, 1979).

GML fulfils the RAMSAR criteria (RAMSAR, 2007) for internationally significant wetlands as a representative of lagoons within the southern Mediterranean and furnishing a habitat for migrating birds (Ayache *et al.*, 2009).

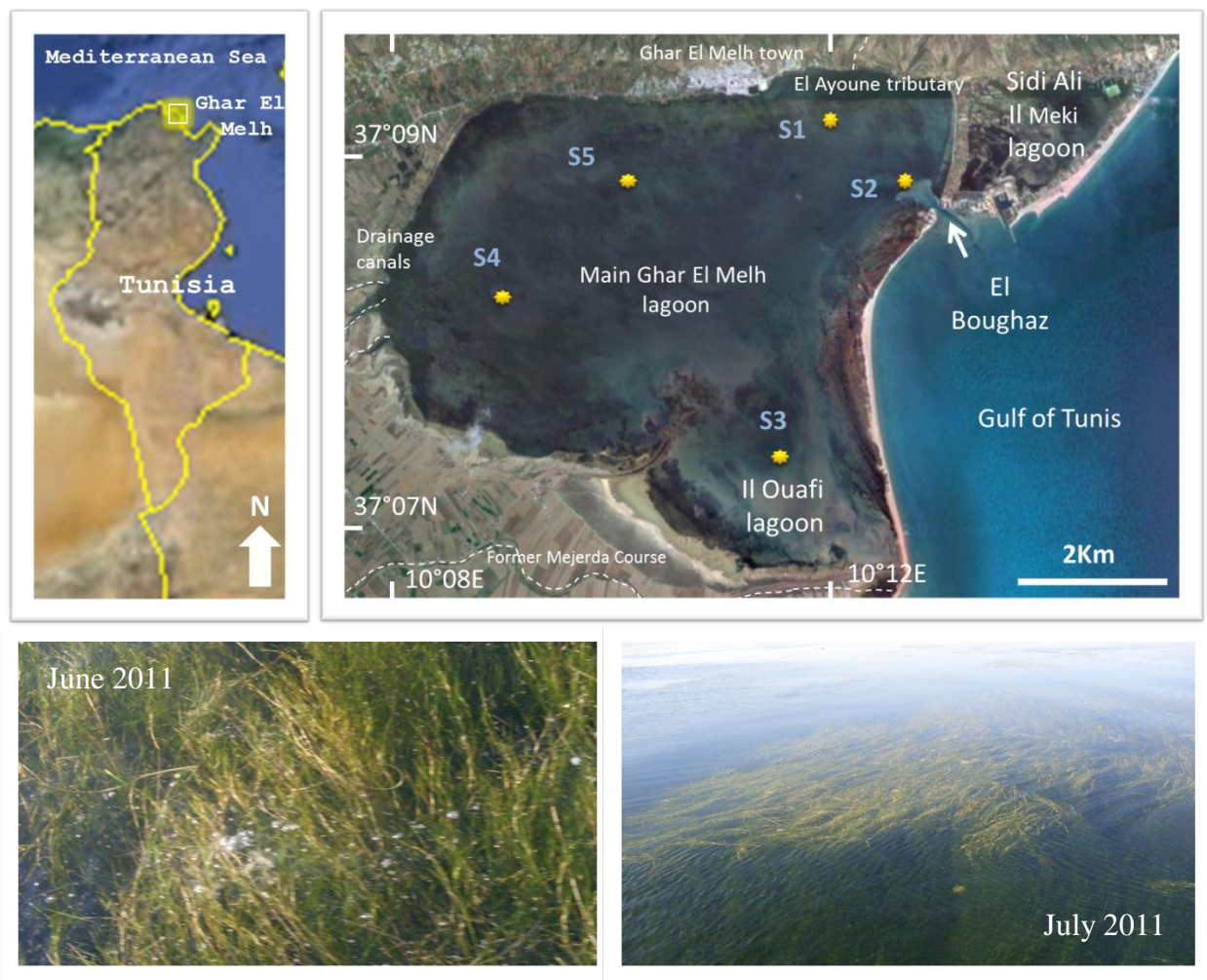


Figure 13. Geographic situation of the Ghar El Melh lagoon, location of the sampling stations S1 to S5 and some photographs of *Ruppia cirrhosa* colonies at the site.

Sampling

Five stations (S1 to S5) were selected to include (1) the spring “El Ayoun”, at the foot of Jbel Nadhour, whose waters reach the lagoon as groundwater, (2) the channel “El Boughaz”, the lagoon’s only connection with the sea, (3) the semi-closed area “Sabkhet El Ouafi” (4) the catchment area from agricultural and industrial developments situated on the Utica floodplain and (5) the rural edges of the lagoon, source of runoff from intensively cultivated land and also of raw sewage from human settlements, both of which flow directly into the lagoon.

Shoot density of *R. cirrhosa* was estimated in situ from 45 replicates from a 400 cm² sub-quadrat at each site over the three summer months: in early June, mid-July and late August. The quadrat (40 cm x 40 cm) was randomly placed over the shoots. Meadow density (expressed as shoot m⁻²) was calculated by the number of shoots bearing leaves in each sub-quadrat (Ott, 1980). At each station, the quadrats were separated by 100 m. To examine epiphytes, 100 g of sampled leaves were gathered and placed in plastic bottles. The epiphytic communities were separated from the plants by vigorous shaking and washing with filtered seawater (200 ml) (Short, 1990; Dauby and Poulicek, 1995). The material was then passed through 250 µm mesh sieves to remove large particles, and finally fixed with formaldehyde (5 %).

Environmental variables were measured in the field at the same time as epiphyte sampling. A WTW multiparameter was used for water temperature (°C) and salinity. Transparency was measured using a Secchi disk. Water samples were collected in 1000-ml polypropylene bottles at 30 to 50 cm depth. Nutrients (ammonium, nitrite, nitrate, phosphate, silicate) were analysed in a laboratory with a BRAN and LUEBBE type 3 AutoAnalyser and concentrations were determined calorimetrically using a UV-visible (JENWAY 6705) spectrophotometer (APHA, 1992).

Epiphyte enumeration

Sub-samples (1 ml) of epiphytic communities (Turki, 2005) were counted by means of an inverted microscope according to the Utermöhl method (Utermöhl, 1958). Unicellular taxa (microalgae and protozoans) and metazoans on *R. cirrhosa* leaves were identified according to various keys. For microalgae, we used the keys of Dodge (1982, 1985), Sournia (1986) and Hasle and Syversten (1997). Naked ciliate identification was achieved based on the methods of Lynn and Small (1997), Petz (1999) and Strüder-Kypke and Montagnes (2002). Tintinnids were identified using lorica morphology and species description according to Kofoid and Campbell (1929, 1939) and Balech (1959). Metazoans were counted according to the methods of Tregouboff and Rose (1978).

Species abundance on *R. cirrhosa* leaves was calculated as a number of cells 100 g^{-1} FW (Fresh Weight) *R. cirrhosa*, for unicellular species (microalgae and protozoans: ciliates and fish eggs in this study) or as a number of individuals (ind) 100 g^{-1} FW, for metazoans.

2.4. Statistical analyses

The data recorded for the epiphytes were submitted to permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2005) to analyse differences in community composition between stations and months. Data were transformed where necessary to meet the assumption of homogeneity of variances (homogeneity confirmed by non-significant Cochran's c-test). Similarity percentage analysis (SIMPER) was used to identify the contribution of individual taxa with the pattern of similarity and dissimilarity between habitats in each month. Canonical correspondence analysis (CCA) with Monte Carlo permutation tests (499 permutations) were applied to physical (temperature, salinity, pH and transparency), chemical (NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-} and $\text{Si}(\text{OH})_4$) and biological parameters (epiflora + epifauna) assessed over 45 observations. Potential relationships between variables were tested by Spearman's correlation coefficient.

3. Results

Physico-chemical variables

Throughout the three sampling months, important variations in abiotic variables were noted (Table 8). Water temperature ranged from $20.9 \text{ }^\circ\text{C}$ to $26 \text{ }^\circ\text{C}$ with the lowest values recorded in early June at S2 near the sea outlet, while the highest were detected in late August at S4 near the industrial periphery. Salinity was high, varying from 37 to 48.6, and peaked in mid-July especially at S3, S4 and S5. Water salinity distribution indicated a clear influence of both freshwater and marine inflows towards S1 and S2 respectively. Within these zones we always recorded the lowest salinity (< 41). Water pH values were important (8 - 9) perhaps ascribed to the large stands of *R. cirrhosa* colonising the site. Water transparency was greater during the summer observation ($Z_s = 1.77 \text{ m}$), but to a lesser extent for S1 ($Z_s = 1.3 \text{ m}$). Nutrient concentrations were, in general, weakened in summer, probably due to an increased uptake by microalgae, benthic algae and macrophytes. Total nitrogen was low and homogenous ($17.64 - 22.51 \text{ } \mu\text{mol l}^{-1}$), consisting of 21.56 % of inorganic form (DIN). Nitrate, nitrite and ammonium exhibit large variations and were exhausted by mid-July. Dissolved inorganic nitrogen peaked in late August causing P limitation ($\text{DIN/DIP} = 35.29$). Total phosphorus concentration was higher and homogenous ($3.08 - 4.65 \text{ } \mu\text{mol l}^{-1}$). Orthophosphate levels were

maximal in mid-July, especially at S1 and S2. Silicates ranged from $3.21 \mu\text{mol l}^{-1}$ to $13.09 \mu\text{mol l}^{-1}$ and peaked in mid-July.

***Ruppia cirrhosa* features**

The mean shoot abundance of *R. cirrhosa* ranged between $41.66 \pm 11.78 \text{ shoot m}^{-2}$ and $1266.67 \pm 208.17 \text{ shoot m}^{-2}$ with the highest density recorded in early June at S5 whereas the lowest was detected in late August at S1 (Figure 14). In accordance with PERMANOVA analyses, significant interaction between months and stations was recorded and shoot density was similar between June and July (Table 9). Indeed, *Ruppia* beds colonised the site from early June to mid-July at all stations except at S1 near the spring “El Ayoune” where only small patches of *Ruppia* were observed during the three sampled months (maximal density = 150 shoot m^{-2}). In late August, the beginning of decay of the stem bases of *R. cirrhosa* was noted at different stations (maximal density = 350 shoot m^{-2} at S5).

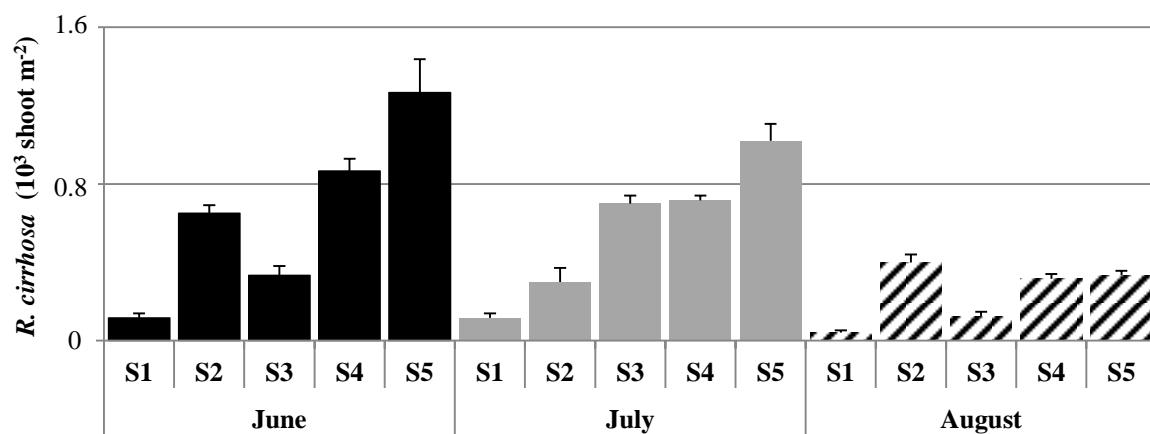


Figure 14. Mean abundance of *Ruppia cirrhosa* (shoot m^{-2}) at the five stations and for each month.

Tableau 8. Summary data for environmental variables monitored at the five stations during the three months. Orange and red refer respectively to poor and bad water quality according to IFREMER (2012).

Time samples	June					July					August				
Site samples	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Physical variables															
Temperature (°C)	23.4	20.9	23.7	23.7	23.6	24.6	25	24.6	23.2	24.8	25.3	25.7	25.6	26	25.9
Salinity	39	37	43.8	45.7	44.1	38.2	38	44.3	48.6	47.5	39.6	41.1	45	46.7	44.7
pH	8.3	8.3	8.6	8.5	9	8	8	8.11	8.2	8	8.2	8.3	8.7	8.6	8.5
Transparency (m)	1.3	1.77	1.45	1.33	1.27	1.4	1.58	1.45	1.3	1.35	1.27	1.53	1.7	1.38	1.2
Depth (m)	1.4	1.77	1.45	1.33	1.27	1.4	1.58	1.45	1.3	1.35	1.27	1.53	1.7	1.38	1.2
Chemical parameters															
Si(OH) ₄ (μmol l ⁻¹)	5.97	4.41	6.48	9.86	8.30	11.89	12	12.54	13.09	12.44	5.2	6.71	8.34	4.66	3.21
NO ₂ ⁻ (μmol l ⁻¹)	0.20	0.22	0.23	0.22	0.34	0.20	0.20	0.18	0.18	0.17	0.25	0.34	0.31	0.20	0.18
NO ₃ ⁻ (μmol l ⁻¹)	1.18	0.94	1.01	0.98	1.70	1.77	1.69	1.64	1.34	1.8	3.14	4.58	5.2	3.11	2.98
NH ₄ ⁺ (μmol l ⁻¹)	2.34	2.10	1.6	2.52	1.63	0.57	0.33	0.27	0.77	0.44	1.25	3.25	4.55	3.05	4.19
PO ₄ ³⁻ (μmol l ⁻¹)	0.13	0.21	0.23	0.25	0.18	0.35	0.3	0.18	0.24	0.25	0.31	0.18	0.16	0.24	0.23
T-N (μmol l ⁻¹)	19.71	20.91	19.52	21.26	22.07	19.96	18.01	17.64	20.67	21.46	20.45	21.65	22.51	18.30	19.99
T-P (μmol l ⁻¹)	3.70	3.23	3.86	4.25	3.19	4.53	4.05	3.08	3.68	4.54	4.65	4.57	3.95	3.547	4.12
DIN/DIP	26.92	15.24	11.92	14.86	19.54	7.14	7.34	11.25	9.35	9.65	14.84	43.31	60.35	26.02	31.90
DIN	3.73	3.26	2.85	3.74	3.69	2.56	2.23	2.1	2.31	2.42	4.66	8.18	10.08	6.37	7.37

Tableau 9. PERMANOVA results for *Ruppia cirrhosa* density between sites and for the three months. Bold numbers indicate significant effects.

Source of variation	df	MS	F	Perm
Month	2	332.05	81.68	0.001
Site	4	575.19	141.49	0.001
Month*Site	8	43.96	10.81	0.001
Residual	30	121.95	4.06	
Cochran's C-test		C= 0.574		
Transformation		Ln (x+1)		
SNK test		August < June = July		

No significant correlation was found between *R. cirrhosa* coverage and the different abiotic variables monitored during this study, though close relationships were detected between meadow density and several epiphytic communities such as dinoflagellates ($r = 0.91$; $p = 0$), diatoms ($r = 0.75$; $p = 0.001$), cyanobacteria ($r = 0.73$; $p = 0.002$), ciliates ($r = 0.61$; $p = 0.015$), nematodes ($r = 0.6$; $p = 0.017$) and gastropod larvae ($r = 0.64$; $p = 0.01$) (Figures 4 and 6).

Epiphytic communities on *Ruppia* leaves

Eleven epiphytic groups (3 epiflora + 8 epifauna) were recognised at the five sampled stations of Ghar El Melh lagoon over the three studied months (Table 10). Epiphytic assemblage was largely dominated by the epiflora community (98.6 % of total abundance) among which diatoms represented 49.65 % of total abundance followed respectively by dinoflagellates (46.93 %) and cyanobacteria (2.01 %). Several species of the microalgae group have been cited in the literature as harmful and/or as potential toxin producers (Table 11).

Epifauna accounted for only 1.4 % of total abundance, consisting of nematodes (47.51 %), ciliates (32.59 %), fish eggs (7.2 %), mollusc larvae (3.22 %), ostracods (2.98 %), copepods (2.85 %), rotifers (2.84 %) and annelid larvae (0.77 %). Based on PERMANOVA analyses, significant interactions between months and sites were detected for all epiphytic groups ($p < 0.01$; Table 12 and 13). SIMPER test revealed an average dissimilarity for epiphytic communities during the three months with dinoflagellates and diatoms being the greatest contributors to this dissimilarity. In June and July, dinoflagellates contribute to this dissimilarity with 56.4 % and diatoms with 37.19 %. From June to August, dinoflagellates contribute 62.79 % while diatoms and cyanobacteria comprise 18.32 % and 11.59 % respectively. In July and August dinoflagellates contribute 44.81 % and diatoms 42.14 % to the dissimilarity.

Tableau 11. Nuisance types of the harmful species identified on *R. cirrhosa* leaves. DIAT: diatoms; DINO: dinoflagellates; CYANO: cyanobacteria; Max: maximal concentrations; DSP: diarrhetic shellfish poisoning; OA: okadaic acid; Pl: proro-centroid; FAT: fast-acting toxin; PSP: paralytic shellfish poisoning; BF: bloom forming

	Taxonomic groups	Nuisance types	Max (cells 100 g⁻¹ FW)
Potentially toxic species¹			
<i>Prorocentrum lima</i>	DINO	DSP, OA, Pl, FAT	6 10 ⁵
<i>Prorocentrum concavum</i>	DINO	DSP, OA, FAT	6 10 ³
<i>Dinophysis acuminata</i>	DINO	DSP, OA	2 10 ²
<i>Oscillatoria</i> sp.*	CYANO	Cytotoxin	9.06 10 ³
<i>Lyngbya</i> sp.*	CYANO	Lyngbyatoxin	4 10 ³
<i>Anabaena</i> sp.*	CYANO	PSP, Anatoxin	5 10 ³
Potentially bloom forming species²			
<i>Prorocentrum micans</i>	DINO	BF	7.09 10 ⁴
<i>Thalassionema nitzshoides</i>	DIAT	BF	1.92 10 ⁵
<i>Cylindrotheca closterium</i>	DIAT	BF	8.63 10 ²
<i>Leptocylindrus danicus</i>	DIAT	BF	1.27 10 ³

¹Potentially toxic species according to Kat (1985); Marr *et al.* (1992); Hu *et al.* (1993); Hallegraeff *et al.* (1995) and Anderson *et al.* (2001).

² Potential bloom-forming species according to Anderson *et al.* (2001); Vila and Maso (2005) and Balkis (2009).

* Genera known as toxin producers.

Tableau 12. PERMANOVA results for epiphytic microalgae on *Ruppia cirrhosa* during the three months. Bold numbers indicate significant effects.

Source of variation	Diatoms			Dinoflagellates			Cyanobacteria			
	<i>df</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>
Month	2	657.39	306.88	0.001	332.05	81.63	0.001	8187.72	3621.68	0.001
Site	4	529.41	247.14	0.001	575.19	141.49	0.001	3.32.48	1337.37	0.001
Month * Site	8	254.62	118.86	0.001	43.96	10.82	0.001	3065.07	1355.77	0.001
Residual	30	2.14			4.06			2.26		
Cochran's C-test		C = 0.988			C = 0.736			C = 0.836		
Transformation		Ln (x+1)			None			None		
SNK test		July > June = August			June > August July = August / July = June			June > July = August		

Tableau 13. PERMANOVA results for epiphytic fauna on *Ruppia cirrhosa* during the three months. Bold numbers indicate significant effects.

		Ciliates			Rotifers			Copepods		
Variation source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>
Month	2	12767.52	28.53	0.001	7989	8520.13	0.001	224.99	0.998	0.349
Site	4	7055.24	15.76	0.001	3000.4	3199.87	0.001	8879.08	39.41	0.001
Month*Site	8	5168.25	11.55	0.001	3000.4	3199.88	0.001	4384.86	19.46	0.001
Residual	30	447.41			0.94			225.3		
Cochran's C-test		C = 0.981			C = 0.988			C = 0.521		
Transformation		Ln (x+1)			Ln (x+1)			None		
SNK test		June > August = July			July > June = August			July = June = August		
		Ostracods			Nematodes			Annelid larvae		
Variation source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>
Month	2	6009.98	7127.27	0.001	11508.33	51.52	0.001	1755.73	7.87	0.001
Site	4	3006.01	3564.85	0.001	8722.92	39.05	0.001	1988.29	8.91	0.001
Month *Site	8	5995.39	7109.97	0.001	4124.6	18.46	0.001	2336.98	10.48	0.002
Residual	30	0.84			223.38			222.96		
Cochran's C-test		C = 0.789			C = 0.527			C = 0.991		
Transformation		None			None			Ln (x+1)		
SNK test		July < August = June			June > August = July			June > August = July		
		Bivalve larvae			Gastropod larvae			Fish eggs		
Variation source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>
Month	2	7990.41	7485.43	0.001	2827.16	6.02	0.015	6024.64	4313.62	0.001
Site	4	2999.73	2810.15	0.001	2185.18	4.66	0.011	3012.46	2156.91	0.001
Month *Site	8	2999.72	2810.11	0.001	2009.25	4.28	0.010	5990.35	4289.06	0.001
Residual	30	1.06			469.14			1.39		
Cochran's C-test		C = 0.998			C = 0.842			C = 0.965		
Transformation		Ln (x+1)			None			Ln (x+1)		
SNK test		August > June = July			July > June = August			August > June = July		

- *Diatoms*

Diatoms were represented by eleven species (Table 10) belonging to five orders namely Fragilariales (44.59 %), Naviculales (35.06 %), Licmophorales (15.54 %), Bacillariales (4.7 %) and Leptocylindrales (0.08 %). The highest mean diatom abundance ($9.76 \cdot 10^5 \pm 1.12 \cdot 10^5$ cells 100 g^{-1} FW) was registered in July at S4, while the lowest (200 ± 0.58 cells 100 g^{-1} FW) was recorded in August at S1 (Figure 15). SNK test revealed that no significant difference was recorded between June and August (Table 12). Indeed, the diatom peak was exclusively detected in mid-July and was mainly awarded to *Thalassionema nitzschioides* (maximal abundance = $1.92 \cdot 10^5$ cells 100 g^{-1} FW at S4), *Navicula phyllepta* (maximal abundance = $2 \cdot 10^5$ cells 100 g^{-1} FW at S4), *Licmophora gracilis* (maximal abundance = $2 \cdot 10^5$ cells 100 g^{-1} FW at S4), *Navicula duerrenbergiana* (maximal abundance = $0.5 \cdot 10^5$ cells 100 g^{-1} FW at S5) and *Denticulopsis* sp. (maximal abundance = $0.5 \cdot 10^5$ cells 100 g^{-1} FW at S4). According to the SIMPER test, the main diatom species contributing to temporal dissimilarity was *T. nitzschioides* which contributed 13.93 % and 15.45 % in June vs July and July vs August respectively. Other diatom species appear in the monthly dissimilarity but to a lesser extent, for example *N. duerrenbergiana*, *L. gracilis*, *N. phyllepta*, *Gyrosigma* sp. and *Climacosphenia* sp.

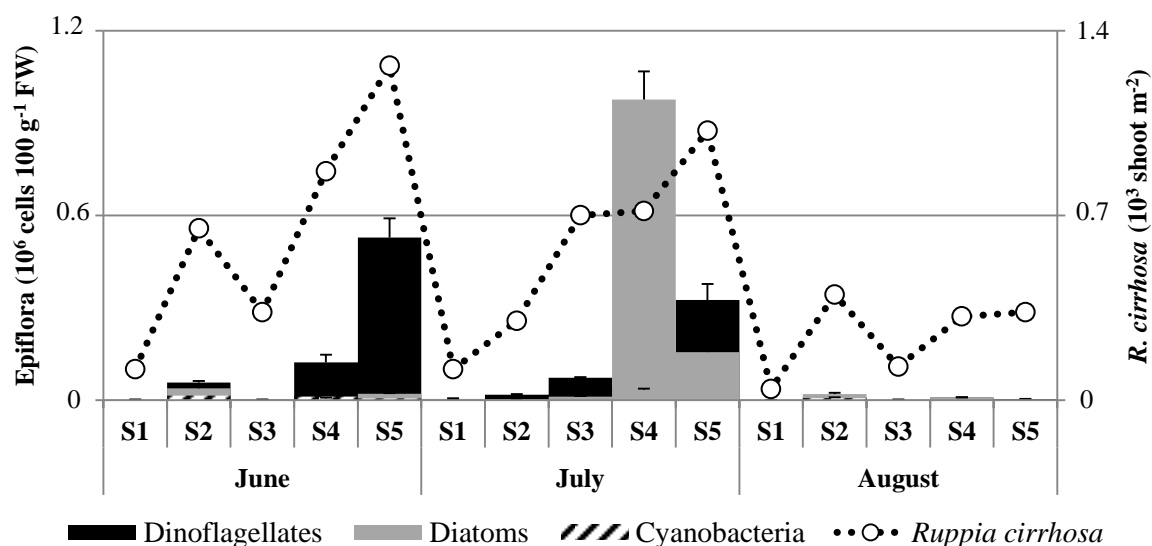


Figure 15. Mean abundance of epiflora (cells 100 g^{-1} FW) and *Ruppia cirrhosa* (shoot m^{-2}) at the five stations and for each month.

- *Dinoflagellates*

Dinoflagellates were exclusively represented by four harmful species namely *Prorocentrum lima*, *Prorocentrum concavum*, *Prorocentrum micans* and *Dinophysis acuminata* (Table 10). The high mean abundance of harmful dinoflagellates ($5.28 \cdot 10^5 \pm 0.76 \cdot 10^5$ cells 100 g^{-1} FW) was recorded in early June at S5, whereas the low abundance (200.33 ± 0.58 cells 100 g^{-1} FW) was detected in late August at S1 (Figure 15). SNK test revealed that similar results were recorded in June-July and in July-August, whereas a significant difference was recorded from June to August (Table 12). Indeed, the high dinoflagellate abundances were recorded in early June and mid-July, mainly attributed to *P. lima* which represents 95.26 % of total dinoflagellate abundance. In late August, a decline in dinoflagellate concentrations was associated with numerous *P. lima* valves left behind by this species (Figure 16). *P. micans* and *P. concavum* were frequently associated with *P. lima* but in slight concentrations that did not exceed $7.09 \cdot 10^4$ cells 100 g^{-1} FW and $6 \cdot 10^3$ cells 100 g^{-1} FW respectively. *D. acuminata* was detected sporadically in July in a single sample (200 cells 100 g^{-1} FW at S4). Simper test showed that the main dinoflagellate species contributing to temporal dissimilarity of the epiphytic community was *P. lima* with 50.88 %, 49.96 % and 44.81 % in June-July, from June to August and in July-August respectively. *P. micans* contributes to the monthly dissimilarity as well, especially in June-July and from June to August with 3.56 % and 5.27 % respectively. *P. concavum* contributes from June to August with only 2.32 %.

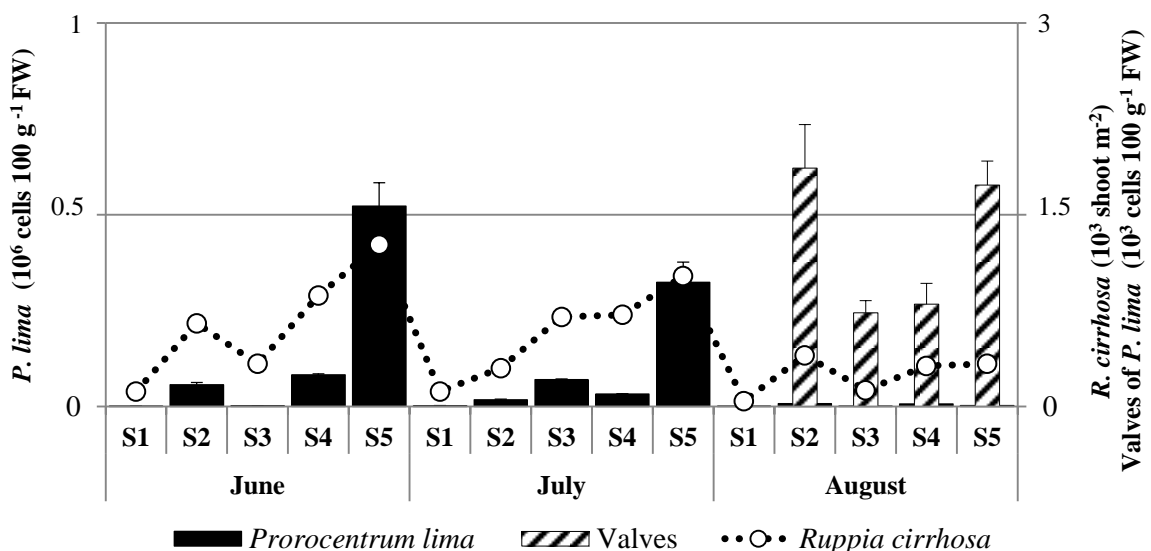


Figure 16. Mean abundance of *Prorocentrum lima* (cells 100 g^{-1} FW) and *Ruppia cirrhosa* (shoot m^{-2}) at the five stations and for each month.

- *Cyanobacteria*

Cyanobacteria were represented by five species (Table 10) belonging to two orders, Oscillatoriales and Nostocales. The highest mean abundance of cyanobacteria ($1.41 \cdot 10^4 \pm 5.45 \cdot 10^3$ cells 100 g^{-1} FW) was recorded at S2 in June whereas the lowest (0 cells 100 g^{-1} FW) was detected in August at S1 and S3 (Figure 15). No significant difference was recorded in July-August (Table 12). In fact, maximum cyanobacteria proliferation was detected in early June, mainly awarded to *Oscillatoria* sp. (maximal abundance = $9.06 \cdot 10^3$ cells 100 g^{-1} FW at S2), *Oscillatoria salina* (maximal abundance = $9.12 \cdot 10^3$ cells 100 g^{-1} FW at S4), *Lyngbya* sp. (maximal abundance = $4 \cdot 10^3$ cells 100 g^{-1} FW at S2) and *Spirulina* sp. (maximal abundance = $1.4 \cdot 10^3$ cells 100 g^{-1} FW at S4). Maximum concentrations of *Anabaena* sp. ($5 \cdot 10^3$ cells 100 g^{-1} FW) was detected in late August at S2. Both *Oscillatoria* sp. and *O. salina* are responsible for epiphytic dissimilarity from month to month. *Oscillatoria* sp. contribute 6.92 % and 2 % from June to August, and in July-August respectively, whereas *O. salina* was responsible for 3.25 % of dissimilarity from June to August.

- *Protozoans and metazoans*

A total of nine epifauna species were identified (Table 10). The highest numbers of collected species were those of ciliates (4), copepods (2), nematodes (1), rotifers (1) and ostracods (1). The remaining groups (bivalves, gastropods and annelids) were only represented at the larval stage. Epiphytic ciliates on *R. cirrhosa* leaves were *Tintinnopsis campanula*, *Aspidisca* sp., *Strombidium acutum* and *Amphorides amphora*. The highest mean abundance of ciliates ($7.78 \cdot 10^3 \pm 1.33 \cdot 10^3$ cells 100 g^{-1} FW) was registered at S5 in June while the lowest (0 cells 100 g^{-1} FW) was detected in June at S1 and S3, in August at S1 and 5 and in July at S1, S2, S3 and S5 (Figure 17). No significant difference between August and July was detected (Table 13).

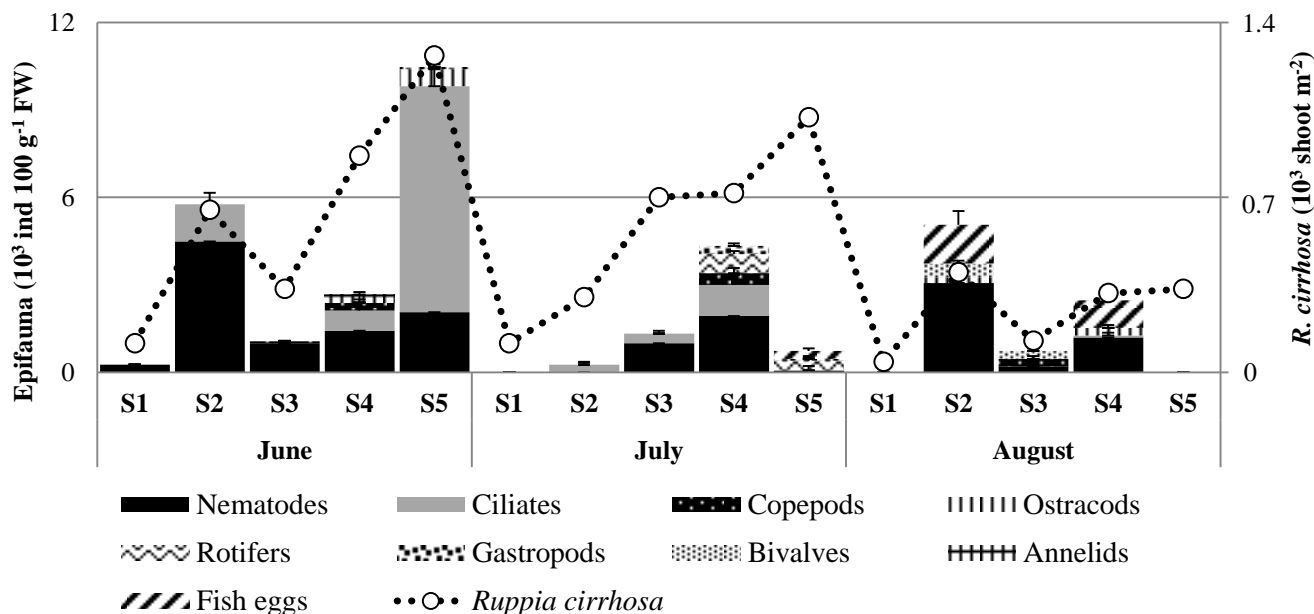


Figure 17. Mean abundance of epifauna groups (ind 100 g⁻¹ FW) and *Ruppia cirrhosa* (shoot m⁻²) at the five stations and for each month.

Maximum ciliate abundance was ascribed mainly to *T. campanula* (maximal abundance = 7.64 10³ cells 100 g⁻¹ FW at S5) and *Aspidisca* sp. (maximal abundance = 1.82 10³ cells 100 g⁻¹ FW at S2). *S. acutum* and *A. amphora* were only detected in mid-July with maximal concentrations of 800 cells per 100 g FW at S4. Nematodes were only represented by *Oncholaimus campyloceroïdes* with the highest average abundance (4.49 10³ ± 849 ind 100 g⁻¹ FW) recorded at S2 in early June, whereas the lowest (0 ind 100 g⁻¹ FW) was detected in July at S1 and S2 and in August at S5 (Figure 17) with no significant difference between August and July detected (Table 13). Two copepod species were identified namely *Acartia clausi* and *Euterpina acutifrons* associated with taxa in the larval stage. Copepods were only detected at S3 and S4. The highest mean abundance was detected in July (400 ± 200 ind 100 g⁻¹ FW at S4) and in June (272 ± 111 ind 100 g⁻¹ FW at S3). Late August copepod abundance reached 266 ± 115 ind 100 g⁻¹ FW at S3 (Figure 13) while no significant difference in copepod abundance was detected between the three months (Table 13). Rotifers were represented by *Synchaeta* sp. which was only recorded in mid-July at S4 and S5. The highest mean abundance (666 ± 115 ind 100 g⁻¹ FW) was recorded at S4 (Figure 17) with no significant difference in rotiferan abundance detected between June and August (Table 13). Ostracods were represented by *Cypridina* sp. with high abundance recorded in early June at S5 while none were observed in July (Figure 17). SNK test revealed that no significant

difference existed between June and August (Table 13). Molluscs were represented by gastropods and bivalves in their larva stage. The highest mean abundance of gastropod larvae (266 ± 115 ind 100 g^{-1} FW) was detected in July at S4 (Figure 17) with no significant difference observed between June and August (Table 13). Bivalve larvae were detected in late August at S2 and S3, with the highest mean abundance (466 ± 115 ind 100 g^{-1} FW) recorded at S2 (Figure 17) while no significant difference in abundance between June and July was detected (Table 13). Annelids consisted of polychaete larvae which were only detected in June at S3 with 272 ± 111 ind 100 g^{-1} FW (Figure 17), with no significant difference between August and July recorded (Table 6). The last group consisted of fish eggs whose highest mean abundance ($1.33 \cdot 10^3 \pm 577$ cells 100 g^{-1} FW) was registered in late August at S2 (Figure 17), with no significant difference detected between June and July, but with July exhibiting the lowest egg numbers (maximal abundance = 400 cells 100 g^{-1} FW at S5) (Table 13).

Epiphyte - abiotic variable interactions

The relationships between epiphytic communities and the environmental variables are illustrated in Figure 18. Canonical Correspondence Analysis (CCA) indicates that axis I and II expressed high cumulative variance of epiphyte-environmental variables (99.6 %). The eigen values calculated with the CCA associated with the Monte Carlo test showed that NH_4^+ ($F = 18.32$, $p = 0.002$), NO_3^- ($F = 10.05$, $p = 0.002$), T-P ($F = 5.96$, $p = 0.002$), salinity ($F = 5.33$, $p = 0.002$) and $\text{Si}(\text{OH})_4^-$ ($F = 2.25$, $p = 0.04$) were the environmental variables which accounted for most of the differences in epiphyte composition. The first CCA component axis positively selected the diatom group with nitrate, total phosphorus and salinity. The CCA showed a relationship between biotic variables, namely cyanobacteria, ciliates and nematodes and high ammonium concentration. Gastropods and rotifers have a negative axis II score correlating with enhanced values of salinity and silicate. The different relationships between epiflora and epifauna communities assessed by Spearman test are summarised in Table 14.

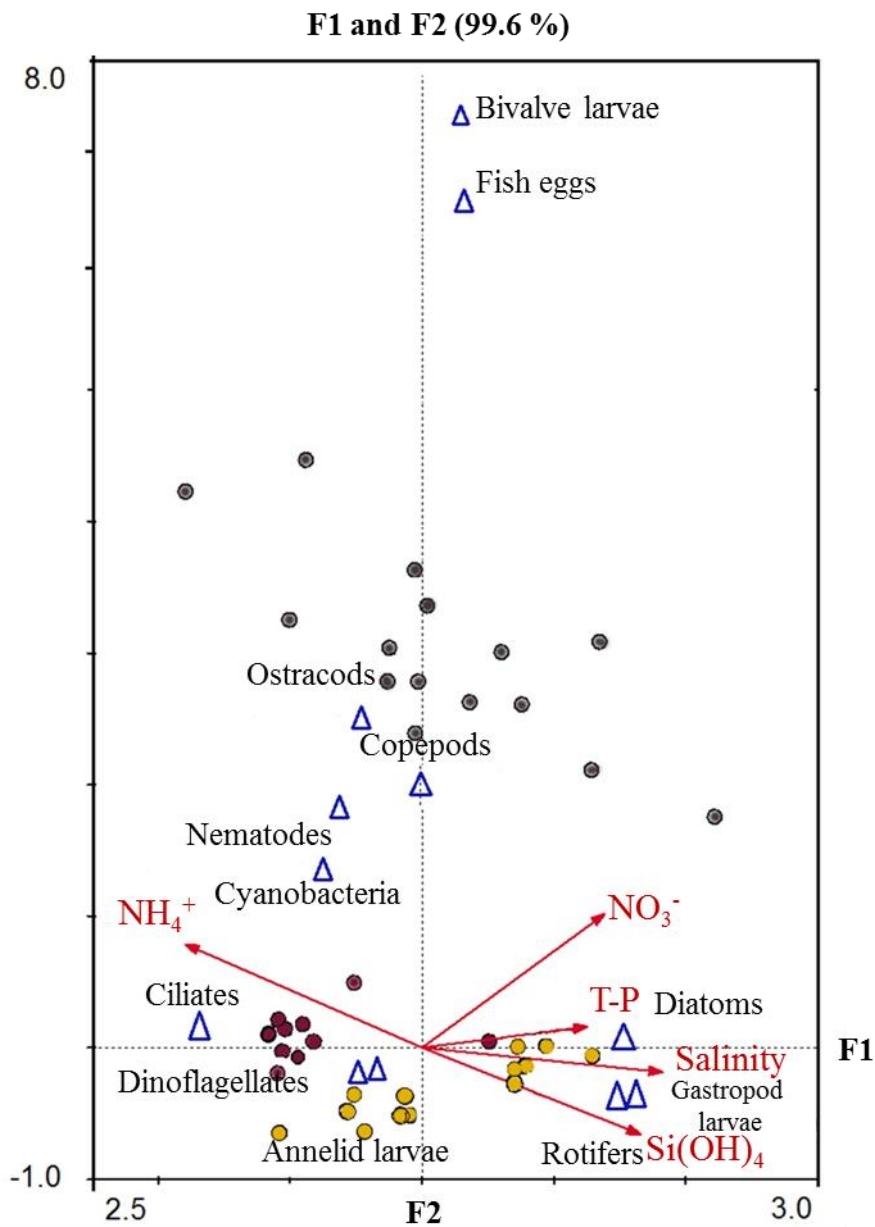


Figure 18. Triplot of Canonical Correspondence Analysis (CCA) showing the effects of environmental variables on abundance of epiphytic taxa for the three months and their order along the first and second axes. Red circle= June; yellow circle = July; black circle = August.

Tableau 14. Results of Spearman correlations between epiflora and epifauna communities on *Ruppia cirrhosa* leaves.

	Diatoms	Dinoflagellates	Cyanobacteria
Ciliates	r = 0.6; p = 0.017	r = 0.71; p = 0.003	r = 0.59; p = 0.021
Rotifers	r = 0.59; p = 0.02	-	-
Copepods	-	-	-
Ostracods	-	-	-
Nematodes	r = 0.63; p = 0.011	-	r = 0.84; p = 0
Annelid larvae	-	-	-
Bivalve larvae	-	-	-
Gastropod larvae	r = 0.66; p = 0.007	r = 0.55; p = 0.033	-

4. Discussion

During this study, *R. cirrhosa* coverage in the lagoon was maximal in early June to mid-July while the beginning of meadow decomposition was noted in late August. Indeed, in spring *R. cirrhosa* begins rapid vegetative growth, with budding rhizomes and branching stems, forming large, dense stands from late spring to early autumn (Calado and Duarte, 2000; Menéndez *et al.*, 2002). In late autumn vertical stems begin to lose contact with the vegetation mat near the bottom and gradually decompose before being washed ashore (Verhoeven, 1980b; Menéndez and Peñuelas, 1993).

No correlation was found between *Ruppia* density and the different abiotic variables monitored. Previous studies in shallow waters link the occurrence and abundance of macrophytes mainly to physical variables (Madsen, 1991; Scheiner, 1993; Carruthers *et al.*, 1999) such as temperature, salinity and light availability for *R. cirrhosa* (Verhoeven *et al.*, 1982; Calado and Durate, 2000; Mannino and Sara, 2006). In our case, though there were no significant differences in salinity as a function of coverage ($r = 0.49$; $p = 0.064$), the data indicated that *R. cirrhosa* growth was highest at water salinity above 40 and that minimal seagrass densities were always detected at S1 (mean \pm s.d. = 91.66 ± 35.35 shoots m^{-2}), influenced by freshwater inflows with salinity less than 40. The negative but non-significant correlation found between *R. cirrhosa* and NO_3^- ($r = -0.11$; $p = 0.689$), NO_2^- ($r = -0.34$; $p = 0.218$), NH_4^+ ($r = -0.13$; $p = 0.643$), PO_4^{3-} ($r = -0.14$; $p = 0.624$) and T-P ($r = -0.32$; $p = 0.245$) may be explained by the simultaneous massive presence of epiphytes on its leaves which can decrease *Ruppia* nutrient uptake. As indicated in the introduction, though no study of *Ruppia* epiphytes is available to allow comparison with our data, our assumptions may be supported by other findings, but in the case of *P. oceanica*, whose epiphytes (1) contribute 30

% of nitrogen uptake at shoot level (Lepoint *et al.*, 2007) and (2) may block active leaf uptake sites, the shade and extreme competition for nutrients, especially during the main seagrass growth period in summer, will result in reduced seagrass production at shoot level (Ruiz *et al.*, 2001; Cornelisen and Thomas, 2004). Another indicator of the implication of *Ruppia* beds and their epiphytes on nutrient uptake is the enhanced concentrations of DIN recorded in late August coinciding with the beginning of the disappearance of *Ruppia* beds and their epiphytic microalgae.

A total of 29 epiphytic species was detected in *R. cirrhosa* leaves which is much lower than those found on *P. oceanica* leaves in Tunisia (Mabrouk *et al.*, 2011; Ben Brahim *et al.*, 2013) and other localities (Borowitzka *et al.*, 2006, and references therein) and possibly explained by the characteristic feature of *Ruppia* which usually harbours an abundant but species-poor fauna (Verhoeven, 1980a). This feature was also highlighted by Mannino and Sara (2006) who reported low diversity of epiphytic macroalgae on *R. cirrhosa* leaves, epiphytic microalgae on *Ruppia*, which, as previously mentioned, had never before been studied.

Significant correlations were found between *R. cirrhosa* and most of the epiphytic communities recorded during this study (dinoflagellates, diatoms, cyanobacteria, ciliates, nematodes and gastropod larvae) (Figures 16 and 18). This may be explained by the fact that most parameters (leaf length, leaf surface and leaf area index) increased in summer (Pergent *et al.*, 1995; Gobert, 2002; Giovannetti *et al.*, 2008), thus augmenting substratum availability for the epiphytic community. For example, diatoms, which lead the epiphytic community, were the dominant group in terms of abundance and diversity as seagrass leaves and algal thalli are ideal habitats for benthic diatoms and other epiphytes (Penhale, 1977; Cummins *et al.*, 2004) representing a larger surface area for the colonisation and growth of this algae (Main and McIntire, 1974; Zieman *et al.*, 1989). In this study, diatoms were mainly pennate, with Fragilariales and Naviculales being the most dominant. Several studies have shown the prevalence of pennate diatoms, especially Naviculales on the leaves of *P. oceanica* (Mazzella *et al.*, 1994; De Stefano *et al.*, 2000; Mabrouk *et al.*, 2011) (since this type of data is lacking for *Ruppia* epiphytes), while others have reported higher abundance of centric over pennate diatoms (Xivanand *et al.*, 2006; Raveesha *et al.*, 2009; Ben brahim *et al.*, 2013). Among the 11 diatoms identified, 3 taxa, namely *T. nitzshioides*, *C. closterium* and *L. danicus*, were reported as harmful as they can form extensive blooms (Anderson *et al.*, 2001; Vila and Maso, 2005; Balkis, 2009).

Dinoflagellates were the second most abundant group in this study, but were represented by 4 species only among which *P. lima* and *P. concavum* are typically benthic. Prorocentrales, the

predominant dinoflagellate order, dominated in the most stagnant areas such as stations 4 and 5, in accordance with other findings indicating that this group, when abundant far from wave action and water motion, is highly abundant and is found mostly in low- or moderately low-energy environments (Foden *et al.*, 2005). On the other hand, *P. lima* may attach itself by a coating of mucus (Tindall and Morton, 1998; Vila *et al.*, 2001a) which may explain its presence, though very limited at S2 (El Boughaz Channel) characterised by high hydrodynamics. *P. lima* overwhelms all epiphytic species and was the main taxon contributing to temporal dissimilarity over the three studied months with maximal concentrations rising to 6×10^5 cells 100 g^{-1} FW recorded in early June at S5. This abundance was much higher than that found, for example, on *P. oceanica* leaves in Tunisian coastal ecosystems (Turki, 2005; Mabrouk *et al.*, 2011; Ben Brahim *et al.*, 2013) and in other areas (Foden *et al.*, 2005). Do *Ruppia* leaves offer the best biotope for epiphytic *P. lima*? And how? In comparison to *P. oceanica* leaves? These questions must be further addressed through in vitro experiments.

Many papers have reported the harmful effects of the four dinoflagellates recorded in this study. *Prorocentrum* species are mainly associated with okadaic acid and the production of analogues (Foden *et al.*, 2005; Nascimento *et al.*, 2005; Paz *et al.*, 2007). Toxic effects have been reported for *P. lima* (Marr *et al.*, 1992), *P. concavum* (Hu *et al.*, 1993) and *D. accuminata* (Kat, 1985).

Concerning cyanobacteria, their presence on *Ruppia* leaves was ubiquitous during the three months under study and mainly comprised of Oscillatoriales. Cyanobacteria density was close to that found for *P. oceanica* on Kerkennah Island (Ben Brahim *et al.*, 2013), but much higher than that found along the Mahdia coast (Mabrouk *et al.*, 2011). It is noteworthy that several species of the three genera of *Oscillatoria*, *Lyngbya* and *Anabaena* found in this study are known for their production of toxins such as cytotoxin, lyngbyatoxin and anatoxin respectively (Hallegraeff *et al.*, 1995; Anderson *et al.*, 2001).

Based on CCA analyses, epiphytic microalgae were positioned near total phosphorus and nitrate for diatoms and near ammonium for cyanobacteria, whereas harmful dinoflagellates did not seem to correlate with any nutrient. While a link between the appearance of planktonic harmful algae and anthropogenic nutrient supply has been proposed in many studies (Heisler *et al.*, 2008; Conley *et al.*, 2009; Feki *et al.*, 2013), this absence of correlation suggests that epiphytic harmful dinoflagellate proliferation seems to be more in relation to the niche advantages offered by *Ruppia* leaves and the plant's seasonal growth cycle. Indeed, both the temporal and spatial distributions of epiphytic dinoflagellate abundance follow roughly those

of *R. cirrhosa* shoot abundance ($r = 0.9$; $p < 0.01$; Figure 16), with maximal abundances found in June and July, especially at stations 2, 3, 4 and 5, associated with massive beds of *Ruppia*, while minimal dinoflagellate abundances are found in late August at the majority of stations at the start of seagrass mat decomposition. This decomposition is confirmed by the high number of valves of dominant *P. lima* indicative of the mortality of this species following *Ruppia* bed decomposition (Figure 17).

CCA analysis illustrates a good association between high ammonium concentrations and epifauna, namely ciliates, nematodes and ostracods. Indeed, Nematodes are known for nutrient cycling through their burrowing and feeding activities (Constable, 1999) and their proliferation as an opportunistic species in eutrophic environments (Karlson *et al.*, 2002; Kemp *et al.*, 2005; Losi *et al.*, 2012). Ciliates as well are known for their high sensitivity to anthropogenic inputs making them excellent bioindicators of water quality (Aleya *et al.*, 1992; Irizuki *et al.*, 2011; Moreno *et al.*, 2011). They were the only group to correlate with all microalgae groups thus suggesting potential feeding on algae (Gismervik *et al.*, 1996; Loder *et al.*, 2011), attaining even optimal growth rates when fed with some HAB species (Kamiyama, 1997; Jeong *et al.*, 1999). Since the GML is currently subject to HABs, we postulate that the ciliate potential to graze on HAB species may at least serve to lessen their magnitude.

Copepods, ostracods and bivalve larvae did not follow the *Ruppia* coverage and were enormously present in late August at the moment of *Ruppia* decomposition. This suggests that they contribute to seagrass decomposition by feeding preferentially on leaf tips where the epiphyte biomass reaches its maximum (Alcoverro *et al.*, 1997; Ruiz and Romero, 2003; Peterson *et al.*, 2007).

The abundant presence of fish eggs and larvae (12.15 %) revealed the key role of *Ruppia* beds as a nursery habitat for epiphytic communities as reported from other seagrass meadows (Heck *et al.*, 2003). The most abundant quantity of fish eggs (2000 cells 100 g⁻¹ FW) was detected at S2 opposite El Boughaz Channel and may be attributed to migration from the Mediterranean Sea. Indeed, GML contains a variety of fish species with different behaviour patterns (sedentary, migratory, benthic, pelagic) and provides habitats for shelter, food, reproduction and growth (Dumay *et al.*, 2004; Kraiem *et al.*, 2009).

Larvae of gastropods, bivalves, annelids and copepods were mainly recorded in stagnant areas of the lagoon at S3 and S4, in accordance with their preference for reduced water velocity (Donelle *et al.*, 1999; Bologna and Heck, 2000). Absence of epifauna from some stations may be attributed to predation known to have a strong effect on the spatial pattern of the epiphytic

community (Sarnelle *et al.*, 1993; Bostrom *et al.*, 2006). In GML, which shelters many birds, *Ruppia* seeds and habitat-associated invertebrates provide them with a source of food, as reported earlier (Verhoeven, 1980a). The birds, in return, may contribute to dispersal of *Ruppia* seeds at short distances.

Do *Ruppia cirrhosa* and its epiphytic organisms function as early warning indicators of the health status of GML waters?

As indicators of the health status of aquatic ecosystems, seagrass and their epiphytes appear to be sounding the alarm as to the critical situation of Ghar El Melh lagoon. Indeed (1) the overwhelming quantity of harmful microalgae detected on *R. cirrhosa* leaves reflect the eutrophication of the lagoon, also confirmed by the frequently reported fish mortality, and (2) harmful epiphytic microalgae, heavily present on *R. cirrhosa* may contribute to exacerbate HAB. Unfortunately, ecological information regarding toxicity of epiphytic microalgae is limited (Gilbert *et al.*, 2012), possibly due to the lack of sampling of these communities (Marr *et al.*, 1992; Levasseur *et al.*, 2003). The large development of epiphytes may also have negative effects on the host plant: they reduce the light availability at the leaf surface, creating a physical barrier to light absorption (Sand-Jensen, 1977; Dalla Via *et al.*, 1998) and thus decrease plant uptake of carbon and other nutrients (Orth and van Montfrans, 1984). (3) Early decomposition of *Ruppia* beds in late August rather than late autumn, and dominance of benthic algae at S1 during the three sampled months (personal observation) were both noted. It is well known that under eutrophication conditions, species composition shifts from the dominance of angiosperms to blooms of opportunistic macroalgae (Cloern, 2001; Orfanidis *et al.*, 2008; Viaroli *et al.*, 2008), due to their more efficient nutrient assimilation (Thompson and Valiela, 1999). Rasmussen *et al.* (2009) have also observed the diminished *Ruppia* coverage in GML waters and suggested that the nutrient load be reduced by 25 % in order to bring the algae biomass down to an acceptable level. Globally, seagrass are in serious decline with their loss accelerating from about 1 % per year before 1940 to 7 % per year at present (Waycott *et al.*, 2009).

5. Conclusion

R. cirrhosa may play two contrasting key roles in GML waters providing a useful reservoir for biodiversity offering the substrata, nursery, food and canopy to many microalgae, protozoans and metazoans, but is also a shelter for many harmful species that may have negative effects on the plant itself and on the entire aquatic food web. *R. cirrhosa* and its epiphytes appear to be good bioindicators of the deterioration of the Ghar El Melh lagoon, a

RAMSAR site which, unfortunately is not getting the attention it deserves in comparison to other such sites that benefit from specific protective measures (e.g. Camargue Natural Reserve (France), Ebro Natural Park (Spain), Ichkeul National Park (Tunisia)) (Carasp, 1997; Pasqualini *et al.*, 2006). Given the increased eutrophication in the GML and the decline in *Ruppia* coverage in its waters along with the loss of the lagoon's *Cymodocea* and *Zoostera* meadows, one wonders whether or not the *R. cirrhosa* beds will suffer the same fate as the aforementioned meadows. Will the beautiful and spectacular development of *Ruppia* at this site will be lost?

Acknowledgments This study was conducted by Amel DHIB as a part of her PhD research (co-directed at the University of Franche-Comté, CNRS 6249, Besançon, France, and at the Institut National des Sciences et Technologie de la Mer, Tunisia).

Chapitre IV. Distribution saisonnière du phytoplancton et des kystes des dinoflagellés dans la lagune de Ghar El Melh

Ce chapitre a été principalement consacré à l'étude du cycle annuel du phytoplancton de la lagune de Ghar El Melh et la relation des microalgues épiphytes dans la colonisation de la colonne d'eau (Article 2 publié).

Compte tenu de l'importance des dinoflagellés dans la lagune de Ghar El Melh dont plusieurs espèces sont responsables des efflorescences nuisibles, une deuxième partie de ce chapitre a été consacrée à l'étude de la distribution des kystes des dinoflagellés (Projet d'article) afin d'associer leur présence à l'initiation des proliférations des populations phytoplanctoniques des espèces dominantes.

La troisième partie de ce chapitre a été développée par une étude réalisée sur l'ultraphytoplancton et les hétérotrophes procaryotes (article soumis), afin de mettre en évidence l'importance de cette classe de taille du plancton d'une part et sa relation avec le microplancton de la lagune de Ghar El Melh d'autre part.

1. Distribution spatio-temporelle des microalgues planctoniques et épiphytes dans la lagune de Ghar El Melh

Article 2. Harmful planktonic and epiphytic microalgae in a Mediterranean Lagoon: The contribution of the macrophyte *Ruppia cirrhosa* to microalgae dissemination

Article publié dans le journal 'Harmful Algae' (IF = 3.87)

Harmful Algae 45 (2015) 1–13



Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal



Harmful planktonic and epiphytic microalgae in a Mediterranean Lagoon: The contribution of the macrophyte *Ruppia cirrhosa* to microalgae dissemination



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Résumé

Il s'agit de l'étude des microalgues planctoniques et épiphytes ainsi que des paramètres physico-chimiques dans la lagune de Ghar el Melh au niveau de 5 stations. Le prélèvement du phytoplancton a été effectué pendant une année (de janvier 2011 à janvier 2012) et le prélèvement des épiphytes a été réalisé durant la saison estivale de l'année 2011, parallèlement avec les proliférations massives du macrophyte « *Ruppia cirrhosa* » dans le milieu.

La lagune a varié d'euryhaline à hyperhaline (26.6-51.2) et peut être considérée comme hypertrophique avec des concentrations exceptionnelles du phosphore total (0.74-6.93 $\mu\text{mol. l}^{-1}$) et d'azote inorganique dissous (0.83-22.38 $\mu\text{mol. l}^{-1}$).

Pour le phytoplancton, On a identifié 61 espèces qui se répartissent sur 5 groupes : dinoflagellés, diatomées, cyanobactéries, chlorophycées et euglénophycées. Le phytoplancton est représenté à plus de 28 % par le dinoflagellé nuisible « *Prorocentrum micans* ». On a enregistré (1) des proliférations microalgales maximales dans les parties internes de la lagune, (2) une association printemps-hiver de la majorité des espèces, y compris l'espèce dominante *Prorocentrum micans*, et (3) une prolifération maximale des diatomées au mois d'été. L'Analyse de Redondance a montré l'implication de la température et des nutriments dans la distribution spatio-temporelle du phytoplancton.

Concernant les épiphytes, on a identifié 20 espèces qui se répartissent sur 3 groupes : dinoflagellés, diatomées, cyanobactéries. Les microalgues épiphytes sont représentées à plus de 45 % par le dinoflagellé toxique « *Prorocentrum lima* ». La distribution des épiphytes suit largement le développement du macrophyte *R. cirrhosa* ($r = 0.89$; $p = 0$).

Pour les deux peuplements, planctonique et épiphyte, il y a une dominance d'espèces nuisibles et/ou toxiques avec un pourcentage de plus que 70 %.

On note le rôle de *R. cirrhosa* dans la dissémination des espèces épiphytes nuisibles, notamment *P. lima* et *Thalassionema nitzschioides*, et des diatomées dans la colonne d'eau de la lagune. En effet, des corrélations hautement significatives ont été trouvées entre ces espèces dans la colonne d'eau et sur les feuilles du *R. cirrhosa*.

Abstract

The temporal and spatial distribution of planktonic and epiphytic microalgae coupled with environmental factors was studied at five stations in Ghar El Melh Lagoon (Tunisia). Phytoplankton was monitored for a year and epiphytic microalgae were sampled during phases of proliferation of the seagrass *Ruppia cirrhosa* in summer 2011. Microalgae assemblages were dominated by dinoflagellates and diatoms both in the water column and on *Ruppia* leaves. A significant difference was found in phytoplankton distribution among stations and seasons with (i) maximum proliferation in the inner parts of the lagoon, (ii) spring-winter association of the majority of species including the dominant harmful species *Prorocentrum micans* and (iii) summer diatom proliferation. Redundancy analysis indicates that temperature and nutrients have a substantial effect on distribution. Epiphyte distribution roughly follows the development cycle of *R. cirrhosa*. A list of harmful planktonic and epiphytic microalgae found in the water column and *Ruppia* leaves was compiled. This study highlights the role of *Ruppia* in the dissemination of harmful species in the lagoon's water column.

Keywords: Microalgae, environmental factors, harmful species, *Ruppia cirrhosa*

1.1. Introduction

The harmful algae blooms (HAB) with which we are most familiar tend to be planktonic and every coastal nation is affected by their increasing frequency (Hallegraeff, 1993). Some of this apparent increase may be attributed to the recent proliferation of benthic microalgae (Hallegraeff, 1993; Landsberg, 2002; James *et al.*, 2003) which have spread during the last decade from tropical–subtropical waters to temperate zones such as the Mediterranean Sea (Tognetto *et al.*, 1995; Vila *et al.*, 2001a, b; Ismael and Halim, 2006; Monti *et al.*, 2007; Aligizaki *et al.*, 2008; Mangialajo *et al.*, 2008). While a link between the appearance of harmful algae and physical, chemical and biological factors has tentatively been proposed (Hallegraeff, 1993; Anderson *et al.*, 2002; Heisler *et al.*, 2008; Conley *et al.*, 2009), gaps in our general knowledge concerning harmful benthic microalgae remain (Glibert *et al.*, 2012), possibly due to the lack of sampling of these communities (Marr *et al.*, 1992; Levasseur *et al.*, 2003). According to Fraga *et al.* (2012), no clear-cut limit is observed differentiating benthic from planktonic dinoflagellates; there is a continuum from planktonic to benthic behavior, which is also seen in many of their associated characteristics (eg. morphology, habitat, nutrient uptake...). These circumstances of harmful microalgae events can be encountered in the semi-enclosed Ghar El Melh Lagoon (GML) (northeastern Tunisia) where *Ruppia cirrhosa*, a macrophyte bioindicator of water deterioration, proliferates spectacularly in summer with its leaves playing a role as both a shelter for and putative supplier of harmful benthic algae in the water column. HABs in GML remain poorly reported with only occasional reference to them in reports on fish mortality: one kill took place in 1995-1996, caused mainly by *Alexandrium minutum* (Romdhane *et al.*, 1998) and another in 2007 due to the dinoflagellate *Kryptoperidinium foliaceum* (Turki *et al.*, 2007).

The aim of this study was to explore the continuum from planktonic (over a year) to benthic behavior (summer only) of HAB species at 5 stations in GML along with physical and chemical variables.

1.2. Material and methods

Study area

Ghar El Melh Lagoon (GML) is a RAMSAR site (RAMSAR, 2007) located in the southern Mediterranean Sea in the northern part of the Gulf of Tunis, Tunisia (37°06–37°10 N and 10°08'–10°15 E). It has a surface of 35 km² and contains three wetlands: the main lagoon and two smaller ones, Il Ouafi and Sidi Ali Il Meki (Figure 19).

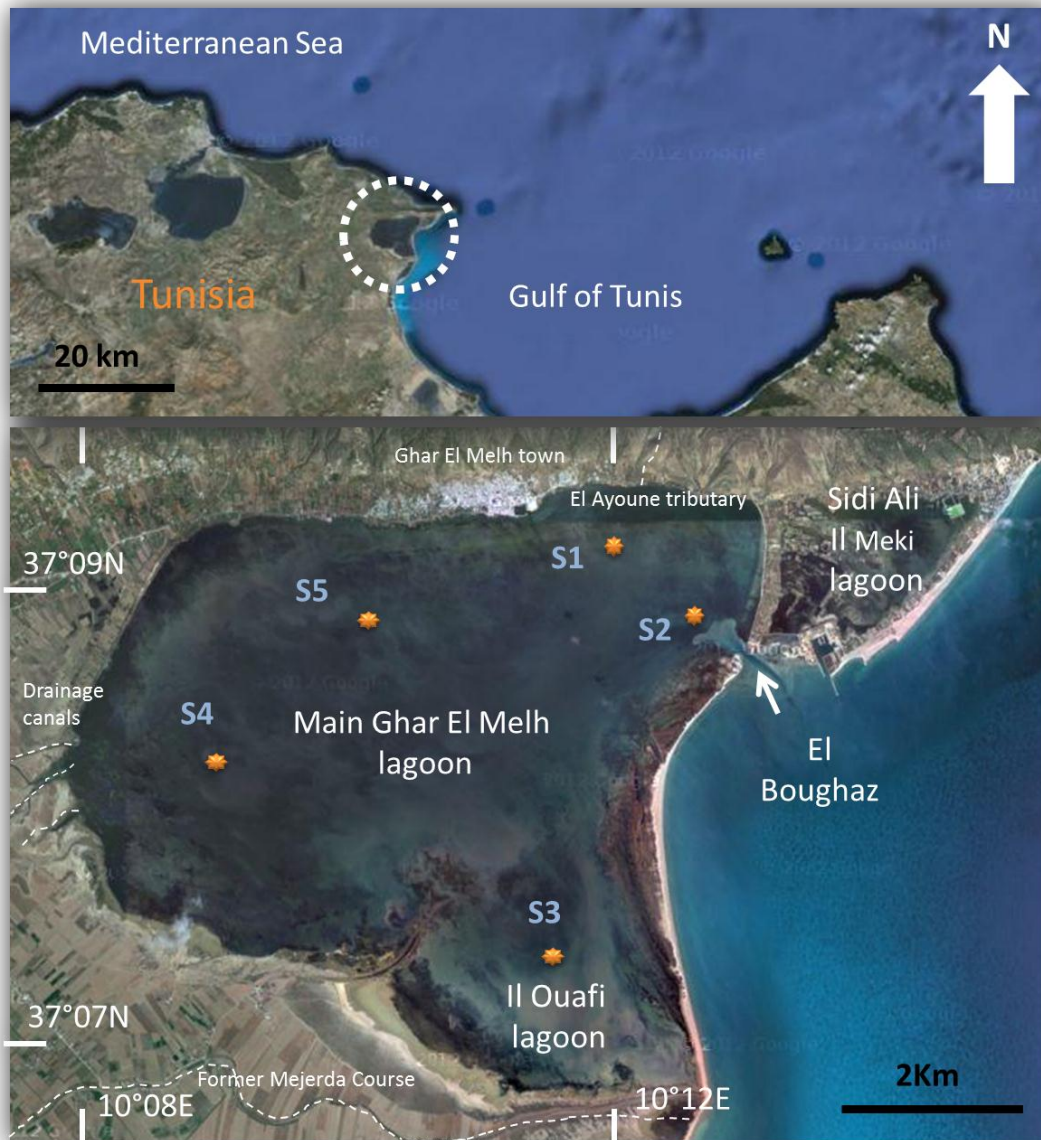


Figure 19. Geographic situation of Ghar El Melh Lagoon and location of the sampling stations from S1 to S5.

The main lagoon is permanently connected with Il Ouafi Lagoon, but is isolated from Sidi Ali Il Meki by embankments. The average depth is 0.8 m with a maximum of 2 m at the channel entrance (Chakroun, 2004). Connected to the Mediterranean Sea by a narrow channel called “El Boughaz”, GML is fed by the Utica floodplain (via drainage canals at the southwest of the main lagoon), several springs (located to the northeast of the main lagoon), seasonal run-off from the Djebel Nadhour and Demina hills in the north and sometimes by the Mejerda

River with the occurrence of occasional floods that may create a connection between the river and the lagoon (Ayache *et al.*, 2009). Thompson *et al.* (2009) have demonstrated that, in summer, the amount of fresh water provided to the lagoon is relatively small, bodies of fresh water at that time of year being stagnant and nearly non-existent. Under these conditions, and due to high evaporation rates and small marine inflows through the narrow connection to the sea, the lagoon's salinity increases substantially (Ktari-Chakroun and Romdhane, 1985; Beyrem *et al.*, 2002). Ultimately, *R. cirrhosa* spread widely, with its extremely high resistance to variations in environmental conditions and its ability to survive and exhibit healthy growth in hypersaline aquatic environments (Verhoeven, 1979, Pergent *et al.*, 2006) such as salt pans or in certain marginal secondary shallow ecosystems (Mannino and Sarra, 2006).

The site fulfills the Ramsar criteria as a wetland of international importance, representative of lagoons within the Southern Mediterranean and as a habitat for migrating fish and birds (Ayache *et al.*, 2009). Birds, including grebes, herons, sterns, flamingos and gulls, are frequent in the shallow southeastern part of GML. Benthic vegetation currently consists of *R. cirrhosa* (Shili *et al.*, 2002). The small fishery in GML produces eel, mullet, sea-bass and sole (Kraïem *et al.*, 2009). A marine hatchery, for fish, alevins and post-larval prawns, was installed at GML in 1973, but has now been abandoned for several years. Most of the lagoon area is exploited for fishing except for an area about 1 km from the western and northern shores where poor environmental conditions prevail (Kraïem *et al.*, 2009).

A consequence of rising water temperatures, salinity has increased as eutrophic conditions have been continuously reported in GML (Table 15). Some harmful events have also been reported, especially coinciding with fish mortality (Table 15), but no study has been undertaken with respect to epiphytic microalgae.

Like some other lagoons of northern Africa, such as Merja Zerga (Morocco) and Lake Manzala (Egypt), GML shares common features such as shallowness, limited sea connection and low freshwater inflow, but differs radically where physical and biological characteristics are concerned (Ahmed *et al.*, 2009), which confirms how important it is to study lagoons with such complex ecosystems.

Tableau 15. Summary data of physico-chemical characteristics and harmful microalgae species detected in GML during previous studies. Cell concentrations are given for important proliferations. (-): no data; **a, d**: summer investigation after fish mortality; **b, c**: annual surveys; **e**: autumn-winter study.

	Romdhane <i>et al.</i> (1998) ^a	SCET-ERI (2000) ^b	Ramdani <i>et al.</i> (2004) ^c	Turki <i>et al.</i> (2007) ^d	Dhib <i>et al.</i> (2013a) ^e
Temperature range	-	13 – 30	12.5 – 32.7	27 – 29.5	10 – 28
Salinity range	-	36 – 51	26.5 – 46.8	38.9 – 44	30 – 43
Eutrophic conditions	-	High total phosphorus High total nitrogen	High total phosphorus High total nitrogen	-	-
Harmful microalgae species	<ul style="list-style-type: none"> • <i>Alexandrium</i> sp. ($\approx 1.5 \cdot 10^6$ cells l^{-1}) 	<ul style="list-style-type: none"> • <i>Akashiwo sanguinea</i> • <i>Gonyaulax polyedra</i> • <i>Prorocentrum micans</i>, <i>P. lima</i>, <i>P. triestinum</i>, <i>P. minimum</i> • <i>Scrippsiella trochoidea</i> • <i>Cylindrotheca closterium</i> • <i>Pseudo-nitzschia</i> spp. 	<ul style="list-style-type: none"> • <i>Alexandrium</i> spp. • <i>Tripos furca</i> • <i>Coolia monotis</i> • <i>Dinophysis</i> spp. • <i>Gonyaulax spinifera</i> • <i>Karenia brevis</i> • <i>Prorocentrum</i> spp. (eg. <i>P.lima</i>, <i>P. micans</i>) • <i>Chaetoceros decipiens</i> • <i>Leptocylindrus danicus</i> • <i>Pseudo-nitzschia</i> spp. • <i>Thalassionema nitzschioides</i> 	<ul style="list-style-type: none"> • <i>Kryptoperidinium foliaceum</i> ($1.83 \cdot 10^5$ cells l^{-1}) • <i>Prorocentrum micans</i> • <i>Anabaena</i> sp. 	<ul style="list-style-type: none"> • <i>Akashiwo sanguinea</i> • <i>Tripos furca</i> • <i>Tripos fusus</i> • <i>Dinophysis</i> spp. • <i>Gonyaulax polyedra</i> • <i>Gonyaulax spinifera</i> • <i>Prorocentrum micans</i> ($1.2 \cdot 10^5$ cells l^{-1}), <i>P.lima</i>, <i>P. concavum</i>, <i>P. gracile</i>, <i>P.triestinum</i>, <i>P.minimum</i> • <i>Scrippsiella trochoidea</i>

Sampling

Five stations (S1–S5) were selected for investigation of the different environmental conditions found within GML (Figure 19). S1 is located in the northeastern part of the lagoon, a shallow area influenced by a supply of fresh water from ‘El Ayoune’ which reaches the lagoon as groundwater. S2 faces El Boughaz Channel which is the lagoon’s only connection to the sea. As this is the deepest part of GML, it is influenced by strong hydrodynamics despite continuous silting of the channel. S3, located in the southeast of the lagoon in the middle of Il Ouafi lagoon, is a semi-closed zone and one of the most stagnant areas. S4, situated in the south of GML near the Utica floodplain, is affected by heavy industrial discharge (≈ 43 industrial units) and is drained by streams mainly in winter, and sometimes by the Mejerda River when exceptional floods create a connection between the river and the lagoon. S5 is located in the northern part of the lagoon, along its rural shores which constitute a source of runoff from intensively cultivated land and also of raw sewage from human habitations, both of which flow directly into the lagoon.

Samples for phytoplankton identification and enumeration were collected using a polyvinylchloride (PVC) tube twice a month, from January 2011 to January 2012 at the five sampling sites ($N = 110$). Additionally, three replicates of epiphytic microalgae of *R. cirrhosa* were collected ($N = 45$) at each station during the summer (early June, mid-July and late August). To detach the microphytobenthic organisms, 100 to 300 g of leaf samples were hand-collected from surface waters and placed in plastic bottles. In the laboratory the plants were removed from the bottles and weighed with precision (100 g); epiphytes were separated by vigorous shaking and washing, then mixed with 200 ml of filtered seawater (through a 0.2 μm pore-size membrane). The solution thus obtained was strained through 250 μm mesh sieves to remove large particles and then fixed with formaldehyde (final concentration 2 %). Concomitantly with epiphyte sampling, the shoot density of *R. cirrhosa* was measured in situ using a quadrat (40 cm x 40 cm) that was randomly placed over the shoots before carefully collecting them. At each station, three replicate quadrats were sampled. Meadow density (shoot m^{-2}) was calculated as the number of shoots bearing leaves in each quadrat (Ott, 1980). Environmental variables were measured in the field concomitantly with microalgae sampling. A WTW multiparametric probe was used to record water temperature ($^{\circ}\text{C}$) and salinity. Transparency was measured using a Secchi disk. Water samples were collected in 1000-ml polypropylene bottles at 30–50 cm depth. In the laboratory, nutrients (ammonium, nitrite, nitrate, phosphate, silicate) were analyzed with a BRAN and LUEBBE AutoAnalyzer 3 and

concentrations were determined via colorimetry using a UV–visible (JENWAY 6705) spectrophotometer (APHA, 1992).

Microalgae enumeration

Sub-samples (25 ml) of phytoplankton communities were counted under an inverted microscope after fixation with Lugol's iodine solution (4 %) and settling for 24 hours using the Utermöhl method (1958). Sub-samples (1 ml) of epiphytic microalgae communities (Turki, 2005) were also enumerated according to the Utermöhl method by means of an inverted microscope. For epiphytes, the entire bottom of the chamber was numerated and all cells found were counted.

Identification of algal taxa was achieved according to various keys by Dodge (1982, 1985), Sournia (1986), Balech (1988), Tomas *et al.* (1996), Nezan and Piclet (1996). Phytoplankton density was expressed as cells l^{-1} . Epiphyte abundance on *R. cirrhosa* leaves was calculated as a number of cells 100 g^{-1} FW (Fresh Weight).

The mean biovolume of each microalga was estimated from length and width measurements of more than 20 individuals for the abundant taxa, and converted to carbon biomass with the conversion factor proposed by Menden-Deuer and Lessard (2000).

Statistical analysis

We used one-way variance analysis (ANOVA) to assess parameter variability among stations and seasons. Potential relationships between variables were tested by Spearman's correlation coefficient. Redundancy analysis (RDA) was performed to define the structuring effects of sampling sites and seasons, along with physical (temperature, salinity, pH and transparency) and chemical (NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-} and $\text{Si}(\text{OH})_4$) parameters on phytoplankton abundance. To prevent any disproportionate influence of rare species in the subsequent analysis, only species having an abundance $> 0.5\%$ of total abundance were considered. Phytoplankton abundance was normalized prior to analysis. Statistical analysis was produced by R 2.15.0 (R Core Team development, 2012) with “Vegan” (Oksanen *et al.*, 2011) and “Pgirmess” (Giraudoux, 2012) packages. The data recorded for epiphytic algae were subjected to (i) permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2005) to analyze the differences in community composition between stations and months, (ii) Similarity percentage analysis (SIMPER) was used to identify the contribution of individual taxa with the pattern of similarity and dissimilarity between habitats in each month., and (iii) Canonical correspondence analysis (CCA) coupled with the Envfit test (fits an environmental vector or factor onto an ordination) (R Core Team development, 2012) (Oksanen *et al.*, 2011)

were applied to assess the variation of epiphyte abundance according to physico-chemical variables.

1.3. Results

Environmental conditions

Water temperature varied significantly from season to season (ANOVA, $F = 101.27$, $p < 0.001$), but not between the sampling sites (ANOVA, $F = 0.08$, $p = 0.98$). The highest temperature ($27.6\text{ }^{\circ}\text{C}$) was recorded in June 2011 at station S5 and the lowest ($11.1\text{ }^{\circ}\text{C}$) in January 2012 at S4. Mean water temperature increased in spring and reached its maximum in summer. Salinity varied significantly according to season (ANOVA, $F = 43.1$, $p < 0.001$) and station (ANOVA, $F = 6.07$, $p < 0.001$), varying from 51.2 in July at S4 to 26.6 in December at S3. Salinity peaked in summer due to the combined effect of high temperature (inducing water evaporation) and low inflow of freshwater to the lagoon. The pH varied significantly according to season (ANOVA, $F = 3.8$, $p = 0.01$) and station (ANOVA, $F = 8.93$, $p < 0.001$), ranging from 7.2 in winter at S1 to 9.11 in summer at S5 where *R. cirrhosa* was massively present. Water transparency did not differ significantly between seasons (ANOVA, $F = 1.47$, $p = 0.22$), but experienced marked variations among the stations (ANOVA, $F = 5.51$, $p < 0.001$). It was high in spring ($Z_s = 1.9\text{ m}$) and low in autumn, especially at S5 ($Z_s = 43\text{ cm}$), the turbid area of the lagoon affected by agricultural and urban discharge, and at S2 ($Z_s = 60\text{ cm}$) opposite El Boughaz Channel, the only access for small fishing boats.

Eutrophic conditions were detected in the field with outstanding concentrations of total phosphorus ($0.74\text{--}6.93\text{ }\mu\text{mol l}^{-1}$) and dissolved inorganic nitrogen ($0.83\text{--}22.38\text{ }\mu\text{mol l}^{-1}$) (Table 16). Nutrients varied significantly among seasons (ANOVA, $p < 0.001$ for each nutrient), but not among sampling stations. Most nutrients decreased in spring and showed a remarkable increase in autumn and winter due to dilution by floods.

Tableau 16. Maximum nutrient concentrations registered at the five stations during the four seasons. Italics and bold values refer respectively to poor and bad water quality according to IFREMER (2012).

Samples	S1	S2	S3	S4	S5	Winter	Spring	Summer	Autumn
Nutriments									
NO ₂ ⁻ (µmol l ⁻¹)	0.624	0.524	0.680	0.734	<i>0.929</i>	<i>0.904</i>	0.68	<i>0.929</i>	0.566
NO ₃ ⁻ (µmol l ⁻¹)	6.098	6.287	5.812	8.656	21.998	21.998	3.504	3.767	6.287
NH ₄ ⁺ (µmol l ⁻¹)	9.498	11.533	9.386	9.559	9.959	11.533	9.559	3.134	6.374
PO ₄ ³⁻ (µmol l ⁻¹)	0.667	0.695	0.521	0.742	0.632	0.742	0.487	0.695	0.632
T-N (µmol l ⁻¹)	24.518	22.697	24.809	24.518	28.734	24.518	22.474	28.733	24.618
T-P (µmol l ⁻¹)	5.287	4.718	4.115	5.847	6.928	5.847	4.718	4.543	6.928
DIN	<i>12.098</i>	<i>14.202</i>	<i>11.223</i>	<i>17.831</i>	22.388	22.388	<i>11.637</i>	5.848	<i>11.272</i>
DIN/DIP	18.137	20.434	21.541	24.031	35.424	15.683	12.008	16.218	18.412

Microalgae dynamics in the plankton

In the water column, 61 microalgae species were observed represented mainly by dinoflagellates (54.88 % of total abundance), then diatoms (38.86 % of total abundance) and finally by cyanobacteria (5.94 % of total abundance) (Table 17). Some of these species are known as epiphytic such as *Coolia monotis* which was exclusively encountered in the water column of GML.

Several species have been cited in the literature as noxious and/or as potential toxin producers (70.27 % of total abundance) (Table18).

Only 21 microalgae species (Table 17) made a considerable contribution to the total phytoplankton abundance (93.64 %) among which 11 harmful species. The prominent species in GML waters was the harmful *Prorocentrum micans* (29 % of total phytoplankton abundance) reaching the highest concentrations in spring (3.62 10⁵ cells l⁻¹).

Tableau 17. List of dominant microalgae species found in the water column of Ghar El Melh Lagoon (- not detected; + 40-1000 cells l⁻¹; *** > 1000 cells l⁻¹). Species marked with an asterisk are known as benthic taxa.

Phytoplankton taxa	Mean length (µm)	Mean biovolume (x10 ³ µm ³)	S1	S2	S3	S4	S5	Winter	Spring	Summer	Autumn
Dinophyceae											
<i>Akashiwo sanguinea</i>	54	58.37	***	+	***	***	***	***	***	+	***
<i>Dinophysis sacculus</i>	37.9	5.84	***	+	***	***	***	***	+	+	+
<i>Prorocentrum lima</i> *	39.81	19.013	+	+	+	***	***	+	***	***	+
<i>Prorocentrum micans</i>	39.75	16.01	***	***	***	***	***	***	***	***	***
<i>Prorocentrum minimum</i>	16.65	1.16	+	-	+	***	+	+	-	-	***
<i>Prorocentrum triestinum</i>	25.29	1.98	***	+	+	-	+	***	+	+	-
<i>Protoperidinium diabolium</i>	44.4	10.08	+	+	***	***	***	+	***	+	+
<i>Scrippsiella cf. trochoidea</i>	18.75	3.23	***	***	***	***	***	***	***	***	***
Bacillariophyceae											
<i>Chaetoceros decipiens</i>	70.2	27.96	***	+	+	+	+	+	***	***	+
<i>Climacosphenia sp.</i> *	208.5	14.71	+	+	***	***	***	***	+	***	***
<i>Cylindrotheca closterium</i>	286.5	4.17	+	+	***	***	***	***	***	***	***
<i>Gyrosigma sp.</i> *	188.4	39.58	***	+	+	***	***	+	+	***	***
<i>Licmophora gracilis</i> *	47.85	1.19	+	+	+	***	***	***	***	+	***
<i>Navicula duerrenbergiana</i> *	39	1.95	***	+	+	***	***	+	-	***	***
<i>Navicula phyllepta</i> *	36.6	2.63	+	-	+	***	***	+	+	***	+
<i>Nitzschia linearis</i> *	121.2	71.95	***	+	***	***	***	+	***	***	***
<i>Rhizosolenia sp.</i>	350	1923.25	+	***	+	+	+	***	***	-	-
<i>Striatella unipunctata</i> *	85.05	253.75	+	+	***	***	***	+	+	***	+
<i>Thalassionema nitzschioides</i>	77.25	29.51	+	+	***	***	***	***	+	***	+
Cyanobacteria											
<i>Oscillatoria salina</i>	100	141.3	+	+	+	+	***	+	***	+	+
<i>Oscillatoria sp.</i>	100	32.49	+	+	***	+	***	***	+	***	+

Tableau 18. List of harmful planktonic and epiphytic microalgae detected in the water column and on *Ruppia* leaves during the study period. DIAT: diatoms; DINO: dinoflagellates; CYANO: cyanobacteria; Max: maximal concentrations; DSP: diarrhetic shellfish poisoning; PSP: paralytic shellfish poisoning; ASP: amnesic shellfish poisoning; BF: bloom forming; mix: mixotroph; auto: autotroph; i: individual; c: chain; P: species found with plankton; E: species found with epiphytes.

Microalgae	Taxa group	Trophic state ^a	Cell types ^b	Nuisance types ^c	Habitat types ^d	Max (cells l ⁻¹)	Max (cells 100 g ⁻¹ FW)
Potentially toxic species							
<i>Alexandrium pacificum</i>	DINO	mix	i	PSP	P	160	-
<i>Coolia monotis</i>	DINO	auto	i	Cooliatoxin	P	840	-
<i>Dinophysis acuminata</i>	DINO	mix	i	DSP	P/E	80	2 10 ²
<i>Dinophysis rotundata</i>	DINO	mix	i	DSP	P	40	-
<i>Dinophysis sacculus</i>	DINO	mix	i	DSP	P	14142	-
<i>Gonyaulax polyedra</i>	DINO	mix	i	Y	P	320	-
<i>Gonyaulax spinifera</i>	DINO	mix	i	Y	P	40	-
<i>Gymnodinium catenatum</i>	DINO	mix	c	PSP	P	400	-
<i>Prorocentrum concavum</i>	DINO	mix	i	DSP	P/E	320	6 10 ³
<i>Prorocentrum lima</i>	DINO	mix	i	DSP	P/E	1400	6 10 ⁵
<i>Prorocentrum rhathymum</i>	DINO	mix	i	DSP	P	200	-
<i>Prorocentrum minimum</i>	DINO	mix	i	Neurotoxin	P	1560	-
<i>Pseudo-nitzschia</i> sp.*	DIAT	mix	c	ASP	P	1120	-
<i>Anabaena</i> sp.*	CYANO	auto	c	PSP, Anatoxin	P/E	200	5 10 ³
<i>Lyngbya</i> sp.*	CYANO	auto	c	Lyngbyatoxin	P/E	320	4 10 ³
<i>Oscillatoria</i> sp.*	CYANO	auto	c	Cytotoxin	P/E	59060	9.06 10 ³
Potentially bloom-forming species							
<i>Akashiwo sanguinea</i>	DINO	mix	i	BF	P	9213	-
<i>Tripos furca</i>	DINO	mix	i	HA	P	40	-
<i>Tripos fusus</i>	DINO	mix	i	HA	P	80	-
<i>Prorocentrum gracile</i>	DINO	mix	i	BF	P	920	-
<i>Prorocentrum micans</i>	DINO	mix	i	BF	P/E	362951	7.09 10 ⁴
<i>Prorocentrum triestinum</i>	DINO	mix	i	BF	P	17145	-
<i>Scrippsiella cf. trochoidea</i>	DINO	mix	i	BF	P	178444	-
<i>Chaetoceros decipiens</i>	DIAT	auto	c	BF	P	12227	-
<i>Chaetoceros socialis</i>	DIAT	auto	c	BF	P	200	-
<i>Cylindrotheca closterium</i>	DIAT	auto	i	BF	P/E	9856	8.63 10 ²
<i>Leptocylindrus danicus</i>	DIAT	auto	c	BF	P/E	2333	1.27 10 ³
<i>Thalassionema nitzschioides</i>	DIAT	auto	c	BF	P/E	11248	1.92 10 ⁵

^aAccording to Burkholder *et al.* (2008) and reference therein; ^{b,d} According to our study; ^c According to Anderson *et al.* (2001), GEOHAB (2001), Vila and Maso (2005) and Balkis (2009); *Genera known as toxin producers.

A significant difference in phytoplankton abundance was recorded between sampling sites (redundancy analysis (RDA), $F = 1.94$, $p = 0.001$). This spatial variability explains 6.91 % of changes in microalgae abundances with only RDA axis 1 supporting a significant effect on this variability ($p < 0.05$; Figure 20). A clear association was observed between stations 1, 3, 4 and 5 and the majority of species, including the harmful ones. The diatoms *Chaetoceros decipiens* and *Gyrosigma* sp. and the cyanobacteria *Oscillatoria* sp. appear to be further associated with station S1 which receives a supply of fresh water. Influenced by strong hydrodynamics, station S2 clearly stands out with a negative RDA axis 1, indicative of no clear correlation with any species, except for the diatom *Rhizosolenia* sp., but to a lesser extent. Indeed, minimum concentrations were always detected at this station, reaching only 40 cells l^{-1} in some cases.

Phytoplankton abundance also undergoes a significant change according to season (RDA, $F = 7.81$, $p < 0.001$). Temporal variability explains 18.11 % of changes in microalgae abundances with RDA axes 1 and 2 both supporting a significant effect on this variability ($p < 0.05$; Figure 21). Spring and winter showed close RDA scores involving the majority of species, including the dominant and harmful species *Prorocentrum micans*. The species *Oscillatoria salina* appears to be more readily observed in spring. Autumn has a positive RDA axis 2 indicative of a close relationship with the diatoms *Cylindrotheca closterium*, *Gyrosigma* sp. and *Nitzschia linearis*. Summer observation has a negative RDA axis 2 associated mainly with the diatoms *Navicula duerrenbergiana*, *Striatella unipunctata* and *Thalassionema nitzschioides*.

Significant correlations were found between phytoplankton abundances and physico-chemical variables such as temperature (RDA, $F = 10.97$, $p < 0.001$), PO_4^{3-} (RDA, $F = 3.61$, $p < 0.001$), NH_4^+ (RDA, $F = 2.51$, $p = 0.008$) and total nitrogen (RDA, $F = 2.49$, $p = 0.009$). Physico-chemical fluctuations explain 15.73 % of changes in phytoplankton abundance. RDA axes 1 and 2 both support a significant effect on the variability of phytoplankton abundance ($p < 0.05$; Figure 22). Along RDA axis 1, the toxic dinoflagellate *Prorocentrum lima* and many diatoms plot with a positive score and are associated with high temperature, whereas the dominant harmful species *P. micans* clearly stands out with a negative score along RDA axis 1 correlating with low temperature and orthophosphate values. The two harmful dinoflagellates *Akashiwo sanguinea* and *Scrippsiella cf. trochoidea*, the noxious cyanobacteria *Oscillatoria* sp. and the diatom *Rhizosolenia* sp. have a positive RDA axis 1 and a negative RDA axis 2 associated with low concentrations of total nitrogen and orthophosphate. The diatom *Climacosphenia* sp. plots with high orthophosphate values. The

two dinoflagellates *Protoperidinium diabolium* and *Dinophysis sacculus* have a negative RDA axis 2 and are thus associated with high nutrient concentrations: total nitrogen and ammonia respectively. The remaining microalgae species shows a RDA score near 0 and did not appear to exhibit a specific feature according to environmental conditions. Similarly, the remaining abiotic factors monitored in this study did not correlate with any of the observed microalgae. Salinity reached high levels in summer (maximal salinity = 51.2 at S4), a characteristic feature of GML waters, but was related neither to species abundance nor to total microalgae abundance (Spearman's test, $p = 0.11$).

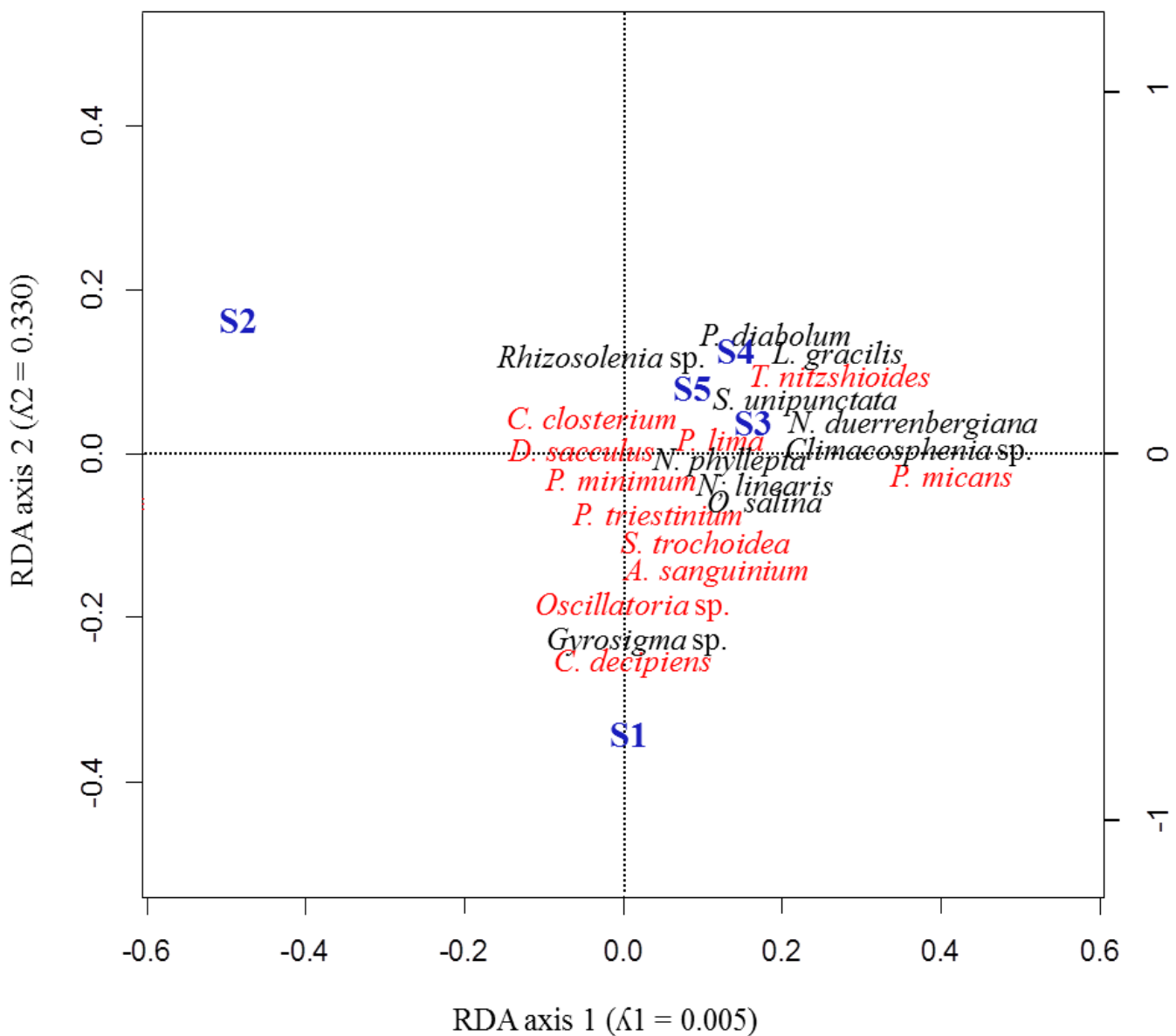


Figure 20. RDA TriPlot depicting the association between phytoplankton dominant species and sampling station in Ghar El Melh Lagoon. Eigenvalues of the first two axes are indicated by λ_1 and λ_2 . Harmful species were mentioned in red color.

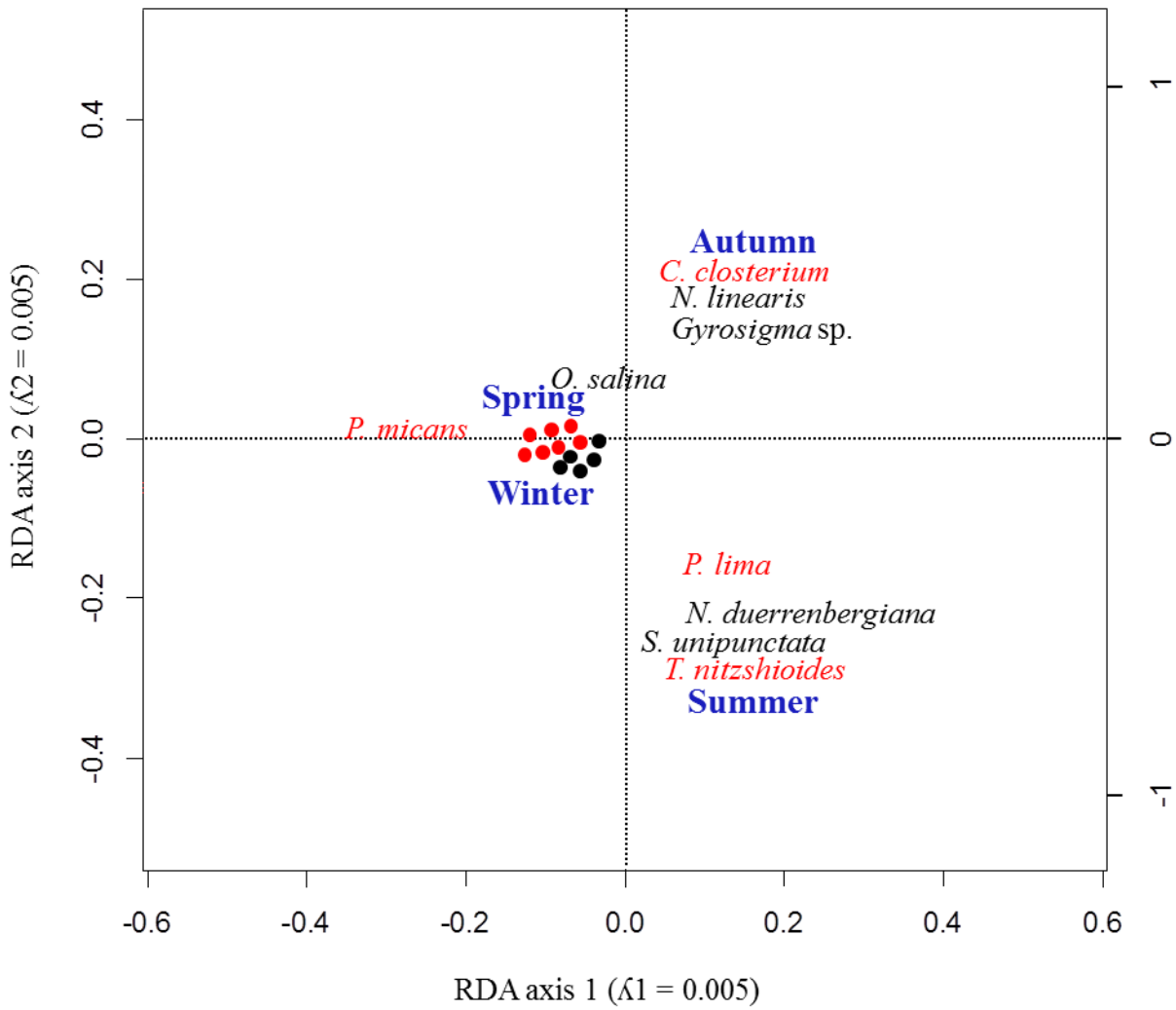


Figure 21. RDA TriPlot depicting the association between phytoplankton dominant species and sampling season in Ghar El Melh Lagoon. Eigenvalues of the first two axes are indicated by λ_1 and λ_2 . Harmful species were mentioned in red color. Red circles refer to the remaining harmful species. Black circles refer to the remaining non harmful species.

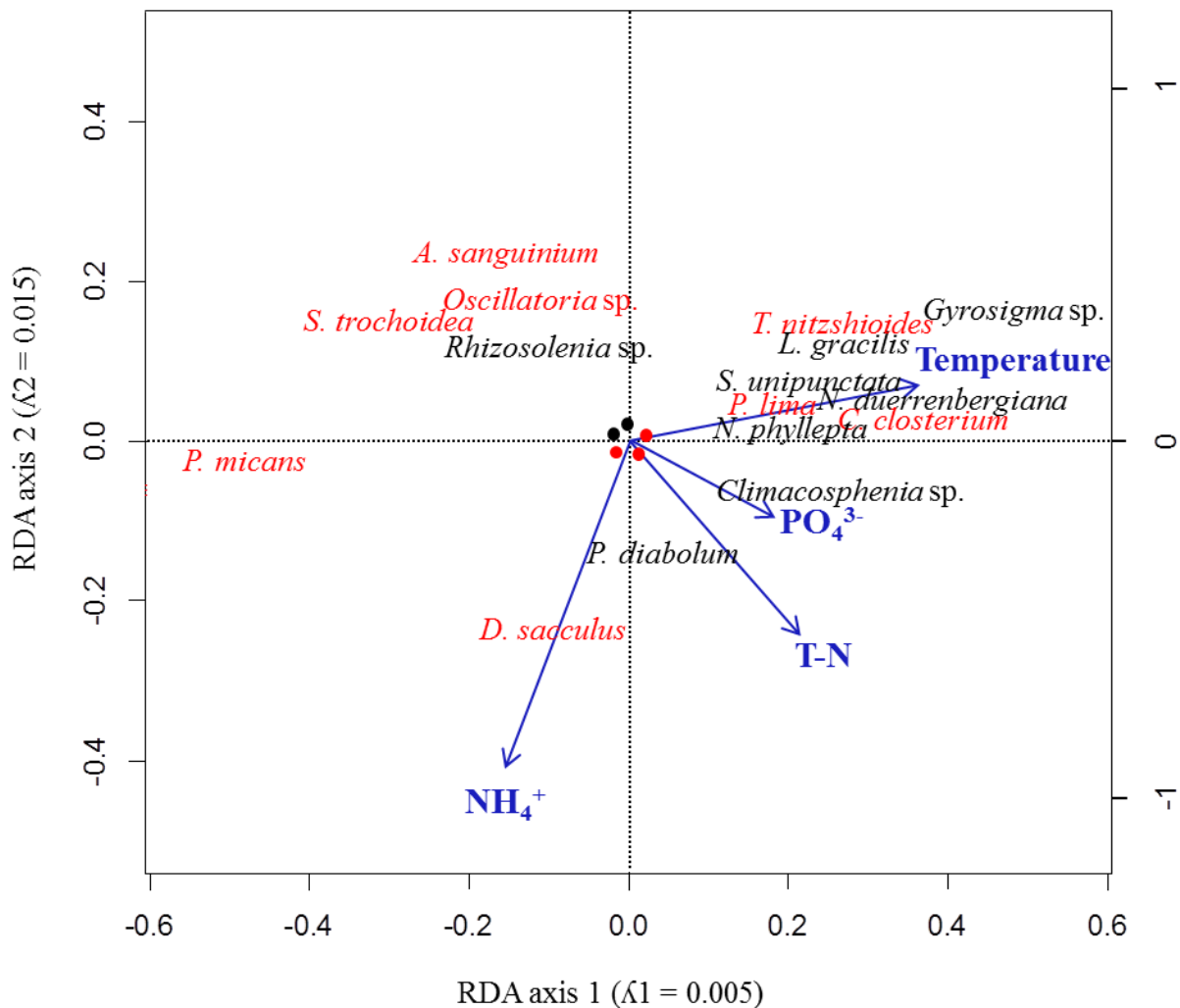


Figure 22. RDA TriPlot depicting the association between phytoplankton dominant species and environmental factors in Ghar El Melh Lagoon. Eigenvalues of the first two axes are indicated by λ_1 and λ_2 . Harmful species were mentioned in red color. Red circles refer to the remaining harmful species. Black circles refer to the remaining non harmful species.

Microalgae species on leaves of *R. cirrhosa*

Twenty microalgae species were recognized on *R. cirrhosa* leaves represented by 11 diatoms (50.36 % of total abundance), 4 dinoflagellates (47.60 % of total abundance) and 5 cyanobacteria (2.04 % of total abundance) (Table 19). Nine species made a global contribution to the epiphytic community:

- the dinoflagellates: *Prorocentrum lima* (45.34 %) and *P. concavum* (0.67 %),
- the diatoms: *T. nitzschoides* (21.39 %), *N. phyllepta* (8.51 %), *N. duerrenbergiana* (8.09 %) and *L. gracilis* (7.82 %)

-the cyanobacteria: *O. salina* (0.84 %) *Oscillatoria* sp. (0.5 %) and *Anabaena* sp. (0.5 %).

All of the epiphytes found over the three months of sampling were also detected within the planktonic community and involved 10 harmful species (> 70 % of total abundance) (Table 19). Almost all epiphytic algae have a greater length and width than those found in the water column. This was highly pronounced in the case of the dominant large-sized *P. lima* on leaves, especially in width: 45.1 μm for epiphytes, but 30.21 μm for planktonic forms (Tables 17 and 19).

Microalgae abundance ranged from 400 to $1.14 \cdot 10^6$ cells 100 g^{-1} FW (mean \pm sd = $1.65 \cdot 10^5 \pm 2.85 \cdot 10^5$ cells 100 g^{-1} FW). According to PERMANOVA analysis, microalgae abundance exhibits a significant correlation to both station and month (Table 20). The highest abundance of microalgae was registered in July at S4, while the lowest was recorded in August at S1 (Figure 23). SNK test revealed no significant difference between July and June (Table 20). Indeed, two peaks of dinoflagellate abundance were recorded in June ($6.05 \cdot 10^5$ cells 100 g^{-1} FW at S5) and July ($3.72 \cdot 10^5$ cells 100 g^{-1} FW at S5), mainly associated with *P. lima* proliferations. One peak of diatoms was detected in July, reaching $1.1 \cdot 10^6$ cells 100 g^{-1} FW at S4 and characterized by the development of *Navicula phyllepta*, *Thalassionema nitzschioides* and *Licmophora gracilis*. According to the SIMPER test, the main microalgae species contributing to temporal dissimilarity was the toxic dinoflagellate *P. lima* which contributed 57.35 %, 63.73 % and 49.91 % in June vs. July, June vs. August and July vs. August, respectively. Other microalgae appear in the monthly dissimilarity, but to a lesser extent, such as the diatom *T. nitzschioides* with: a) 15.52 % and 17.55 % in June vs. July and July vs. August, respectively, b) the cyanobacteria *Oscillatoria* sp. with 10.54 % and *N. phyllepta* with 6.43 %, both in June vs. August, and c) the diatom *N. duerrenbergiana* with 9.46 % in July vs. August.

Tableau 19. List of microalgae species found on *Ruppia cirrhosa* leaves (- not detected; + 40-1000 cells 100 g⁻¹ FW; *** > 1000 cells 100 g⁻¹ FW). Species marked with an asterisk were known as planktonic taxa.

Microalgae on <i>Ruppia</i> leaves	Mean length (µm)	Mean width (µm)	S1	S2	S3	S4	S5	June	July	August
Dinophyceae										
<i>Dinophysis acuminata</i> *	36	33.2	-	-	-	+	-	-	+	-
<i>Prorocentrum concavum</i>	50	50	-	***	***	***	***	***	+	-
<i>Prorocentrum lima</i>	50.2	45.1	***	***	***	***	***	***	***	***
<i>Prorocentrum micans</i> *	40.2	30	-	-	+	***	***	***	+	-
Bacillariophyceae										
<i>Climacosphenia</i> sp.	220	25	+	***	+	***	-	***	+	***
<i>Cylindrotheca closterium</i>	290	6.4	-	-	-	+	+	+	-	-
<i>Denticulopsis</i> sp.	32	20	-	***	+	***	***	***	***	-
<i>Gyrosigma</i> sp.	202	27	***	***	+	***	***	***	***	***
<i>Leptocylindrus danicus</i> *	250	20	-	-	-	-	***	***	-	-
<i>Licmophora gracilis</i>	46	10	***	***	+	***	+	***	***	-
<i>Navicula duerrenbergiana</i>	39	12	+	***	***	***	***	***	***	-
<i>Navicula phyllepta</i>	37	20	+	***	***	***	***	***	***	***
<i>Nitzschia linearis</i>	130	6.5	-	***	+	***	+	***	+	***
<i>Striatella unipunctata</i>	85	61.65	-	+	***	-	+	+	***	-
<i>Thalassionema nitzschioides</i> *	82.5	7	***	***	***	***	***	***	***	-
Cyanobacteria										
<i>Anabaena</i> sp.	85	8	-	***	+	-	***	***	+	***
<i>Lyngbya</i> sp.	120	20	+	***	+	+	***	***	+	+
<i>Oscillatoria salina</i>	120	20	+	***	***	***	***	***	***	***
<i>Oscillatoria</i> sp.	120	6	***	***	***	+	+	***	+	+
<i>Spirulina</i> sp.	82	6	-	-	-	***	***	+	-	-

Tableau 20. PERMANOVA results for microalgae abundance on *Ruppia cirrhosa* leaves. Bold numbers indicate significant effects

Source of variance	df	MS	F	Perm
Month	2	858.37	7.00	0.017
Station	4	674.18	635.57	0.001
Month * Station	8	122.62	115.59	0.001
Residual	30	1.07		
Cochran's C test		C = 0.782 ns		
Transformation		Ln (x+1)		
SNK test		June = July		
		July > August		

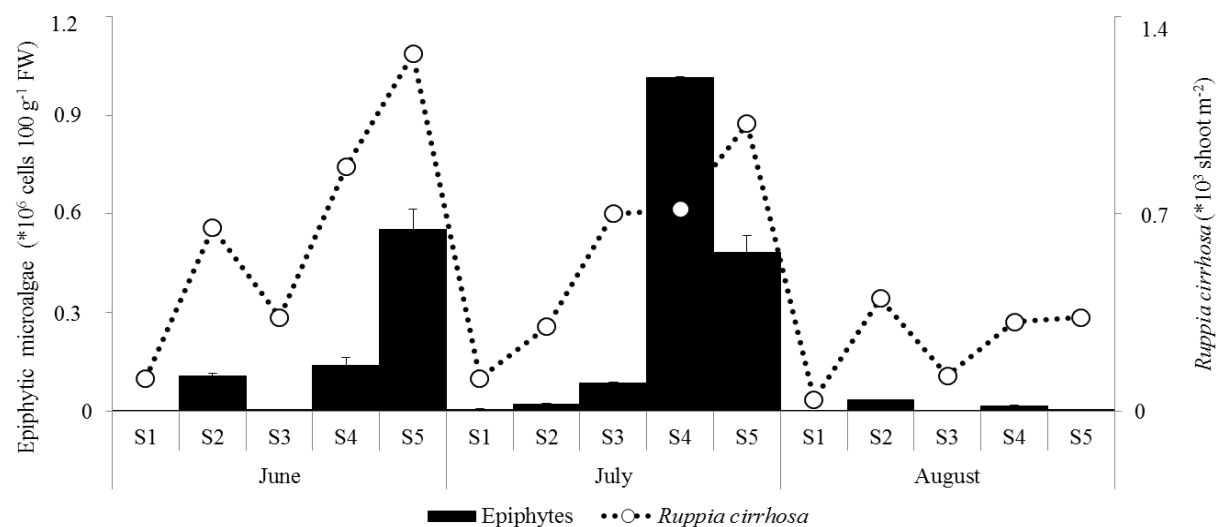


Figure 23. Mean abundance of epiphytic microalgae (cells 100 g⁻¹ FW) and *Ruppia cirrhosa* (shoot m⁻²) at the five stations and for each month.

The relationship between the dominant microalgae species on *Ruppia* leaves and environmental variables is illustrated in the CCA (Figure 24) whose two component axes explain 54.11 % of total variance. The first component axis explains 30.93 % of total variability while the second explains 23.18 % of total variance. The eigen values calculated with the CCA associated with Envfit function showed that NO_3^- ($r^2 = 0.4709$; $p = 0.038$) was the only environmental variable which accounted for most of the differences in epiphyte composition. The first and the second CCA component axes selected a positively high nitrate concentration and the dinoflagellate *P. concavum*, the diatom *N. phyllepta* and the cyanobacteria *O. salina* and *Anabaena* sp.

According to the Spearman test, the epiphytic microalgae abundance follows roughly that of *R. cirrhosa* ($r = 0.89$; $p = 0$) (Figure 23). The main significant correlations with *Ruppia* coverage were those of *P. lima* ($r = 0.91$; $p = 0$) and *N. duerrenbergiana* ($r = 0.59$; $p = 0.02$). Furthermore, remarkable correlations were found between the epiphytic and planktonic taxa of the same species such as *P. lima* ($r = 0.73$; $p = 0$), *T. nitzschioides* ($r = 0.38$; $p = 0.009$) and *N. phyllepta* ($r = 0.34$; $p = 0.02$) (Figure 25).

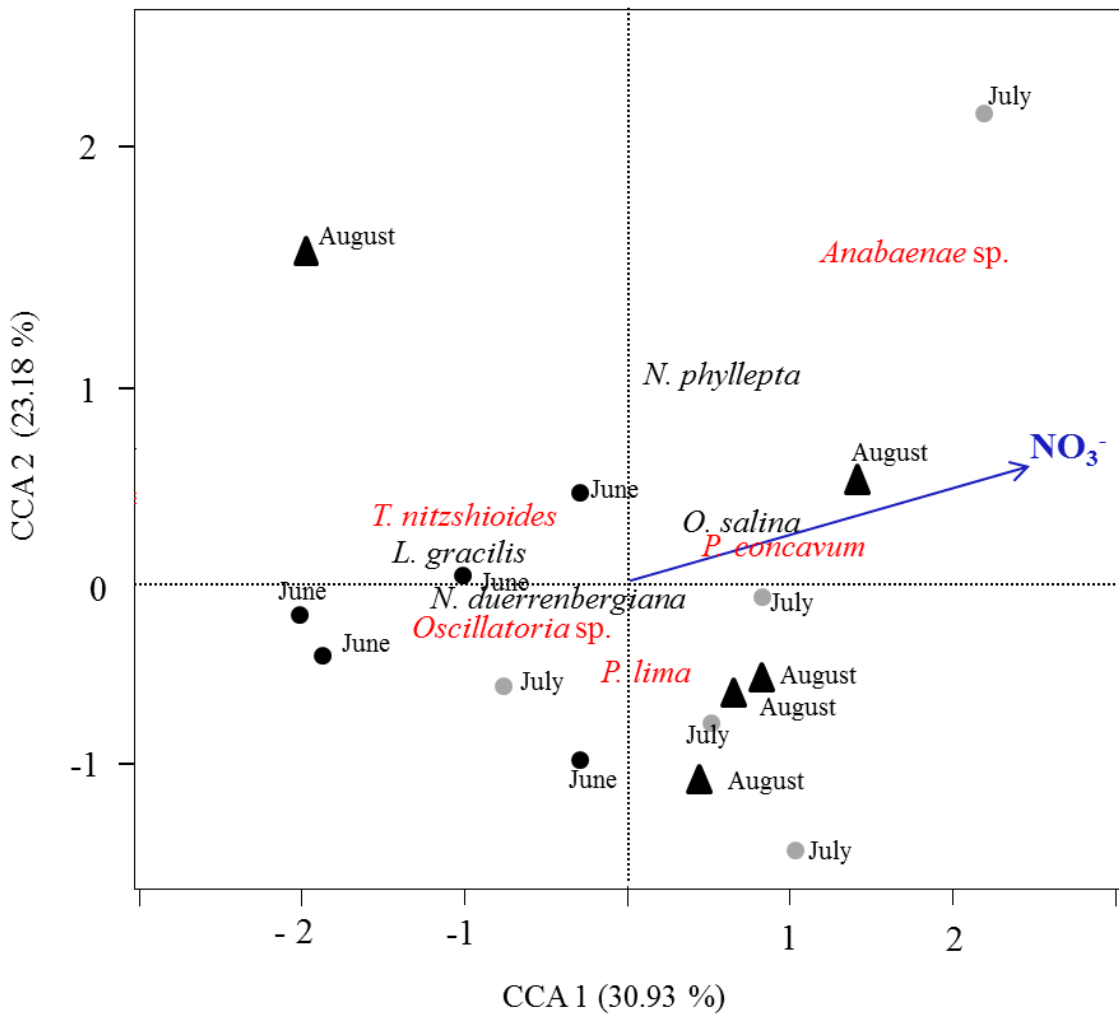


Figure 24. Triplot of Canonical Correspondence Analysis (CCA) showing the effects of environmental variables on abundance of epiphytic taxa for the 3 months and their order along the first and second axes. Harmful species were mentioned in red color.

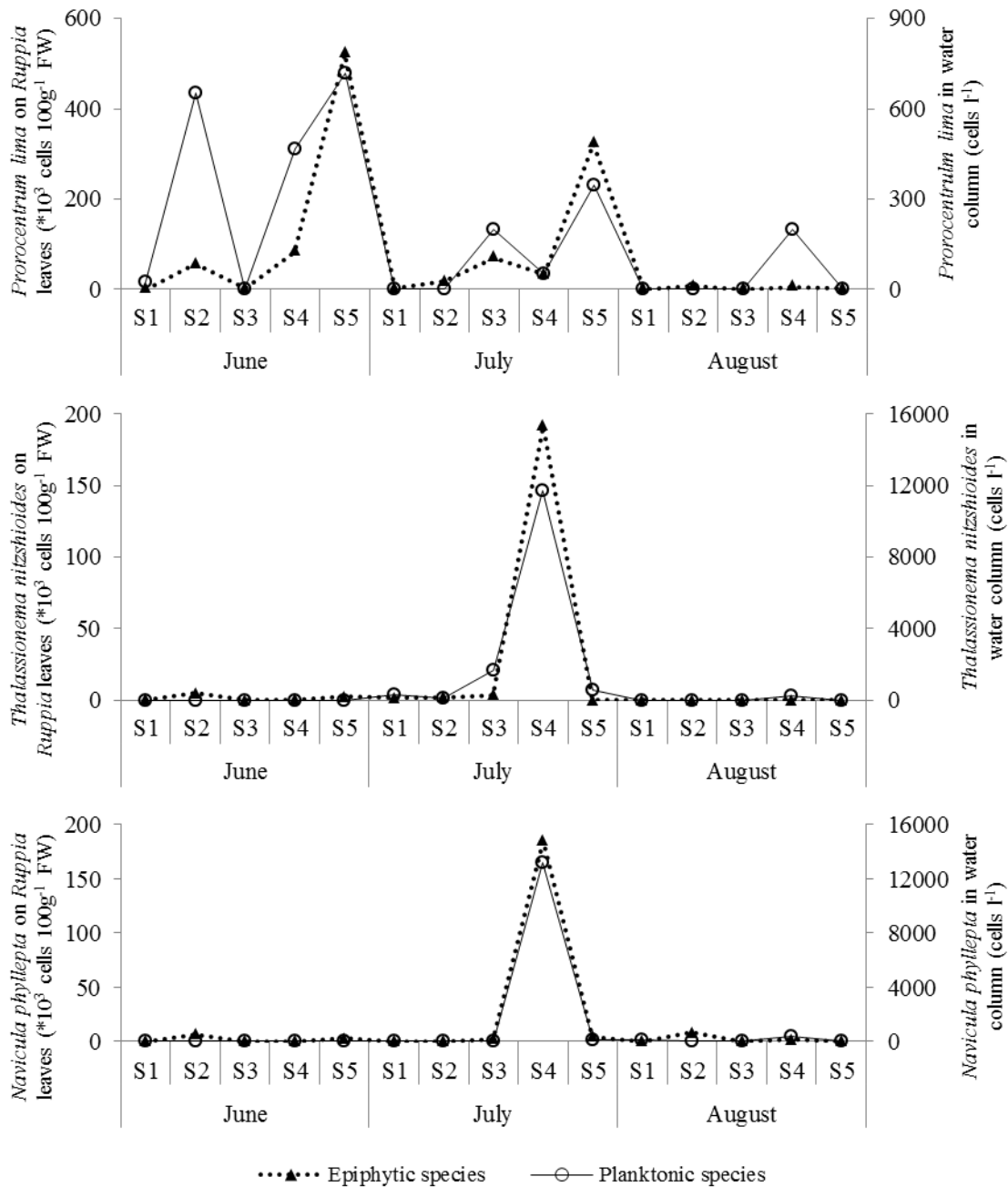


Figure 25. Spatio-temporal variability of some microalgae species in water column and on *Ruppia* leaves.

1.4. Discussion

In this study, dinoflagellates and diatoms appeared as the dominant groups, both in the water column of Ghar El Melh Lagoon and on *Ruppia* leaves as seagrasses are an ideal habitat for benthic diatoms and other epiphytes (Penhale, 1977; Cummins *et al.*, 2004). Diatom dominance was noted in summer, both in the water column (70.53 %) and on *Ruppia* leaves (50.36 %), which may be the result of the presence of many tytoplanktonic diatoms which are

benthic species such as members of the genera *Navicula*, *Licmophora* and *Gyrosigma* and which can detach from the substrate for some time. This illustrates the great role of *R. cirrhosa* as an important source of diatoms to the water column during this period. All epiphytes were also found in the water column, a feature often noted in coastal and shallow waters due to a strong wind impact facilitating the intensive mixing of water masses (Klinkenberg and Schumann, 1994; Telesh, 2004; Telesh *et al.*, 2008). Some planktonic species such as the dominant planktonic dinoflagellate *P. micans* (maximal abundance = 7.09×10^4 cells 100 g^{-1} FW of *R. cirrhosa*) were also found on *Ruppia* leaves. However, the benthic dinoflagellate *Coolia monotis* was found exclusively in the water column of GML. The absence of *Coolia* on *Ruppia* leaves may be due to (i) its small size (mean length = $30 \mu\text{m}$) which suggests that this dinoflagellate was missed during counting as epiphytic samples were overloaded with debris, or (ii) the dominance of the benthic *P. lima* (maximal abundance = 6×10^5 cells 100 g^{-1} FW of *R. cirrhosa*) which is flatter (mean width = $45.1 \mu\text{m}$) and more prone to the benthic life style than *Coolia*; it can attach itself with a coating of mucus (Tindall and Morton, 1998; Vila *et al.*, 2001a) which may explain its presence on *Ruppia* leaves at S2 despite the strong hydrodynamics in this zone. It therefore appears that *Coolia* was loosely attached to the substrata and may have been easily removed and re-suspended in the water column.

A remarkable presence of noxious and/or toxic species (28 taxa, over 70 %) was noted both in the water column and on *Ruppia* leaves, with 16 species able to cause DSP, ASP, PSP and several other toxic symptoms, whereas the remaining species were potentially bloom-forming taxa which can lead to hypoxia and anoxia events in the field. Within GML's harmful community, the genus *Prorocentrum* was a crucial group which included the dominant planktonic *P. micans*, the dominant epiphytic *P. lima* species and was composed exclusively of harmful species: *P. minimum*, *P. concavum*, *P. gracile*, *P. rhathymum*, *P. triestinum* (Table 17). Many authors have reported the harmful effects of the dominant taxon *P. micans* which has caused red tides in many parts of the world (Anderson *et al.*, 1985; Graneli *et al.*, 1990; Fukuyo *et al.*, 1990; Faust *et al.*, 1999). Early reports on *P. micans*, indicating that this strain produces a paralytic shellfish poisoning toxin (Pinto and Silva, 1956), have remained unconfirmed since recent incidents involving shellfish mortality have been attributed to oxygen depletion (Lassus and Berthome, 1988). In contrast to most of the other benthic species, *P. lima* is capable of developing populations in temperate waters in numbers that could justify DSP events (Quilliam *et al.*, 1993; Nascimento *et al.*, 2005). *P. lima* abundance on *Ruppia* leaves (6×10^5 cells 100 g^{-1} FW) was much higher than that found, for example, on

P. oceanica leaves along the Tunisian coast (Turki, 2005; Mabrouk *et al.*, 2011; Ben Brahim *et al.*, 2013) and in other areas (Foden *et al.*, 2005). With this warning value, especially in such shallow waters, any water dynamics will detach *P. lima* cells from *Ruppia* leaves and thus contribute to harmful events.

Other harmful species were recorded in our survey, but in lower abundance:

- (i) The toxic *D. sacculus* proliferated in winter, reaching a level of more than 14000 cells l⁻¹. High winter concentrations of *Dinophysis* species have previously been noted in GML (Dhib *et al.*, 2013a) and other regions (Koukaras and Nikolaidis, 2004), but *Dinophysis* proliferation is generally favored by the warm season (Smayda, 1980; Reguera *et al.*, 2003). No study has yet shown how the species survives through environmentally disadvantageous circumstances such as those existing in the winter months in temperate regions (Reguera *et al.*, 2012);
- (ii) The bloom-forming *S. cf. trochoidea* showed a steady year-around presence. Generally considered nontoxic, *S. cf. trochoidea* has nevertheless been reported as responsible for fish and shellfish kills (Hallegraeff, 1992; Tang and Gobler, 2012);
- (iii) The bloom-forming *T. nitzshioides* appeared in maximum concentrations on *Ruppia* leaves (192000 cells 100 g⁻¹ FW) with a maximum of only 11248 cells l⁻¹ in the water column which is unusual for this typically planktonic species. According to the Spearman test, a high correlation was found between the presence of this species in the water column and on *Ruppia* leaves. Moreover, based on the SIMPER test, *T. nitzshioides* was one of the discriminating species contributing to the temporal dissimilarity of *Ruppia* microalgae. Thus, the presence and the dominance of this species on the macrophyte were probably neither by chance nor the result of simple settling. In any case, in this period of the year (summer), the presence of *Ruppia* was important to the presence of *T. nitzshioides* either for settling (as refuge, food availability, bacterial mucilage..) or for resuspension.
- (iv) Several other potentially toxic species were observed in the water column, but in low concentrations: the dinoflagellates *Alexandrium pacificum*, *Dinophysis acuminata* and the diatom *Pseudo-nitzschia* sp. Blooms of the *Alexandrium* species ($\approx 1.5 \cdot 10^6$) have been previously detected in GML (Romdhane *et al.*, 1998), but were not observed during this year of investigation, nor have they been reported in other studies carried out in the lagoon (Ramdani *et al.*, 2004; Dhib *et al.*, 2013a), which suggests that the conditions there are basically non-favorable to *Alexandrium* bloom initiation. *A. pacificum*, with a maximum concentration at station S2, may be a non-indigenous species introduced by ballast water discharges, as has been demonstrated in other Tunisian lagoons (Fertouna-Bellakhal *et al.*, 2014). The few existing studies of GML's microalgae have signaled the presence of *Pseudo-*

nitzschia spp., but in non-critical concentrations (SCET-ERI, 2000; Ramdani *et al.*, 2004). Concerning the *Dinophysis* species, it must be remembered that, as pointed out by Maestrini (1998), Aubry *et al.* (2000) and Aissaoui *et al.* (2014) these represent only a fraction of total phytoplankton assemblages, a conclusion largely confirmed in our study.

Epiphytic microalgae were quantitatively important only in summer as they obviously coincided with the strong presence of *R. cirrhosa* which is an adequate habitat for them. Indeed, significant temporal and spatial microalgae fluctuations were recorded (PERMANOVA, $p < 0.01$), roughly following those of *R. cirrhosa* ($r = 0.89$; $p = 0$), with maximal abundances found in June and July, especially at stations 3, 4 and 5 which are associated with massive beds of *Ruppia* seagrass. These fluctuations were also recorded in the minimal microalgae abundances found at the majority of stations in August, the beginning of seagrass mat decomposition (Figure 23). *Ruppia*-epiphyte symbiosis was highly pronounced in the case of the dominant toxic *P. lima*, whose abundance roughly follows that of *R. cirrhosa*, with a high number of valves noted in the August samples, indicative of the species' mortality following *Ruppia* bed decomposition. Though *P. lima* is present at S2, the strong hydrodynamics there illustrate the role of *Ruppia* as a refuge for this species. Indeed, it is well known that seagrass is typically considered to act as an ecosystem engineer by playing an important role in structuring benthic assemblages, serving to reduce physical stress and enhance food availability (Orth *et al.*, 1984; Boström *et al.*, 2006; Bos *et al.*, 2007).

The absence of correlation between the majority of epiphytic microalgae and any environmental factors monitored in this study indicate that epiphytic structure may depend more on the niche advantages offered by *Ruppia* leaves and the plant's seasonal growth cycle (Mazzella *et al.*, 1994; Alcoverro *et al.*, 1997; Prado *et al.*, 2007). According to CCA, only nitrate concentrations have an influence on some epiphytic species, especially *P. concavum* and *O. salina* (Figure 24).

In contrast, environmental factors have an important influence over the spatio-temporal variations of the dominant species in in the water column of GML. Indeed, many diatoms, namely *C. closterium*, *Gyrosigma* sp., *N. linearis*, *N. duerrenbergiana*, *S. unipunctata*, *L. gracilis* and *T. nitzschoides*, along with the toxic dinoflagellate *P. lima*, respond to high temperatures, their maximum abundance being recorded in summer. However, it is a low water temperature which appears to enhance the proliferation of our dominant species *P. micans*. The preference of the latter species for low temperatures has been previously noted in GML with species proliferation at a temperature below 22.5 °C (Dhib *et al.*, 2013a). Several studies report a winter (Sarno *et al.*, 1993; Nezan and Piclet, 1996), a winter-spring (Vila *et*

al., 2005) or a summer and early autumn *P. micans* blooming (De Angelis, 1962; Giacobbe and Maimone, 1990; Horner, 2002). We believe that the different seasonal peaks reported for this species are mainly related to its extraordinary capacity to adapt, especially to water temperature, from cold to medium to warm. Indeed, *P. micans* is a cosmopolitan species recorded in most of the world's lagoons, seas and oceans, including the Mediterranean (Gomez, 2003; Kraberg *et al.*, 2010). In GML, the proliferation of *P. micans* in the water column appears to be favored by a low temperature.

Though high in summer, salinity (maximal salinity = 51.2 at S4) did not appear to structure microalgae distribution, suggesting that the species present in GML adapted well to niche properties. Our hypothesis may be supported by the total absence of *Ostreopsis* species in GML, known to prefer salinity in the range 26 – 40 in the Mediterranean Sea (Monti *et al.*, 2007) and to be negatively correlated with salinity along the Hawaiian coast (Parsons and Preskitt, 2007). It is of note that *Ostreopsis* is known as an epiphyte mainly associated with *P. lima* along Tunisian coasts (Turki, 2005; Mabrouk *et al.*, 2011) and other regions (Bomber and Aikman, 1989, Bourdeau *et al.*, 1995, Faust, 1995; Vila *et al.*, 2001a). We should stress the fact that these observations were provided from other macrophytes and that no study of *Ruppia* epiphytes is currently available to allow comparison with our data (especially for *Ostreopsis* populations).

Nutrient concentrations appear to play a contrasting role in the proliferation of some of the lagoon's dominant species (RDA, Figure 22), with certain ones enhancing the proliferation of the diatom *Climacosphenia* sp. and the two dinoflagellates *P. diabolium* and *D. sacculus*. Indeed, changes in the N/P ratio will result in the environmental selection of certain phytoplankters, potentially favoring harmful species (Smayda, 1990; Smayda and Reynolds, 2003; Heisler *et al.*, 2008). However, in all cases, an unequivocal causal link is established between an increase in HAB (frequency, magnitude or duration) and a change in N or P as the limiting nutrient still difficult to establish. Davidson *et al.* (2012) propose that if the concentration of each nutrient contributing to the ratio is not low enough to limit growth, then the value of the nutrient ratio is not important in determining species competition and its use may be misleading. This could explain the non-correlation of the remaining harmful species with nutrient enrichment. Another explanation could be proposed in relation to the mixotrophic behavior of the majority of harmful species (19 mixotrophic species; Table 18) and mixotrophy is recognized as a major mode of feeding for harmful algal species in eutrophic waters (Nygaard and Tobiesen, 1993; Jeong *et al.*, 2004; Burkholder *et al.*, 2008).

Scenario of harmful events in GML

Considering the seasonality of the different planktonic species, it appears that harmful events can happen throughout the year (RDA, Figure 21). The lagoon's inner areas also appear to be at a higher risk of harmful phytoplankton events (RDA, Figure 20). For epiphytic microalgae, the presence of harmful species was clearly related to the presence of *Ruppia* at the site. On the basis of statistical analyses, a schematic scenario of harmful events in Ghar El Melh Lagoon can be proposed (Figure 26):

(1) winter-spring proliferation of the majority of the dominant harmful phytoplankton. The decreased water temperature during this period, with an average of 15.54 °C, seems to favor the proliferation of the dominant taxon *P. micans*. The low concentrations of total nitrogen, with an average of 18.35 $\mu\text{mol l}^{-1}$, and orthophosphate, with an average of 0.27 $\mu\text{mol l}^{-1}$ in these seasons, appears to favor the development of the two dinoflagellates *S. cf. trochoidea* and *A. sanguinea* and the cyanobacteria *Oscillatoria* sp. However, the high concentrations of ammonium, with an average of 4.08 $\mu\text{mol l}^{-1}$, appear to favor the proliferation of *D. sacculus*. It should be noted that maximal concentrations of *P. micans* and *A. sanguinea* were detected in spring while higher proliferations of *D. sacculus* and *Oscillatoria* sp. were noted in winter. The occurrence of the remaining harmful species, namely *P. triestinum*, *P. minimum* and *C. decepiens* in winter-spring did not appear related to any environmental factors monitored during our study. This might have been for the reasons discussed above as regards the eutrophication–bloom relationship and mixotrophy. Also, blooms may be controlled by grazers such as ciliates (Gismervik *et al.*, 1996; Loder *et al.*, 2011), which might attain even optimal growth rates when fed with some HAB species (Kamiyama, 1997; Jeong *et al.*, 1999). In fact, among the samples collected concomitantly with microalgae, we found that ciliates are heavily present in GML waters and have significant positive correlations with harmful microalgae (Dhib *et al.*, 2013b);

(2) summer proliferation of the toxic *P. lima* and the bloom forming *T. nitzschioides*. The proliferation of the former species in the GML water column was significant according to the high water temperature in summer. Yet, in addition to this apparent affinity for warm temperatures, especially *P. lima*, *R. cirrhosa* was an important source of these species and undoubtedly plays a role in their dissemination in the water column in summer. This was confirmed by the highly significant correlations found between these species present in the water column and on *Ruppia* leaves (Figure 25). The slight presence of epiphytic species in other seasons may be ascribed to other macrophytes such as *Cladophora* species (Shili *et al.*, 2002). *Ruppia* leaves were the habitat for other harmful microalgae such as *P. concavum*

(Table 17) which were also detected in the water column in the same period but with non-significant correlations;

(3) autumn development of the harmful *C. closterium* related with the high temperatures still recorded during this period (an average of 21.01 °C).

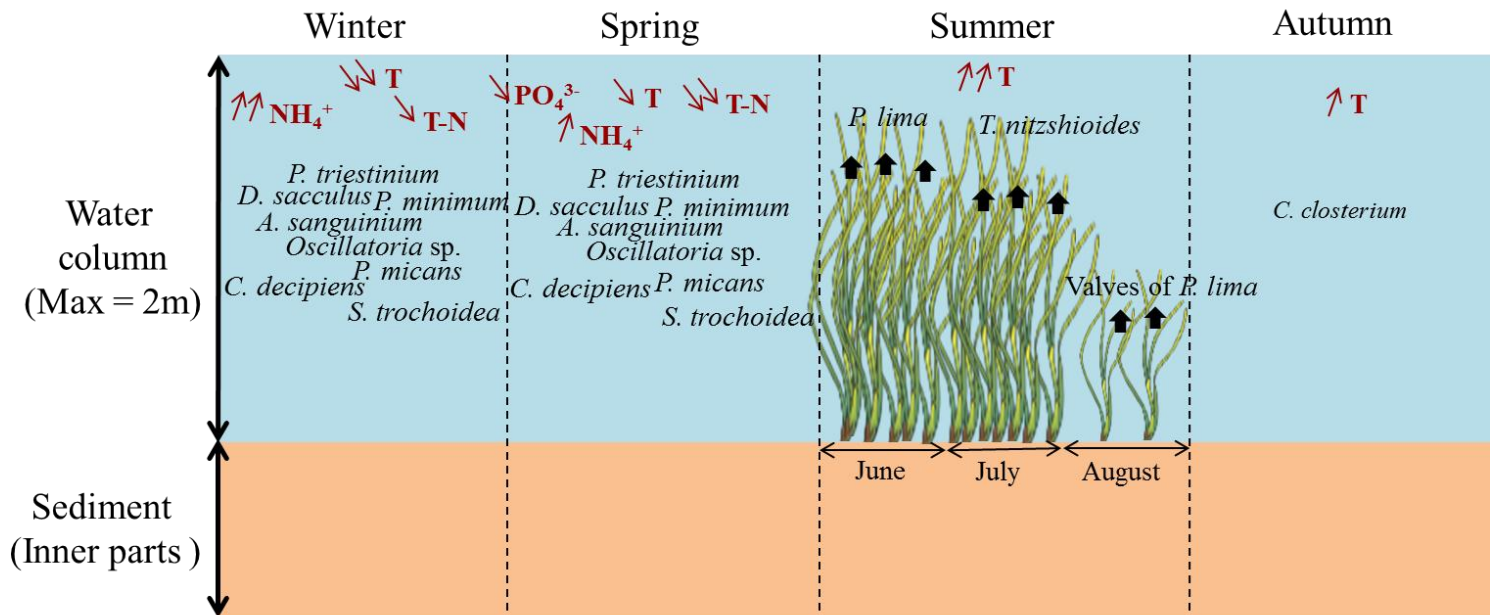


Figure 26. Scenario of harmful events in Ghar El Melh lagoon.

1.5. Conclusion

This study illustrates (1) dinoflagellates and diatoms as the typical microalgae groups both in the water column and on *Ruppia* leaves, (2) low microalgae diversity and monospecific trends with a remarkably high abundance of *P. micans* in the water column and of *P. lima* on *Ruppia* leaves, and (3) the role of *Ruppia* in the dissemination in the water column of *P. lima* and diatom species. Monitoring programs, especially in shallow waters and lagoons, should include epiphytic microalgae studies for early warning of harmful algae presence. This study also illustrates (4) that the inner parts of the lagoon run a higher risk of harmful events and that studies of the toxic profile of both *P. lima* and *P. micans*, together with ecotoxicological tests, may provide insight into the real role of the former species in harmful events and their possible implication in the fish mortality still detected in Ghar El Melh Lagoon.

In summary, Ghar El Melh Lagoon, is a highly disturbed area suffering from increases in salinity, in temperatures, in nutrient gradients and from *Ruppia* invasion (as a macrophyte

bioindicator of water deterioration) which, all combined, are the main inducers of algal blooms, especially of harmful dinoflagellates. Unfortunately, these circumstances have weakened the economic value of the lagoon with, for example, a decline in aquaculture. It has therefore become absolutely urgent to protect this RAMSAR site from environmental pressures and to adopt concrete measures such as the installation of a sewage treatment plant and the dredging of the areas near El Bougaz Channel, the only connection between the lagoon and the sea.

Acknowledgements

This study was conducted by Amel DHIB as a part of her PhD research (co-directed at the University of Franche-Comté, CNRS 6249, Besançon, France, and at the Institut National des Sciences et Technologies de la Mer, Tunisia). The authors would like to thank the lagoon's managers and the local fishermen for their logistic help. We express our appreciation to the editor, Dr. Sandra E. Shumway, and to the anonymous reviewers for helping to improve our paper.

2. Distribution spatio-temporelle des kystes de dinoflagellés et relation avec les communautés microphytoplanctoniques

Projet d'article. On the presence of dinoflagellates cysts in the surface sediments of a Mediterranean lagoon

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Résumé

Dans ce projet d'article, il s'agit de l'étude des kystes de dinoflagellés ainsi que le microphytoplancton dans la lagune de Ghar el Melh. Le prélèvement des différentes communautés a été effectué au niveau de 5 stations pendant quatre campagnes saisonnières (automne 2012 - hiver 2012 - printemps 2013 - été 2013).

Aussi durant ce deuxième suivi, la lagune a varié d'euryhaline à hyperhaline (35.5-51) et peut être considéré comme hypertrophique avec des concentrations exceptionnelles du phosphore total (1.78-3.75 $\mu\text{mol. l}^{-1}$) et d'azote inorganique dissous (2.99 - 7.04 $\mu\text{mol l}^{-1}$).

Les pourcentages d'eau (% H₂O) calculés dans le sédiment ont été homogènes (73.1 - 87.9 %) et élevés reflétant la domination des zones vaseuses dans la lagune de Ghar El Melh.

Le nombre des kystes de dinoflagellés a oscillé entre 0 et 229 kystes par gramme de sédiment sec. Selon l'Analyse de Redondance, l'abondance des kystes n'a pas changé avec les stations, mais elle a changé significativement avec les saisons et ceci a été principalement lié aux concentrations importantes enregistrées en automne. L'absence des kystes a été enregistrée au niveau de 5 échantillons appartenant aux différentes saisons sauf l'automne.

Les kystes de dinoflagellés ont été principalement représentés par les morphotypes suivants : *Protoperidinium* spp. (48.82 %), *Scrippsiella trochoidea* complex (9.56 %), RBC (round brown cysts) (7.22 %), *Lingulodinium machaerophorum* (5.36 %), *Alexandrium* spp. (5.35 %) et *Gymnodinium* spp. (4.03 %). Parmi ces groupes, on a enregistré quelques espèces connues pour être toxiques telles que : *Alexandrium pacificum*, *A. minutum*, *A. pseudogonyaulax* et *Lingulodinium polyedrum*.

Aucune corrélation significative n'a été trouvée entre la distribution des kystes de dinoflagellés et les différents paramètres physico-chimiques mesurés dans l'eau et le sédiment.

Les faibles concentrations des kystes de dinoflagellés enregistrées reflètent la composition du phytoplancton qui a été caractérisée par la dominance des espèces dinoflagellés, ne produisant pas de kystes au cours de leur développement.

Abstract

Seasonal distribution of dinoflagellate cysts in relation to environmental factors were studied at five stations of surface sediment in Ghar El Melh lagoon (GML). At least sixteen dinocysts types were identified mainly dominated by *Protoperidinium* spp., *Scrippsiella trochoidea* complex, *Lingulodinium machaerophorum*, *Alexandrium* spp., *Gymnodinium* spp. along with many round brown cysts. Cyst ranged from 0 to 229 cysts g⁻¹ dry weight sediment. No significant cyst distribution was found among stations although a significant cyst variation was seen among seasons with cysts dominance in autumn observation. No significant variation was found between cyst abundance and the different abiotic factors monitored neither in the water column (physic-chemical parameters) nor in the sediment (% H₂O). As well, no significant difference in cyst abundance was found in relation to phytoplankton abundance. The weak dinocysts abundance consistent with the dominance of dinoflagellates non-cyst formers in the water column of GML.

Keywords: Dinoflagellate cysts; enviromental factors; Ghar El Melh lagoon

2.1. Introduction

About 10 % of 2000 dinoflagellate species produces benthic resting cysts (Dale, 1983) whose excystment constitutes the starting point of their planktonic life cycle. Resting cysts are mainly distributed at the sediment surface (Irwin *et al.*, 2003; Garcés *et al.*, 2004; Mizushima and Matsuoka, 2004) with increasing concentrations in muddy sediment with water and organic matter contents (Erard-Le-Denn *et al.*, 1993; Yamaguchi *et al.*, 1996). However, the observed variations in resting cysts distribution and density could be linked to the encystment capability of planktonic species in the water column, sedimentation, transport and bioturbation processes (Anderson, 1997, Garcés *et al.*, 2004; Joyce *et al.*, 2005).

"Cyst banks" of sediments play a key role in the bloom initiation phase (Anderson *et al.*, 1982; Garcés *et al.*, 1999; McGillicuddy *et al.*, 2003) and the toxicity of dinoflagellate cysts may be even greater than that of the vegetative cells. For instance, *Alexandrium* cysts contain PSP toxin levels several-fold higher than those in motile cells (Oshima *et al.*, 1992).

In the Mediterranean Sea, the composition of dinoflagellate resting cyst assemblages in recent sediments has been examined in only a few studies (Rubino *et al.*, 2000, 2002; Giannakourou *et al.*, 2005; Zonneveld *et al.*, 2009; Satta *et al.*, 2013).

In this context, dinoflagellate cysts were studied during four seasons in surface sediments of five stations in Ghar El Melh Lagoon (Northeast Mediterranean Sea) in relation to both environmental factors and microphytoplankton community. GML was a shallow water body prone for microalage proliferations including harmful dinoflagellates species (Romdhane *et al.*, 1998; Turki *et al.*, 2007; Dhib *et al.*, 2013a) and which the dinocysts community should be explored. The purpose of this paper is to (i) provide information, for the first time in this lagoon, on the composition and the abundance of dinoflagellates cysts and (ii) to identify factors driving their distribution.

2.2. Materials and Methods

Study area

Ghar El Melh Lagoon is a RAMSAR site (RAMSAR, 2007), located in the southern Mediterranean Sea on the north-eastern coast of Tunisia (37°06'-37°10' N and 10°08'-10°15' E) and influenced by the regional water circulation (Ben Ismail *et al.*, 2012) (Figure 19, Chap IV.1). GML has an area of about 3000 ha including 2 small sub-lagoons, namely Sabkhet El Ouafi and Sabkhet Sidi Ali El Mekki. The main lagoon is permanently connected with Sabkhet El Ouafi but isolated from Sabkhet Sidi Ali El Mekki by embankments. It is

connected to the Mediterranean Sea via a permanent channel called El Boughaz that passes through the coastal sand bars.

According to Chakroun (2004), three sediment fractions could be identified (i) sandy facies located only at “El Boughaz” mouth and ranged from 93 to 100 % (ii) silty-sandy facies restricted to the extreme north east of GML with a respective percentage of 52.8 and 33.2 % and (iii) muddy-silty facies at the majority of GML areas with dominance of muddy fractions which ranged from 45.5 to 87.1 %.

Sampling

Sampling was conducted during four seasons (autumn 2012, winter 2012, spring 2013 and summer 2013) at five sampling sites. Stations (S1–S5) were chosen to cope with the different environmental conditions found within GML (Figure 19, Chap IV.1). S1 is located in the north-eastern part of the lagoon, a shallow area influenced by a supply of freshwater from ‘El Ayoune’, with a mat of limnetic plants (reeds) around the edges. S2 near “El Boughaz” Channel which is a permanent 70-m wide connection with the Gulf of Tunis. The deepest area of GML, it is influenced by strong hydrodynamics despite continuous silting of the channel. S3, located in the south-east of the lagoon in the middle of Sabkhet El Ouafi, is a semi-closed zone and one of the most stagnant areas. S4 in the south-west of GML is affected by heavy industrial discharge (from 43 industrial units) and is drained by tributaries mainly in winter. S5, located in the northern part of the lagoon, is affected by agricultural and urban discharge. Leachate from rural zones, rainwater and the wastewater from the city of Ghar El Melh arrive directly into this part of the lagoon.

Surface sediment was collected from the top 3 cm with a Van Veen Grab Sampler (Yamaguchi *et al.*, 1995). Sediment samples were placed in dark plastic containers, hermetically sealed to prevent any germination and stored in the dark at 4°C. Samples for phytoplankton and ciliates identification and enumeration were collected using a polyvinylchloride (PVC) tube in the same period and stations. Environmental variables were measured in the field concomitantly with all samples. A WTW multiparameter was used for water temperature (°C) and salinity. Transparency was measured using a Secchi disk. Water samples were collected in 1000-ml polypropylene bottles at 30 to 50 cm depth. In the laboratory, nutrients (ammonium, nitrite, nitrate, phosphate, silicate) were analysed with a BRAN and LUEBBE type 3 autoanalyser and concentrations were determined colourimetrically using a UV-visible (JENWAY 6705) spectrophotometer (APHA, 1992).

Cyst processing and identification

Dinocysts were separated from the sediment fraction according to a gradient density method using Ludox CLX as described in Genovesi *et al.* (2007) modified from Erard-Le Denn and Boulay (1995) and Yamaguchi *et al.* (1995). We checked only the surface sediment and empty cysts were not considered. Subsamples (1 g) were suspended in 20 ml of sucrose 24 % and sonicated for 3 min at 100 Hz in a Wiseclean DAIHAN sonicator. The sonicated suspension was sieved through 120 µm and 20 µm mesh sieves. The slurry remaining on the 20 µm mesh sieve was washed with sucrose solution and placed in a 50 ml Falcon tube for sonification (3 min at 100 Hz). It was processed for cyst concentration and separated from the sediment using 20 ml of Ludox CLX (density 1.3 g cm⁻³). The tube was centrifuged for 30 min at 3000 rpm at 16°C. Dinocysts were concentrated in the upper fraction. This unstable phase containing dinocysts was extracted and sieved through a 20 µm nylon membrane. The resulting sample was rinsed with distilled water and collected with 5 ml of filtered seawater and sonicated. An aliquot of the final sample were counted in a sedimentation chamber with an inverted microscope.

For better identification, morphotypes were germinated in f/2 medium (Guillard and Ryther, 1962). Water salinity was adjusted to 34, temperature 20 ± 2°C, photon irradiance 100 µmol photons m⁻² s⁻¹ under a 12/12 light / dark cycle.

Dinocysts were identified according to Head (1996), Zonneveld (1997a,b), Zonneveld and Jurkschat (1999), Pospelova and Head (2002), Kim *et al.* (2007) and Matsuoka *et al.* (2009).

Sediment analysis

To calculate the sediment water content, subsamples of 20 g were oven dried at 60°C for 7 days. Water content was then calculated using the following formula (Cho and Matsuoka, 2001):

$$\text{Water \%} = [(W_w - W_d) * 100] / W_w$$

W_w = wet weight; W_d = dry weight

As suggested by Cho and Matsuoka (2001), dinocysts were expressed as:

$$\text{Cysts g}^{-1} \text{ dry sediment (DS)} = \text{cysts g}^{-1} \text{ wet sediment (WS)} / [1 - (\text{water content}/100)].$$

Statistical analysis

We used one-way variance analysis (ANOVA) to assess the variability of parameters among stations and seasons. Potential relationships between variables were tested by Spearman's correlation coefficient. Redundancy analysis (RDA) was performed to define the structuring effects of sampling station and seasons along with environmental conditions on cyst abundances. To prevent any disproportionate influence of rare species in the subsequent

analysis, the only species considered were the dominant cysts. Cyst abundances were normalised prior to analysis. Statistical analysis was produced by R 2.15.0 (R Core Team development, 2012) with “Vegan” (Oksanen, 2011) and “Pgirmess” (Giraudoux, 2012) packages.

2.3. Results

Environmental conditions

Mean water temperature increased in spring and reached its maximum (26.9 °C) in summer. Temperature varied significantly from season to season (ANOVA, $F = 475.75$, $p < 0.001$), but not among sampling sites (ANOVA, $F = 0.54$, $p = 0.7$). Water salinity varied from 35.5 to 51. Salinity peaked in summer due to the combined effect of high temperature, inducing water evaporation and the low inflow of freshwater to the lagoon. It varied significantly according to season (ANOVA, $F = 9.73$, $p = 0.001$) but not among station (ANOVA, $F = 0.79$, $p = 0.54$), and its seasonal changes roughly followed those of temperature ($r = 0.91$, $n = 20$, $p = 0$). The pH ranged from 8 to 8.6 and did not varied significantly according to season (ANOVA, $F = 0.96$, $p = 0.43$) or station (ANOVA, $F = 0.45$, $p = 0.77$). Water Transparency were closed (1.07-1.78 m) and did not differ significantly between seasons (ANOVA, $F = 0.57$, $p = 0.64$) or stations (ANOVA, $F = 2.42$, $p = 0.1$).

Maximum concentrations of nutrients were illustrated in Table 21. Nutrients varied significantly among seasons (ANOVA, $p < 0.001$ for each nutrient) but not among sampling stations. Most of nutrients concentrations decreased in summer and showed a remarkable increase in winter due to dilution by floods. Total phosphorus (T-P) concentrations were high (1.78 - 3.75 $\mu\text{mol l}^{-1}$). Orthophosphate concentrations were lower (0.069 - 0.316 $\mu\text{mol l}^{-1}$). Total nitrogen (T-N) concentrations showed a regular trend (mean \pm s.d. = 11.67 \pm 0.65 $\mu\text{mol l}^{-1}$). DIN values were higher (2.99 - 7.04 $\mu\text{mol l}^{-1}$). Total nitrogen and phosphorus show similar trends ($r = 0.52$, $p = 0.019$). Silicate concentrations ranged from 3.05 $\mu\text{mol l}^{-1}$ (winter) to 6.27 $\mu\text{mol l}^{-1}$ (summer).

The % H₂O of sediment samples was homogenous (mean \pm s.d = 81.82 \pm 4.71) (Table 22) with no significant variation from station to station (ANOVA, $F = 0.46$, $p = 0.75$) or between the four seasons (ANOVA, $F = 0.77$, $p = 0.52$). The maximum of 87.9 was detected in summer at station 4 and the minimum of 73.1 was registered in winter at station 4. The occurrence of high % H₂O at the different zones of the lagoon was related to the dominant of muddy areas in GML.

Tableau 21. Nutrient concentrations recorded at four successive seasons: autumn 2012, winter 2012, spring 2013 and summer 2013. Maximum and minimum values are reported for each season. Bold values refer to poor water quality according to IFREMER (2012).

Sampling	Autumn		Winter		Spring		Summer	
	Max	Min	Max	Min	Max	Min	Max	Min
Nutriments ($\mu\text{mol l}^{-1}$)								
NO ₂ ⁻	0.215	0.098	0.219	0.143	0.165	0.107	0.156	0.067
NO ₃ ⁻	3.934	1.193	3.267	2.725	3.145	2.122	2.205	1.958
NH ₄ ⁺	4.385	1.647	3.609	1.966	3.672	1.945	3.932	0.874
PO ₄ ³⁻	0.316	0.075	0.229	0.118	0.172	0.096	0.116	0.069
Si(OH) ₄	5.187	3.604	5.405	3.056	5.954	3.087	6.277	5.053
T-N	15.407	10.335	13.283	11.972	11.526	10.977	11.357	10.123
T-P	3.751	1.789	3.704	2.777	3.139	2.142	2.457	1.978
DIN	6.476	4.739	7.042	5.169	6.924	4.726	6.073	2.996
DIN/DIP	75.353	17.962	54.440	22.572	53.010	40.255	88.014	32.017
PO ₄ ³⁻ /PT	8.424	3.617	7.154	3.590	7.220	3.408	4.919	3.488

Tableau 22. Water content (% H₂O) for each sediment sample.

	S1	S2	S3	S4	S5
Autumn	85	82.6	78.55	84.9	82.1
Winter	87.85	73.1	83.45	74.9	76.25
Spring	82.25	78.85	73.7	86.4	86.6
Summer	76.75	85.7	83.15	87.9	86.5

Cyst composition

About 16 morphotypes were identified in the surface sediment of GML spread over 4 orders namely Gonyaulacales (at least 8 species), Peridinales (at least 4 species), Gymnodinales (at least 2 species) and Thoracosphaerales (at least 2 species) (Table 23, Figure 27). The highest cyst density (229 cysts g⁻¹ DS) was recorded in autumn at station 2 near El Boughaz the only access of fisher boats, the lowest (no cysts) was detected at five samples in the different seasons except autumn. The cyst assemblage was largely dominated by *Protoperidinium* spp. (48.82 %), *Scrippsiella trochoidea* complex (9.56 %), round brown cysts (RBC) (7.22 %), *Lingulodinium machaerophorum* (5.36 %), *Alexandrium* spp. (5.35 %) and *Gymnodinium* spp. (4.03 %). The remaining cysts were represented by infrequent morphotypes (Table 23) and some unidentified cysts. Four morphotypes related to potentially noxious/toxic species

were detected in surface sediment, namely *Alexandrium minutum*, *Alexandrium pacificum* complex, *Alexandrium pseudogonyaulax* and *L. machaerophorum* (Table 23; Figure 27).

Tableau 23. Taxonomic citation, biological names and harmful effects of dinoflagellate cysts identified in the present study.

Order	Paleontological names	Biological names	Toxicity	Concentrations (cysts g ⁻¹ DS)
Gonyaulacales	Cysts of <i>Alexandrium pacificum</i> complex (Bravo <i>et al.</i> , 2006)	<i>Alexandrium pacificum</i> complex	Paralytic shellfish poisoning	0-17
	Cysts of <i>Alexandrium affine</i> (Hallegraeff and Bolch, 1991)	<i>Alexandrium affine</i>		0-20
	Cysts of <i>Alexandrium minutum</i> (Bravo <i>et al.</i> , 2006)	<i>Alexandrium minutum</i>	Paralytic shellfish poisoning	0-6
	Cysts of <i>Alexandrium pseudogonyaulax</i> (Bravo <i>et al.</i> , 2006)	<i>Alexandrium pseudogonyaulax</i>	High biomass proliferation	0-6
	<i>Spiniferites ramosus</i> (Ehrenberg, 1838) Mantell, 1854	<i>Gonyaulax spinifera</i> complex		0-5
	<i>Spiniferites</i> spp.	<i>Gonyaulax</i> spp.		0-8
	<i>Lingulodinium machaerophorum</i> (Mertens <i>et al.</i> , 2009)	<i>Lingulodinium polyedrum</i>	Yessotoxin producer	0-17
	<i>Impagidinium</i> sp.	<i>Gonyaulax</i> sp.		
Peridinales	<i>Quinquecuspis concreta</i> (Price and Pospelova, 2011)	<i>Protoperidinium leonis</i>		0-8
	<i>Votadinium spinosum</i> (Repakevich and Pospelova., 2010)	<i>Protoperidinium claudicans</i>		0-5
	<i>Votadinium Calvum</i> (Reid, 1977)	<i>Protoperidinium oblongum</i>		0-10
	Cysts of <i>Protoperidinium</i> spp.	<i>Protoperidinium</i> spp.		0-160
Gymnodinales	Cysts of <i>Polykrikos kofoidii</i> (Matsuoka <i>et al.</i> , 2009)	<i>Polykrikos kofoidii</i>		0-7
	Cysts of <i>Gymnodinium</i> spp.	<i>Gymnodinium</i> spp.		0-20
Thoracosphaerales	Cysts of <i>Scrippsiella trochoidea</i> complex (von Stein, 1883) Loeblich, 1976	<i>Scrippsiella trochoidea</i>		0-33
	Cysts of <i>Scrippsiella</i> spp.	<i>Scrippsiella</i> spp.		0-17

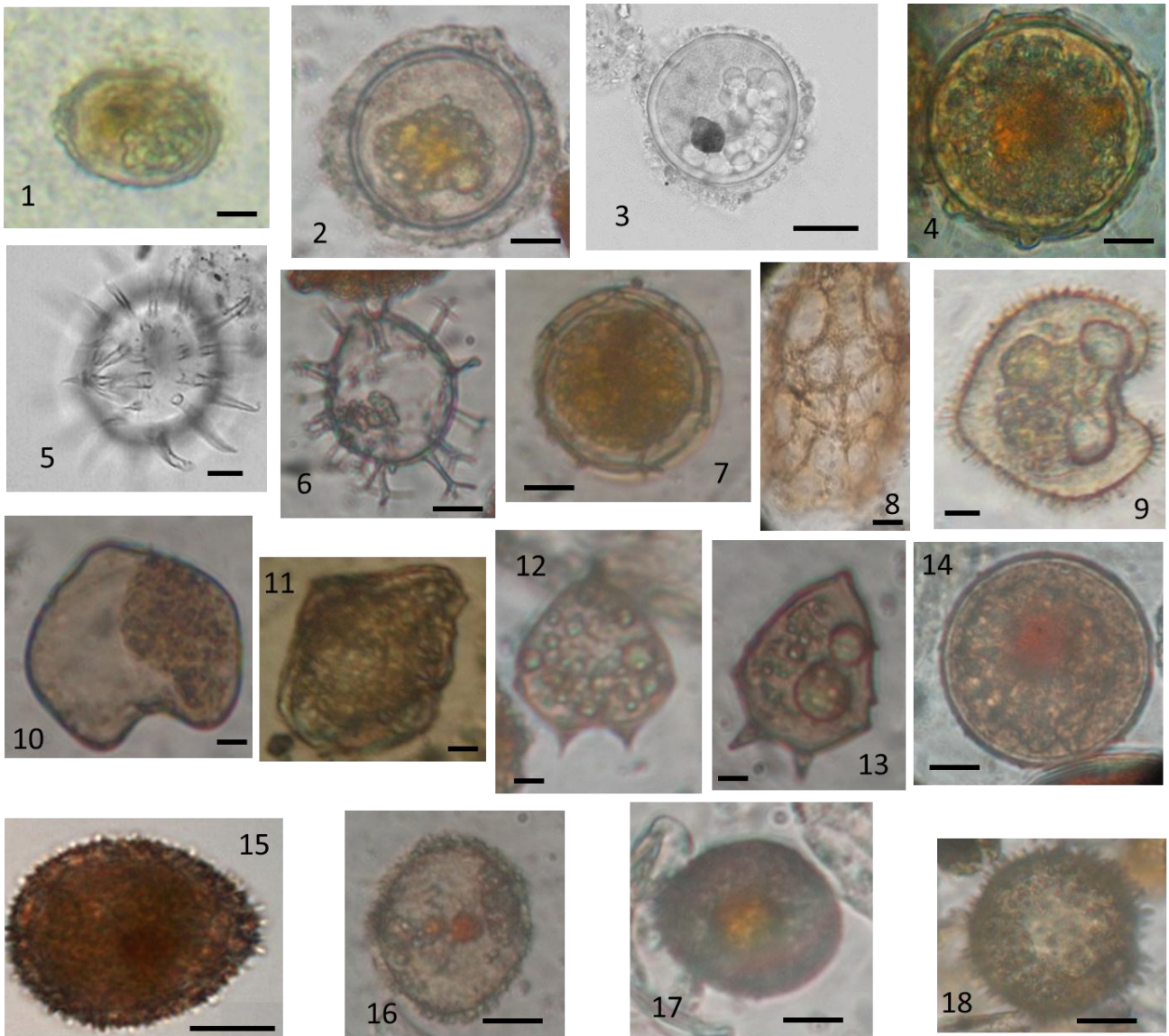


Figure 27. Dinoflagellate cysts isolated from recent sediments in Ghar El Melh lagoon. **1.** Cyst of *Alexandrium catenella* complex **2.** Cyst of *Alexandrium affine* **3.** Cyst of *Alexandrium minutum* **4.** Cyst of *Alexandrium pseudogonyaulax* **5.** *Lingulodinium machaerophorum* **6.** *Spiniferites* sp. **7.** *Impagidinium* sp. **8.** *Polykrikos kofoidii* **9.** *Votadinium spinosum* **10.** *Votadinium Calvum* **11.** *Quinquecuspis concreta* **12-13-14.** Cysts of *Protoperidinium* spp. **15.** Cysts of *Scrippsiella trochoidea* complex **16-17-18.** Cysts of *Scrippsiella* spp. Scale bars 10 μm .

Distribution of dominant cysts

No significant difference in cyst abundance was found among sampling sites (RDA, $F = 0.62$, $p = 0.9$), although a significant change was seen according to seasons (RDA, $F = 4.11$, $p < 0.001$). Both RDA axis 1 and 2 supports a significant effect on cyst variability ($p < 0.05$)

(Figure 28). Autumn clearly stand out with a negative RDA axis 1 score and thus associated mainly with cysts of *Protoperidinium* spp. and *Gymnodinium* spp. and *L. machaerophorum* cysts. Indeed, the highest concentrations of all these cysts were recorded in autumn with respective concentrations of 160, 20 and 17 cysts g⁻¹ DS. RBC cysts have positive RDA axes 1 and 2 scores and thus associated with spring observation. The remaining cysts (cysts of *S. trochoidea* and *Alexandrium* spp. and *L. machaerophorum* cysts) show RDA scores close to 0 indicative of similar distribution in all seasons.

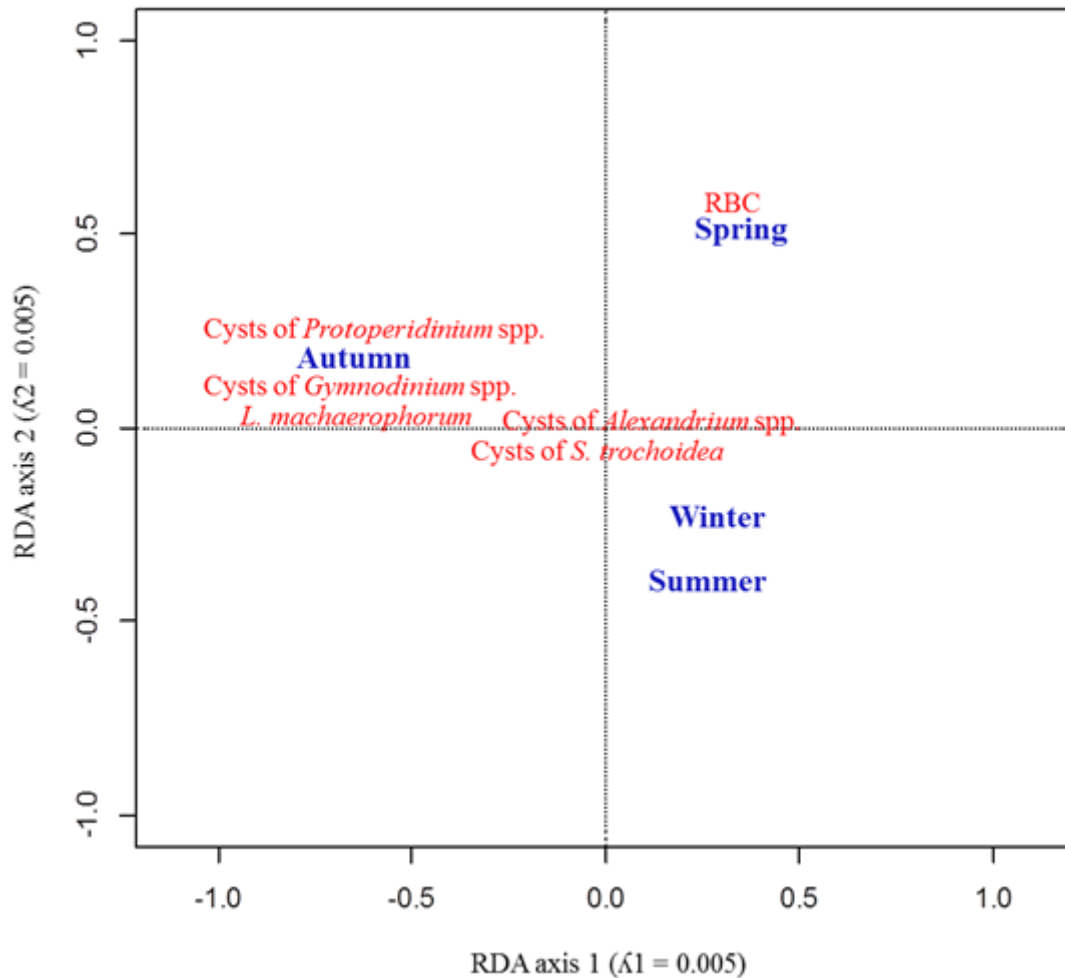


Figure 28. RDA TriPlot depicting the association between dinoflagellates cysts and sampling seasons in Ghar El Melh Lagoon. Eigenvalues of the first two axes are indicated by λ_1 and λ_2 .

No significant variation was found between cyst abundance and the different abiotic factors monitored neither in the water column (physic-chemical parameters) nor in the sediment (% H₂O). As well, no significant difference in cyst abundance was found in relation to phytoplankton abundance.

Microphytolankton

During the four sampling seasons, microphytoplankton community consisted of only three groups identified as Dinophyceae, Bacillariophyceae and Cyanobacteriae. Microphytoplankton abundance ranged from 80 to 31000 cells l⁻¹. Two peaks of microphytoplankton were detected in spring (mean ± sd = 1.48 10⁴ ± 9.7 10³ cells l⁻¹) and winter (mean ± sd = 6.56 10³ ± 7.23 10³ cells l⁻¹) mainly assigned to dinoflagellates community and specially to *Prorocentrum micans* (maximal abundance = 2.95 10⁴ cells l⁻¹). Minimum concentrations were detected in summer (mean ± sd = 169 ± 93 cells l⁻¹).

2.4. Discussion

The results of the present study reveal the weakness of the cyst bank of Ghar El Melh Lagoon. Dinoflagellate cysts abundance in surface sediments did not exceed 229 cysts per gram of dry sediment and was much lower than that reported for other Mediterranean areas (Giannakourou *et al.*, 2005; Elshanawany *et al.*, 2010; Satta *et al.*, 2010; Fertouna-Bellakhal *et al.*, 2014) and temperate zones (Pospelova *et al.*, 2005, 2008; Radi *et al.*, 2007; Harland *et al.*, 2006), but similar to that detected in tropical zones (Lirdwitayaprasit, 1998; Azanza *et al.*, 2004; Kawamura, 2004) which harbour dinoflagellates most of which are non-cyst formers. Interestingly, although the importance of muddy areas in the field revealed by our personal observations, by Chakroun (2004) and also by the high % H₂O measured in all the sampled stations, this parameter (fine sediment particles) seems with no influence on the cyst abundance of GML while it's known to be a favourable for cyst accumulation in other areas (Marcus, 1989; Anglès *et al.*, 2010; Horner *et al.*, 2011, Genovesi *et al.*, 2013).

Among the dinoflagellate cysts, the protoperidinioids, represented by *Votadinium Quinquecuspis* and cysts of *Protopteridinium* and the gonyaulacoids, namely *Lingulodinium*, *Alexandrium*, *Spiniferites* and *Impagidinium* were the most abundant. Dominance by these groups has usually been recorded in eutrophic waters as has been indicated in many studies (Matsuoka, 1999; Matsuoka *et al.*, 2003; Pospelova *et al.*, 2002; Wang *et al.*, 2004). Cysts of *Protopteridinium* spp. are the most abundant and diverse morphotypes in the lagoon. They comprise almost the half of the total population, suggesting a steady sedimentation into the study area. *L. machaerophorum*, the fourth most abundant morphotype in this study, is also often used as a proxy for eutrophication (Dale *et al.*, 1999; Harland *et al.*, 2004). The use of dinoflagellate cysts as indicators of eutrophication has been well documented (Dale *et al.*, 1999; Matsuoka, 1999; Matsuoka *et al.*, 2003; Pospelova *et al.*, 2002; 2005).

Use of the Ludox technique and no acid treatment for cyst identification, allowed us to observe the presence of many morphotypes belonging to the calcareous group represented by the cysts of *Scrippssiella* spp. The most important cysts were *S. trochoidea* with a steady presence in the surface sediment in concordance with its steady presence in the water column of GML (Dhib *et al.*, 2013a; Dhib *et al.*, 2015). The sediment seems to be a continual source for these populations.

Cysts of *Alexandrium* spp. and *L. machaerophorum* cysts, known as producers of harmful motile cells, have a noteworthy presence in GML, representing more than 10 % of the community. *Alexandrium* genus is widespread in the Mediterranean Sea (Penna *et al.*, 2008; Abdennadher *et al.*, 2012), and is considered to be the most important PSP toxin-producing dinoflagellate (Taylor *et al.*, 1995). *L. machaerophorum* cysts are common in Mediterranean sediments (Montresor *et al.*, 1998; Rubino *et al.*, 2010; Giannakourou *et al.*, 2005; Elshanawany *et al.*, 2010; Satta *et al.*, 2010) and are known to cause toxic blooms, mainly in the northern Adriatic Sea (Pistocchi *et al.*, 2012). In GML, blooms of *Alexandrium* species ($\approx 1.5 \cdot 10^6$ cells l^{-1}) have been previously detected (Romdhane *et al.*, 1998), but were not observed during this study, nor have they been reported in other studies carried out in the lagoon (SCET-ERI, 2000; Ramdani *et al.*, 2004; Dhib *et al.*, 2013a; Dhib *et al.*, 2015). The few existing studies of GML's microalgae have signaled the presence of the motile cells *L. polyedrum*, but in few concentrations (SCET-ERI, 2000; Dhib *et al.*, 2013a; Dhib *et al.*, 2015). This could be explained by the non-favorable conditions for excystment of *Alexandrium* and *L. machaerophorum* cysts or by their few concentrations which did not exceed 20 and 17 cysts per gram respectively (no seedbeds for blooms initiation).

No significant variation of cyst distribution was found according to stations or environmental factors. Some hypotheses could explain these results:

- (i) The non-significant variation of all environmental parameters according to stations (ANOVA test) may be the main explanation of the non-significant spatial variation of cyst abundance. Indeed, all the abiotic factors monitored in the water column (temperature, salinity, pH and nutrients) as well as in the sediment (water content) did not varied among the stations suggesting a similar conditions in all the field and thus with no influence on the spatial distribution of cysts.
- (ii) The presence of others processes and factors which can also influence the cysts abundance and not fully assessed in this study, such as, the encystment capability of planktonic species in the water column, sedimentation, transport and bioturbation processes (Anderson, 1997, Garcés *et al.*, 2004; Joyce *et al.*, 2005).

For instance, Mud sediment combined with high sedimentation rate promotes resting cysts burying (Anderson *et al.*, 1982; Godhe and Mcquoid, 2003). In GML, because of its shallowness, the rate of sedimentation is very high in the field ranged from 0.37 to up to 2.44 cm per year (Flower *et al.*, 2009) and could be behind the few cysts abundance at the upper layers of sediment. Optimization of the sampling technique, such as using core sampling, may investigate more cysts in the deepest layers of sediment.

On the other hand, a significant variation of cyst abundances was seen according to seasons with maximum concentrations recorded in autumn. This could be in relation to microphytoplankton community. Indeed, although, there is no significant correlation between cysts and microphytoplankton during our study, some common features could be revealed and explained the abundance and the composition of dinoflagellate cysts

- (i) first, by the dominance of the non-formers cysts in the water column of GML (present study, Dhib *et al.*, 2013a; Dhib *et al.*, 2015) which could explain the scarcity of cyst abundances.
- (ii) second, by the steady presence of *Protoperdinium* spp. and *Scrcpsiella* spp. in the water column of GML (present study, Dhib *et al.*, 2013a; Dhib *et al.*, 2015) which could explain the dominance of these species as cysts, in the sediment surface.
- (iii) third, by the weak presence of phytoplankton, especially dinoflagellates, in the water column during autumn which could be a result of dinoflagellates encystment in concordance with the high cysts abundance recorded in this season.

According to the current statue of dominant cysts, it seems there is no potential threat for likely bloom initiation.

2. 5. Conclusion

Contrary to the different Mediterranean and Tunisian areas, this study illustrates the poverty of dinocysts bank of Ghar El Melh lagoon. The water stagnancy of the majority GML's zones and the weak water current seem do not allow cysts transfer and accumulation in the sediment as has been reported elsewhere (Matsuoka and Fukuyo, 2000; Joyce *et al.*, 2005; Fertouna-Bellakhal *et al.*, 2014). Burying of cysts in the deeper layer of sediment, especially with the high rate of sedimentation and the shallowness of the lagoon, may was the foremost hypothesis. This may be the case of Alexandrium cysts which the blooming of their vegetative cells have been early detected in GML but with no other comeback.

Furthermore, the eutrophication in the lagoon, revealed by the increasing nutrient concentrations as well as the dominance of cysts indicators of eutrophication, was known as responsible for the overwhelming of mixotrophic dinoflagellates such as *Prorocentrum* species (Burkholder *et al.*, 2008; Dhib *et al.*, 2015) and which are non-former cysts. Thus, the eutrophication, also, could indirectly influence the abundance and composition of the cyst beds of Ghar El Melh lagoon.

Finally, this study provides the basis for further studies to match the cysts abundance and composition with the vegetative cells prevailing on the water column especially in peculiar ecosystems such as lagoons.

Acknowledgements

This study was conducted by Amel DHIB as a part of her PhD research (co-directed at the University of Franche-Comté, CNRS 6249, Besançon, France, and at the Institut National des Sciences et Technologie de la Mer, Tunisia).

3. Distribution spatio-temporelle de l'ultraphytoplancton et des procaryotes hétérotrophes et relation avec les communautés microphytoplanctoniques

Article soumis. Ultra- and microplankton assemblages as indicators of trophic status in a Mediterranean lagoon







Article soumis dans le journal 'Ecological Indicators' (IF = 3.44)

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Action 	Manuscript Number 	Title 	Initial Date Submitted 	Status Date 	Current Status 
Action Links	ECOLIND-5238	Ultra- and microplankton assemblages as indicators of trophic status in a Mediterranean lagoon	May 18, 2015	May 23, 2015	Under Review

Résumé

Dans cet article, il s'agit d'étudier les composantes du phytoplancton dont la taille est inférieure à 5 μm s'agissant de l'ultraphytoplancton, des procaryotes hétérotrophes ainsi que les interactions avec le microphytoplancton et les ciliés de la lagune de Ghar el Melh. Le prélèvement des différentes communautés a été effectué au niveau de 5 stations pendant quatre campagnes saisonnières (automne 2012 - hiver 2012 - printemps 2013 - été 2013).

Concernant les procaryotes hétérotrophes, 2 groupes ont été identifiés : HNA (high nucleic acid content) et LNA (low nucleic acid content) avec des pourcentages respectifs de l'ordre 57.47 % et 42.52 %. Selon les analyses de redondance, HNA et LNA ont été corrélés surtout avec les saisons d'été et d'automne. Selon les analyses de Modèles Additifs Généralisés (GAM), l'abondance totale des procaryotes hétérotrophes a varié avec la température de l'eau et la teneur en orthophosphate.

Pour l'ultraphytoplancton, 12 groupes ont été identifiés dont 5 groupes dominants : nanoeucaryotes 64.5 %, cryptophytes 27.7 %, picoeucaryotes 2.79 %, *Synechococcus* 2.74 %, *Prochlorococcus* 0.05 %, et le reste correspondant à des groupes inconnus (2.22 %). Selon les analyses de redondance, *Synechococcus* et picoeucaryotes ont été relevés pendant l'automne. Les nanoeucaryotes et les cryptophytes ont été enregistrés en hiver et en été, alors que *Prochlorococcus* a été relié à la saison hivernale. Selon les analyses de GAM, l'abondance totale d'ultraphytoplancton varie avec la température et la salinité.

Des corrélations positives significatives ont été trouvées entre le microphytoplancton et les ciliés. Il est intéressant de noter des corrélations significatives négatives entre l'ultraplancton et le microplancton notamment entre le microphytoplancton et les picoeucaryotes ($r = -0.59$; $p = 0.006$) et entre les ciliés avec les cryptophytes ($r = -0.52$; $p = 0.019$) et HNA ($r = -0.6$; $p = 0.005$).

Abstract

The seasonal abundance distribution of heterotrophic prokaryotes, pico and nanophytoplankton were investigated in connexion with environmental factors and microplankton abundance at five stations in the Ghar El Melh Lagoon (North Tunisia). Flow cytometry analysis of ultraplankton resolved (i) five heterotrophic prokaryote groups labelled LNA1, LNA2 (low nucleic acid content), HNA1, HNA2 and HNA3 (high nucleic acid content) and (ii) at least 14 ultraphytoplankton groups assigned to picoeukaryotes, picoprokaryotes, nanoeukaryotes, cryptophyte-like cells and some unknown communities. RDA analyses revealed (i) autumn-summer proliferation of heterotrophic prokaryotes dominated by HNA groups and (ii) winter-summer proliferation of ultraphytoplankton dominated by nanophytoplankton groups. GAM analyses highlighted the role of (i) water temperature and orthophosphate concentrations on heterotrophic prokaryote distribution and (ii) water temperature and salinity on ultraphytoplankton abundance variation. Based on Spearman's rank correlation, significant negative correlations were established between ultra and microplankton communities suggesting that microplankton may be behind, through grazing pressure, the drastically decrease of ultraplankton abundances during the spring season.

Keywords: Heterotrophic prokaryotes, ultraphytoplankton, microplankton, abiotic factors, Ghar El Melh Lagoon

3.1. Introduction

For decades, the dynamics of plankton communities and the relationships within food webs in aquatic ecosystems have been shown to be heavily dependent on the size spectrum of species (Rai, 1982; Aleya 1989; Jacquet *et al.*, 2002). Size is frequently used as the basis for defining functional groups within the plankton community (Quinones, 1994; Padišak *et al.*, 2009; Naselli-Flores and Barone, 2012) since species within a given size class tend to respond to environmental changes in a similar manner (Malone, 1980; Reynolds *et al.*, 2002; Aleya *et al.*, 2011). In addition, the contribution of each plankton size class differs from oligotrophic to eutrophic waters with oligotrophic ecosystems dominated by pico- (Platt *et al.*, 1983; Glover *et al.*, 1988; Goericke and Welschmeyer, 1993) and nanoplankton (Booth *et al.*, 1982; Hannah and Boney, 1983). For instance, Weisse (1993) and Rodrigues and Williams (2002) reported a dominance of picoplankton in oligotrophic ecosystems coinciding with maximum concentrations of heterotrophic bacteria. A number of studies have also shown that ultraphytoplankton (<10 µm) make up the most abundant fraction in these waters (Psarra *et al.*, 2000; Vidussi *et al.*, 2001; Siokou-Frangou *et al.*, 2010). This autotrophic ultraphytoplankton is mainly composed of photosynthetic prokaryotes, with, in the Mediterranean Sea, dominance of the cyanobacteria *Synechococcus*, ubiquitous in oceanic areas of varying trophic levels (Li *et al.*, 1992; Rekik *et al.*, 2012), over *Prochlorococcus* (Martin, 1997; Denis *et al.*, 2000; Casotti *et al.*, 2003; Psarra *et al.*, 2005) and of eukaryotes belonging to the group of Chlorophyceae, Prasinophyceae and Prymnesiophyceae (Vidussi *et al.*, 2001; Psarra *et al.*, 2005). In contrast, large cells such as microphytoplankton seem to dominate in eutrophic ecosystems.

It therefore appeared interesting to conduct a survey in the eutrophic Ghar El Melh Lagoon. Our aims are (i) to bring information, for the first time, about the heterotrophic prokaryotes and ultraphytoplankton (pico and nanophytoplankton) in GML waters and the types numerically most important and (ii) to recognize the seasonal distribution of these communities in relation to both abiotic (temperature, salinity and nutrients) and biotic (microalgae + ciliates) factors.

3.2. Materials and methods

Study area

The Ghar El Melh Lagoon (GML) is a RAMSAR site (RAMSAR, 2007), located in the western Mediterranean Basin on the north coast of Tunisia (37° 06'-37°10' N and 10°08'-10°15' E) and influenced by the regional water circulation (Ben Ismail *et al.*, 2012) (Figure 19, Chap

IV.1). GML has an area of about 3000 ha including 2 small sub-lagoons, namely Sabkhet El Ouafi and Sabkhet Sidi Ali El Mekki. The main lagoon is permanently connected with Sabkhet El Ouafi but isolated from Sabkhet Sidi Ali El Mekki by embankments. It is connected to the Mediterranean Sea via a permanent channel called “El Boughaz” that passes through the coastal sand bars.

Sampling

Five stations (S1–S5) were chosen to deal with the different environmental conditions found within GML (Figure 19, Chap IV.1). S1 is located in the north-eastern part of the lagoon, a shallow area influenced by freshwater inputs from ‘El Ayoune’, with a mat of limnetic plants (reeds) around the edges. S2 faces the El Boughaz Channel which is a permanent 70-m-wide connection with the Gulf of Tunis. El Boughaz Channel makes the deepest area of GML and is influenced by strong hydrodynamics despite its continuous silting. S3 is located in the south-east of GML, in the middle of Sabkhet El Ouafi, a semi-closed zone and one of the most stagnant areas. S4, in the south-west of GML, is affected by heavy industrial discharge (from 43 industrial units) and is drained by tributaries mainly in winter. S5, located in the northern part of the lagoon, is affected by agricultural and urban discharges. Leachate from rural zones, rainwater and the wastewater from the city of Ghar El Melh outpour directly into this part of the lagoon.

Sampling was conducted during four seasons (autumn 2012, winter 2012, spring 2013 and summer 2013) at the five sampling sites. Samples for ultraphytoplankton and heterotrophic prokaryotes FCM analysis were collected (in duplicates) from surface waters and filtered through 100 µm mesh size net to prevent clogging of the cytometer flow cell. Microphytoplankton and ciliates were collected using a polyvinylchloride (PVC) tube.

Environmental variables were measured in the field concomitantly with ciliate and phytoplankton sampling. A WTW multi-parameter sensor was used for water temperature (°C) and salinity. Transparency was determined using a Secchi disk. Water samples were collected in 1000 cm³ polypropylene bottles at 30 to 50 cm depth. In the laboratory, nutrients (ammonium, nitrite, nitrate, phosphate, silicate) were analysed with a BRAN and LUEBBE type 3 autoanalyser and concentrations were determined by colourimetry with a UV-visible (JENWAY 6705) spectrometer (APHA, 1992).

Flow cytometry

For determining heterotrophic prokaryote and autotrophic pico-nanoplankton abundances subsamples (1.8 cm³) were immediately fixed with 0.2 cm³ of a 20% (w/v) borate-buffered formalin solution, pH 7, and then freeze-trapped in liquid nitrogen. They were stored at

- 80° C in the laboratory until flow cytometry analysis at the Regional Flow Cytometry Platform for Microbiology (PRECYM, <https://precym.mio.univ-amu.fr/>) at the Mediterranean Institute of Oceanography (MIO) of Marseille (France). To that purpose, frozen samples were transferred in dry ice to MIO where they were analysed with an INFLUX™ (BD) cell sorter flow cytometer. Samples were thawed at room temperature, homogenised and directly analysed for autotrophic pico- and nanophytoplankton. For analysis of heterotrophic prokaryotes, a subsample of 300 mm³ was incubated for 15 min in the dark with a 10 mm³ SYBRGreen solution (Sigma, commercial solution diluted 1000 times) to stain the nucleic acids and distinguish unambiguously the heterotrophic prokaryotes (green fluorescence) from inorganic particles, detritus and free DNA (Marie et al., 2000). The SYBRGreen family dyes are widely used to stain nucleic acids of aquatic bacteria and in particular marine bacteria (Lebaron et al., 1998). SYBRGreen is the dye of choice due to its high fluorescence yield when bound to nucleic acids (<http://www.invitrogen.com>). Data were analysed with the SUMMIT software package (DAKO). Cell groups were resolved on the basis of the cell optical properties regarding scatter and fluorescence. To isolate heterotrophic prokaryotes from overlapping small autotrophic cells, the latter were removed on the basis of their red fluorescence.

Scanning electron microscopy

Sorted samples were filtered onto 0.2 µm nuclepore filters (25 mm diameter), dehydrated through an ethanol bath (70 %), dried out and metal-coated with palladium gold.

The observations of the sorted samples were performed at the SEM facility of the Centre Interdisciplinaire de Nanoscience de Marseille (CINaM) by HRSEM (high resolution scanning electron microscopy) JSM-6320F (JEOL Company). This SEM was a FEGSEM (Field Emission Gun Scanning Electron Microscope) equipped with an EDS (BRUKER Company) for the chemical microanalysis. The FEGSEM resolution was 1.2 nm at 15 kV and 2.5 nm at 1kV (at 6 mm Working Distance) with a magnification from 25 x to 650,000 x. High resolution observations on insulators are possible at low accelerating voltages with this instrument due to its Field Emission Gun (cold cathode) and its Secondary Electrons In Lens Detection. The insulator characteristics of our samples meant that we had to work at very low accelerating voltages and coat them with a very thin conductive gold layer (10 nm). The gold plating step was carried out with an Ion Cathode Sputtering System manufactured by JEOL. The digital image acquisition was carried out with a NEWTEC System.

Microplankton analysis

To establish the composition of microplanktonic communities, sub-samples (25 cm³) were

fixed with a lugol solution (final concentration 1% v/v) and settled for 48 hours according to the Utermöhl method (1958). Then, cells were counted under an inverted microscope.

Identification of algal taxa was achieved according to various keys of Dodge (1982, 1985); Sournia (1986), Balech (1988), Tomas *et al.* (1996). Ciliate identification was performed using the keys of Corliss (1961), Petz (1999) and Struder-Kypke and Montagnes (2002). Microplankton (phytoplankton and ciliates) abundances were expressed as cells dm^{-3} .

Statistical analysis

We used one-way variance analysis (ANOVA) to assess the variability of parameters among stations and seasons. Potential relationships between variables were tested by Spearman's correlation coefficient. Redundancy analysis (RDA) was performed to define the structuring effects of sampling sites and seasons on ultraphytoplankton and heterotrophic prokaryote abundances. Plankton abundances were normalized prior to analysis. The relationships between total abundance of ultraphytoplankton and heterotrophic prokaryotes, and abiotic factors, were assessed using generalised additive models (GAM; Wood 2006). GAMs can be considered as a nonparametric generalisation of linear regressions and are increasingly used in marine ecology. The model validation was assessed according to Züür *et al.* (2010). Statistical analysis and graphical display were produced using R 2.15.0 (R Development Core Team, 2012) with the R packages of "Vegan" (Oksanen *et al.*, 2011), "Pgrimmess" (Giraudoux, 2012) and "mgcv" (Wood, 2006).

3.3. Results

Hydrological conditions

Water temperature ranged from 11 °C in winter to 26.9 °C in summer. It varied significantly from season to season (ANOVA, $F = 475.75$, $p < 0.001$), but not among sampling sites (ANOVA, $F = 0.54$, $p = 0.7$). Water salinity varied from 35.5 to 51. It varied significantly according to season (ANOVA, $F = 9.73$, $p = 0.001$) but not among station (ANOVA, $F = 0.79$, $p = 0.54$), and its seasonal changes roughly followed those of temperature ($r = 0.9$, $p = 0$). Salinity peaked in summer due to the combined effect of high temperature, inducing water evaporation and the low inflow of freshwater to the lagoon. The pH ranged from 8 to 8.6 and did not varied significantly according to seasons (ANOVA, $F = 0.96$, $p = 0.43$) or stations (ANOVA, $F = 0.45$, $p = 0.77$). Water transparency was high (75-100 %) and homogenous (1.07-1.78 m) and did not differ significantly between seasons (ANOVA, $F = 0.57$, $p = 0.64$) or stations (ANOVA, $F = 2.42$, $p = 0.1$).

Nutrient maximum concentrations are reported in Table 21 (Chap IV.2). Nutrient concentration varied significantly among seasons (ANOVA, $p < 0.001$ for each nutrient) but not among sampling stations. Nutrients concentration decreased in summer and showed a remarkable increase in winter and autumn due to the large fresh water input upon flooding. Total phosphorus (T-P) concentrations were high ($1.78 - 3.75 \mu\text{mol l}^{-1}$). Orthophosphate concentrations were lower ($0.069 - 0.316 \mu\text{mol l}^{-1}$), but never exhausted. Total nitrogen (T-N) concentrations showed a regular trend (mean \pm s.d. = 11.67 ± 0.65). Dissolved inorganic nitrogen (DIN) values were high ($2.99 - 7.04 \mu\text{mol l}^{-1}$). T-N and T-P show similar trends ($r = 0.52$, $p = 0.019$). Silicate concentrations ranged from $3.05 \mu\text{mol l}^{-1}$ (winter) to $6.27 \mu\text{mol l}^{-1}$ (summer).

Heterotrophic prokaryotes (HP)

HP were resolved into five groups (Figure 29), distinguished by their fluorescence intensity (nucleic acid content) and labelled LNA1 and LNA2 (low nucleic acid content), HNA1, HNA2 and HNA3 (high nucleic acid content). HNA and LNA groups were detected in GML waters with respective percentages of about 57.47 % and 42.52 %.

HP abundance ranged from $8.06 \cdot 10^5$ to $9.16 \cdot 10^6$ cells cm^{-3} . Maximum concentrations were detected in summer (mean \pm sd = $6.92 \cdot 10^6 \pm 2.33 \cdot 10^6$ cells cm^{-3}) and autumn (mean \pm sd = $5.88 \cdot 10^6 \pm 1.72 \cdot 10^6$ cells cm^{-3}) at all stations. Minimum concentrations were detected in spring (mean \pm sd = $20.1 \cdot 10^5 \pm 6.9 \cdot 10^5$ cells cm^{-3}) (Table 24).

No significant difference was found in HP abundance among sampling sites (RDA, $F = 0.43$, $p = 0.78$); however a significant HP abundance variation was observed with seasons (RDA, $F = 11.58$, $p < 0.001$). This temporal variability explained 68.4 % of changes in HP abundance with only RDA axis 1 supporting a significant effect on this variability ($p < 0.05$; Figure 30). Summer and autumn had a similar RDA axis 1 score, indicative of close relationships with both HNA and LNA groups. Spring and winter HP abundance had a similar RDA axis 1 score and thus correlated with LNA group. Indeed, maximum concentrations of both communities were detected in summer and autumn seasons. Nevertheless, LNA concentrations were also important in spring and winter (mean \pm sd = $1.43 \cdot 10^6 \pm 4.55 \cdot 10^5$ cells cm^{-3}) comparing to those registered in summer and autumn (mean \pm sd = $2.16 \cdot 10^6 \pm 8.21 \cdot 10^5$ cells cm^{-3}). Furthermore, the highest diversity of LNA group was detected in winter and spring with two subgroups for each season (Figure 29).

The Generalized additive model (GAM, Figure 31) selected temperature and orthophosphate concentration as the main driving environmental covariates distinguishing HP abundance. Total HP abundance had significant nonlinear relationships with both parameters as revealed

by the effective degrees of freedom (Table 25). Together, temperature and orthophosphate concentration, accounted for 65.6 % of the deviance in variability in HP abundance. The 3D plot (Figure 31) highlights a positive high temperature effect on HP abundance and an orthophosphate's negative effect in the range of 0.15 – 0.20 $\mu\text{mol l}^{-1}$. Our GAM predicts an optimum of HP abundance, in a context of temperature above 20 °C and two peaks of HP abundance, in a context of orthophosphate below 0.15 $\mu\text{mol l}^{-1}$ and above 0.20 $\mu\text{mol l}^{-1}$.

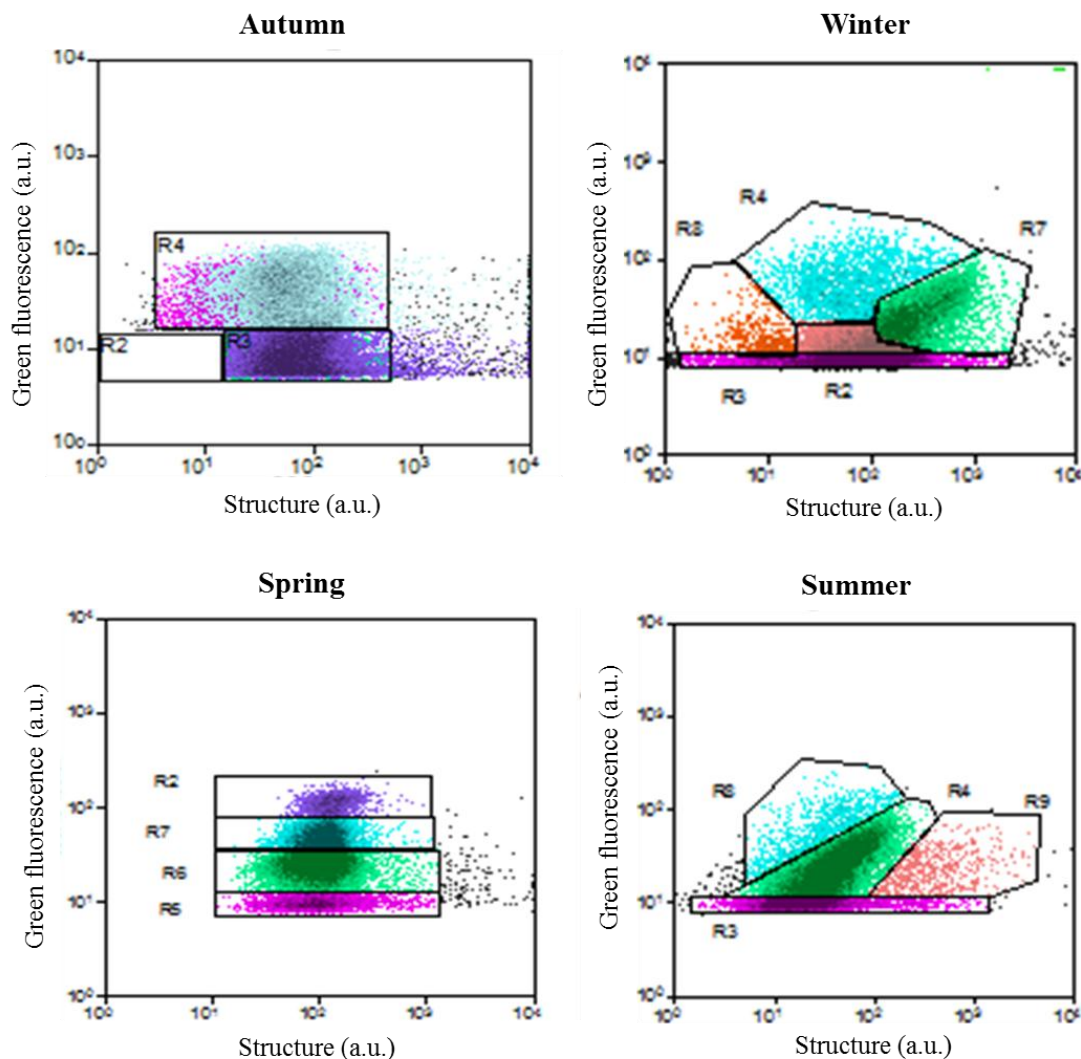


Figure 29. Typical heterotrophic procaryotes group resolution by flow cytometry for each season. Green fluorescence versus side scatter (linked to cell structure) cytogramme resolving HNA and LNA after staining with SYBR Green. a.u.: arbitrary unit. **(Autumn) R3:** LNA, **R4:** HNA. **(Winter) R2:** LNA1, **R8:** LNA2, **R4:** HNA1, **R7:** HNA2. **R3:** detritus. **(Spring) R5:** LNA1, **R6:** LNA2, **R2:** HNA1, **R7:** HNA2. **(Summer) R3:** LNA, **R4:** HNA1, **R8:** HNA2, **R9:** HNA3.

Tableau 24. Ultra and microplankton abundances observed at four successive seasons: autumn 2012, winter 2012, spring 2013 and summer 2013. Maximum and minimum values are reported for each season. HP: heterotrophic prokaryotes; HNA: high nucleic acid (HP); LNA low nucleic acid (HP).

Sampling	Autumn		Winter		Spring		Summer	
	Max	Min	Max	Min	Max	Min	Max	Min
Ultraplankton (cells cm⁻³)								
HP (*10 ⁶)	7.79	3.13	2.76	1.88	2.724	0.80	9.16	2.67
HNA (*10 ⁶)	5.38	1.57	1.90	0.05	0.85	0.26	6.78	2.07
LNA (*10 ⁶)	3.64	1.56	2.05	0.86	1.93	0.54	2.36	0.60
Ultraphytoplankton (*10 ⁵)	1.45	0.41	5.22	2.35	1.01	0.17	6.17	1.96
Nanoeukaryotes (*10 ⁵)	0.05	0.02	4.46	1.52	0.071	0.006	4.42	1.51
Cryptophytes (*10 ⁵)	0.81	0.13	1.15	0.48	0.71	0.06	1.76	0.45
Picoeukaryotes (*10 ⁴)	1.7	0.73	0.2	0	0.61	0.13	1.69	0.32
<i>Synechococcus</i> (*10 ⁴)	8.26	0.23	0	0	0	0	0	0
<i>Prochlorococcus</i> (*10 ³)	0	0	1.69	0	0	0	0	0
Microplankton (cells dm⁻³)								
Microphytoplankton (*10 ⁴)	0.26	0.008	2.06	0.01	3.09	0.05	0.02	0.008
Ciliates	280	0	440	40	600	120	80	280

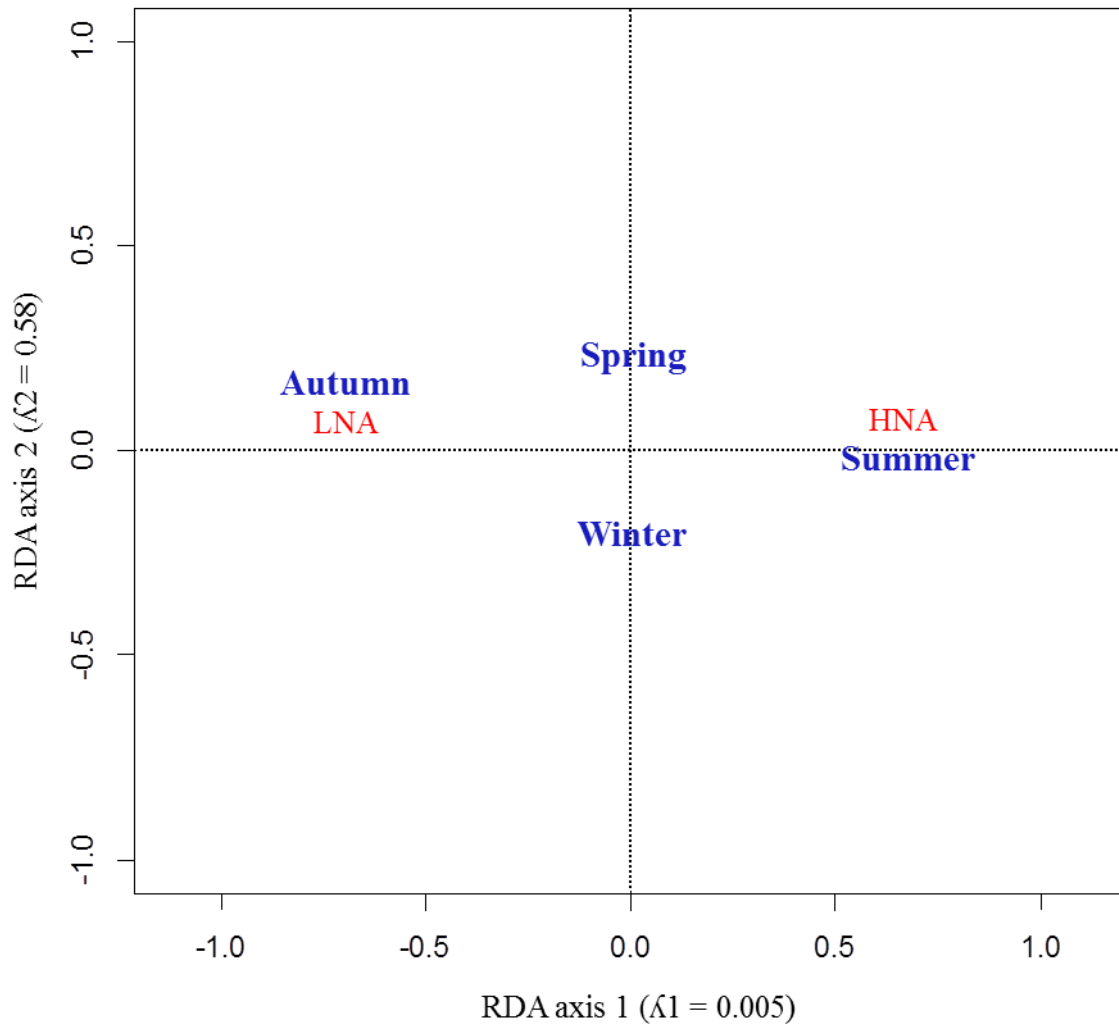


Figure 30. RDA TriPlot depicting the association between heterotrophic prokaryotes groups and sampling seasons in Ghar El Melh Lagoon. Eigenvalues of the first two axes are indicated by λ_1 and λ_2 .

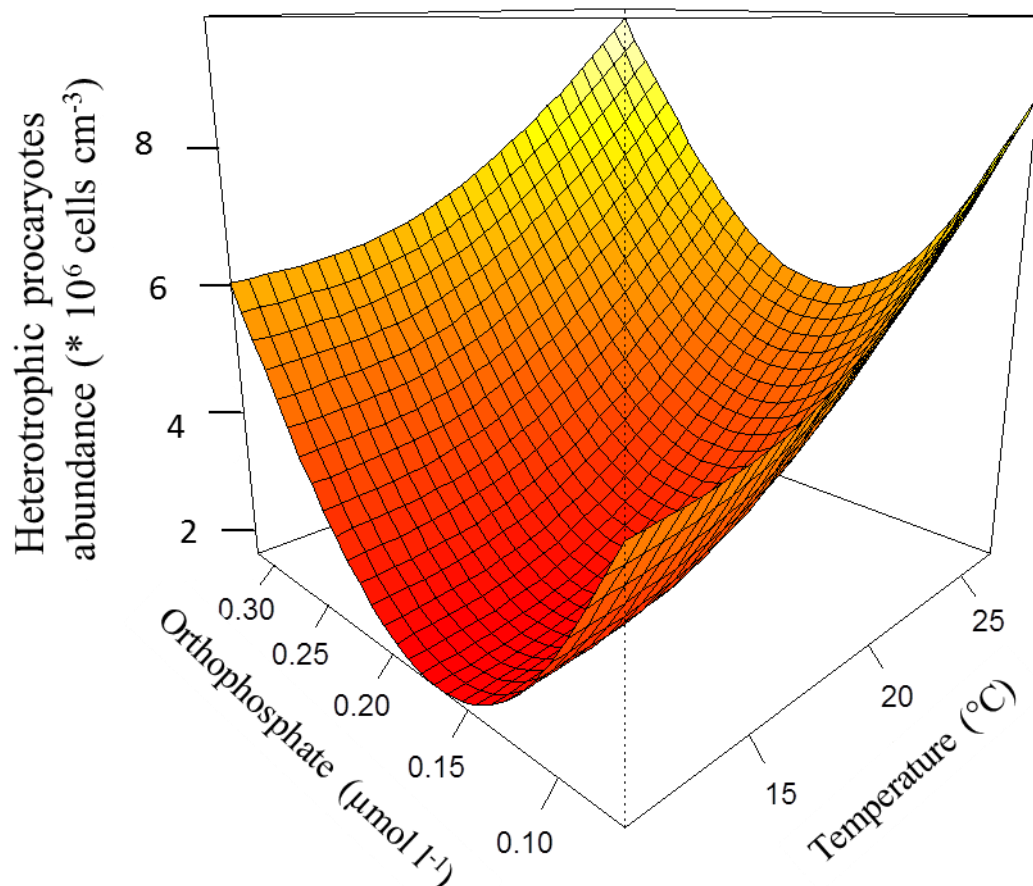


Figure 31. 3D plot showing the nonlinear relationship between heterotrophic prokaryotes abundance and both temperature and orthophosphate concentration. The orange map represents the estimations of the GAM.

Tableau 25. Summary of the GAM statistics that depict the relationship between heterotrophic prokaryote abundance and both temperature and orthophosphate concentration. The effective degree of freedom can be considered as indicative of the degree of nonlinearity of the relationship between heterotrophic prokaryote abundance and either temperature or orthophosphate concentration.

Covariates	Effective degree of freedom	F	<i>p values</i>
Temperature	1.97	13.45	0.001
Orthophosphate	2.94	3.50	0.04

Ultraplankton

At least twelve ultraplankton groups were resolved by flow cytometry in GML waters and assigned to five groups (nanoeukaryotes 64.5 %, cryptophytes 27.7 %, picoeukaryotes 2.79 %, *Synechococcus* 2.74 %, *Prochlorococcus* 0.05 %) and some unknown communities (2.22 %) (Figures 32, 33 and 34).

The best resolution of the different ultraplankton groups were performed by using Red fluorescence versus orange fluorescence. In the absence of species identification, cell groups are referred to by the label of their region boundary. Nanoeukaryotes have high red fluorescence and low orange fluorescence and their size signal is larger than that of the 2 μm beads. Cryptophyte-like cells have net red and orange fluorescence signals. Picoeukaryotes have low red and orange fluorescence signals and their size was $\leq 2 \mu\text{m}$. *Synechococcus*-like cells have low red fluorescence and high orange fluorescence and a size $\leq 2 \mu\text{m}$. *Prochlorococcus*-like cells have a small size and exhibit a very low red fluorescence and no orange fluorescence signal. The former was only detected using Forward Scatter versus Side Scatter parameters (Figures 32 and 33).

Ultraplankton abundance ranged from $1.71 \cdot 10^4$ to $61.8 \cdot 10^4$ cells cm^{-3} . Maximum concentrations were detected in winter (mean \pm sd = $4.2 \cdot 10^5 \pm 1.17 \cdot 10^5$ cells cm^{-3}) and summer (mean \pm sd = $3.41 \cdot 10^5 \pm 1.46 \cdot 10^5$ cells cm^{-3}) at all stations. Minimum concentrations were detected in spring (mean \pm sd = $6.38 \cdot 10^4 \pm 2.95 \cdot 10^4$ cells cm^{-3}).

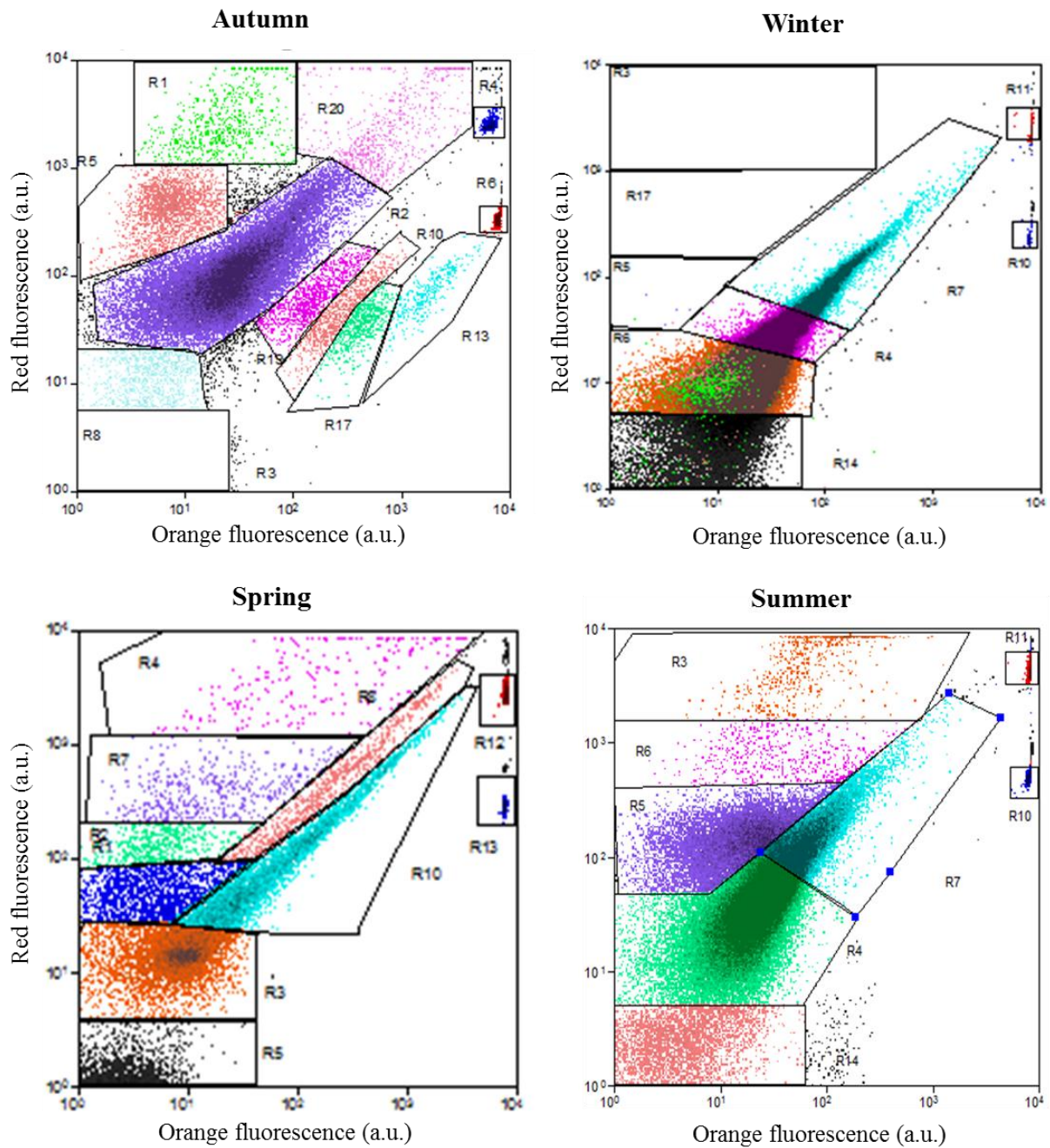


Figure 32. Typical ultraphytoplankton group resolution by flow cytometry for each season. Red fluorescence versus orange fluorescence cytogrammes lead to the perfect resolution of ultraphytoplankton groups. TC: Trucount beads (Becton Dickinson). a.u.: arbitrary unit. **(Autumn)** R1+R20: Nanoeukaryotes, R2: Cryptophytes-like cells, R5+R8: Picoeukaryotes, R10+R13+R17+R19: *Synechococcus*-like cells, R4: TC, R6: 2µm Beads. **(Winter)** R4+R6: Nanoeukaryotes, R7: Cryptophytes-like cells, R14: non-fluorescent particles of about 3 µm, R11: TC, R10: 2µm Beads. **(Spring)** R1: Picoeukaryotes, R8+R10: Cryptophyte-like cells, R2+R4+R7: Nanoeukaryotes, R3: Unidentified group. R12: TC, R13: 2µm Beads. **(Summer)** R3+R5+R6: Nanoeukaryotes, R4: ¼ Picoeukaryotes ¾ Nanoeukaryotes, R7: Cryptophytes-like cells, R11: TC, R10: 2µm Beads.

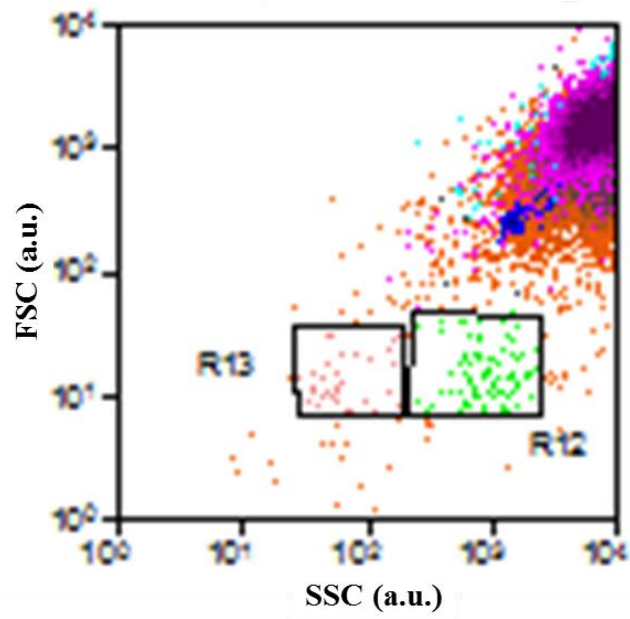


Figure 33. Cell group resolution of winter season using Forward Scatter versus Side Scatter parameters (linked to cell structure). **R12:** *Prochlorococcus*-like cells, **R13:** Picoeukaryotes.

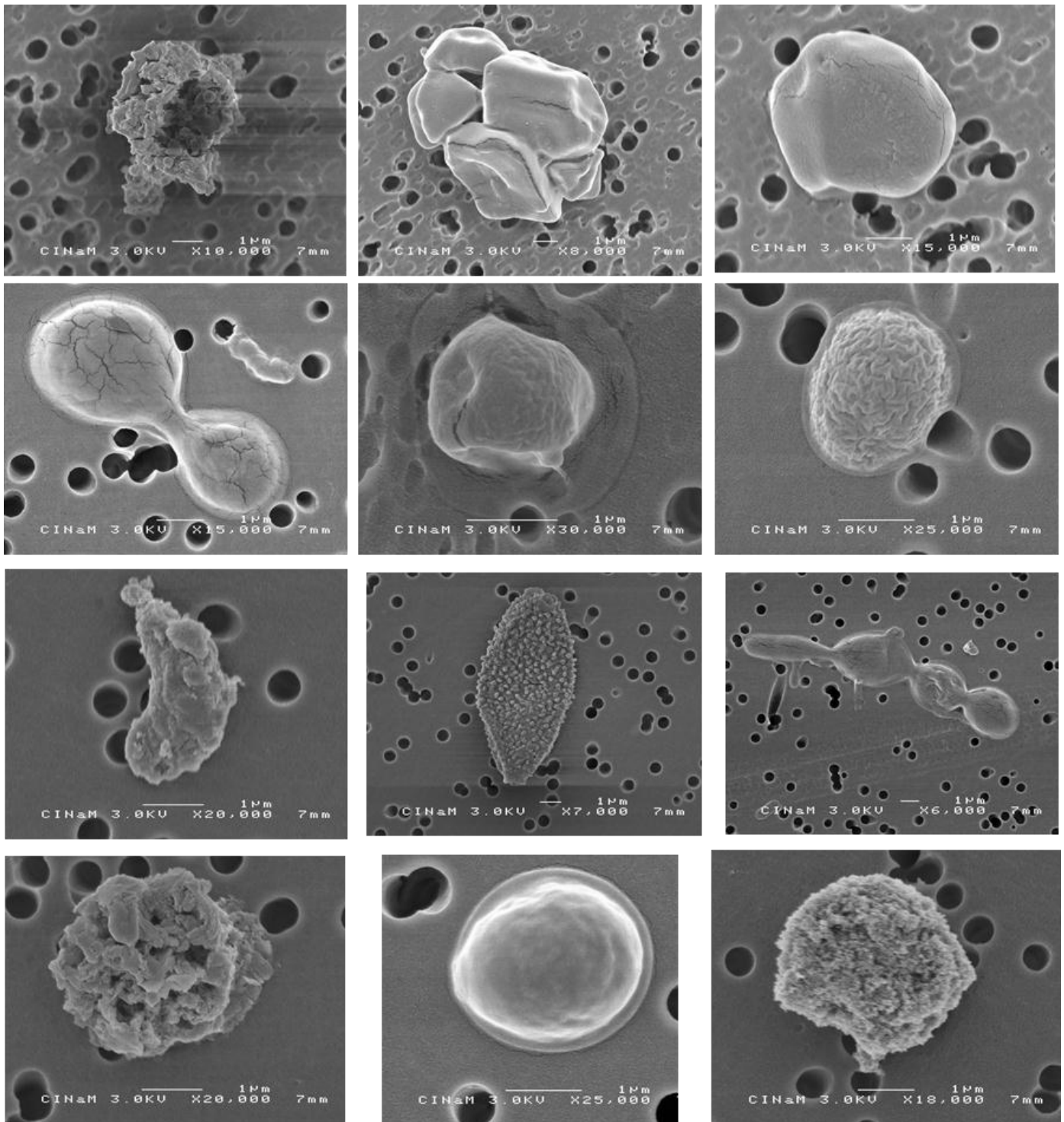


Figure 34. Electron micrograph pictures of sorted ultraphytoplankton cells detected in Ghar El Melh Lagoon.

No significant difference was found in ultraphytoplankton abundance among sampling sites (RDA, $F = 0.13$, $p = 0.99$); however a significant change was observed with seasons (RDA, $F = 30.16$, $p < 0.001$). This temporal variability explained 59 % of changes in ultraphytoplankton abundance. Both RDA axis 1 and 2 supported a significant effect on this variability ($p < 0.05$; Figure 35). Autumn had a negative RDA score, indicative of close relationships with *Synechococcus*-like cells and Picoeucaryote groups. Indeed, *Synechococcus*-like cells appeared only in autumn with a high diversity (4 subgroups) (Figure 32) and, interestingly, they were not observed at other seasons. For picoeukaryotes, the highest concentrations were detected in autumn with an average of about $10.51 \cdot 10^3$ cells cm^{-3} and with a presence of two subgroups in this period (Figure 32). *Prochlorococcus*-like cells have a positive RDA axis 2 score close to 0, associated with winter observations. Indeed, we only observed this group in winter and in low concentrations (maximal abundance = $1.69 \cdot 10^3$ cells cm^{-3}) (Figure 33). Winter and summer had a positive RDA axis 2 score correlating with nanoeukaryotes and cryptophyte-like cell-groups. Indeed, abundance peaks of both groups were recorded in these two seasons. Furthermore, the highest diversity of the nanoeukaryote group was established in summer with 4 subgroups (Figure 32).

The Generalized additive model (GAM, Figure 36) selected temperature and salinity as the main driving environmental covariates distinguishing ultraphytoplankton abundance. Total ultraphytoplankton abundance had significant nonlinear relationships with temperature and salinity as revealed by the effective degrees of freedom (Table 26). Both environmental covariates together accounted for 62.2 % of the deviance in variability in ultraphytoplankton abundance. The 3D plot illustrating these nonlinear relationships (Figure 36) highlights the temperature negative effect on ultraphytoplankton abundance in the 15 - 25 °C range and salinity positive effect in the 40 - 45 range. Our GAM predicts two peaks of ultraphytoplankton abundance, in a context of temperature below 15 °C and above 25 °C, and one peak of ultraphytoplankton abundance, in a context of salinity between 40 and 45. Outside of this salinity range, ultraphytoplankton abundance did not change substantially.

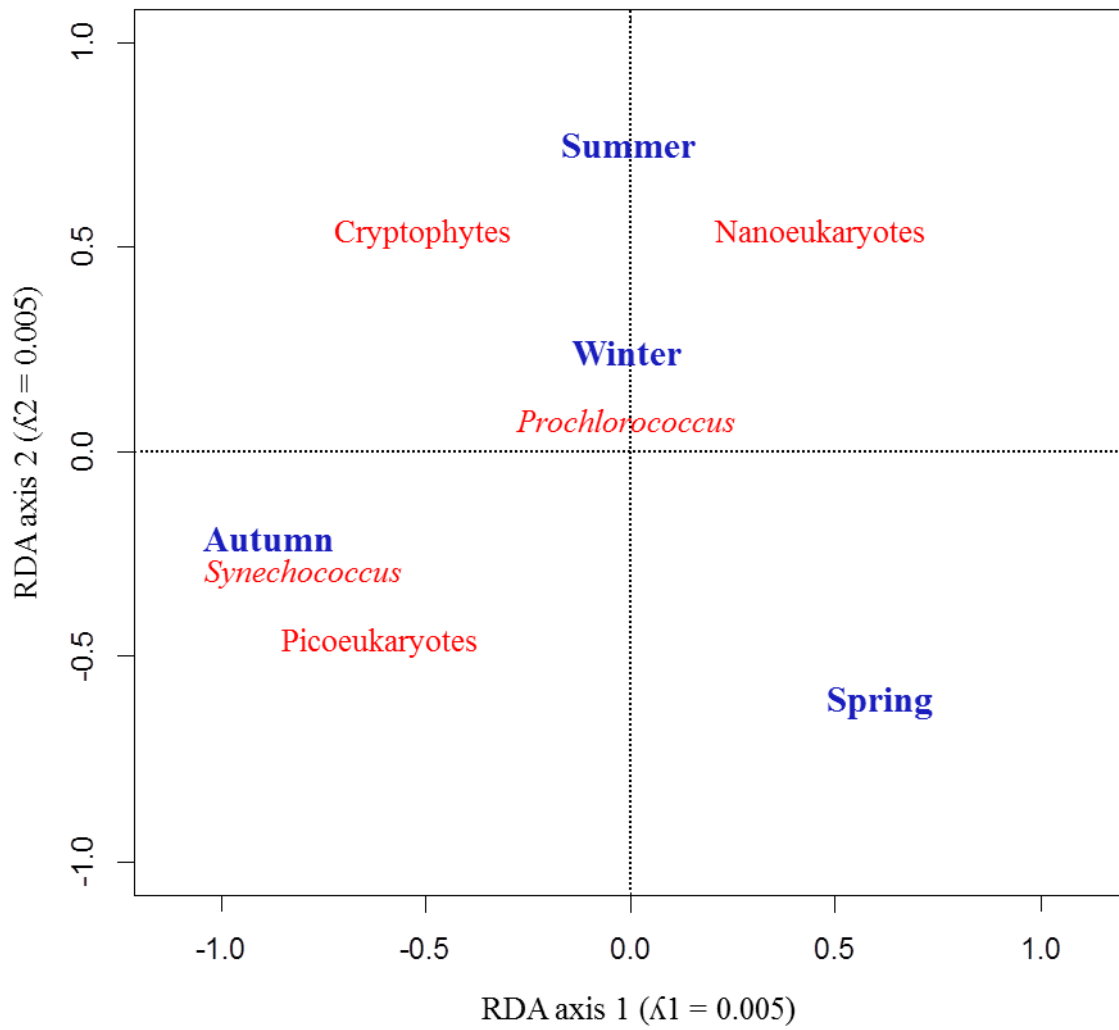


Figure 35. RDA TriPlot depicting the association between ultraphytoplankton dominant groups and sampling seasons in Ghar El Melh Lagoon. Eigenvalues of the first two axes are indicated by λ_1 and λ_2 .

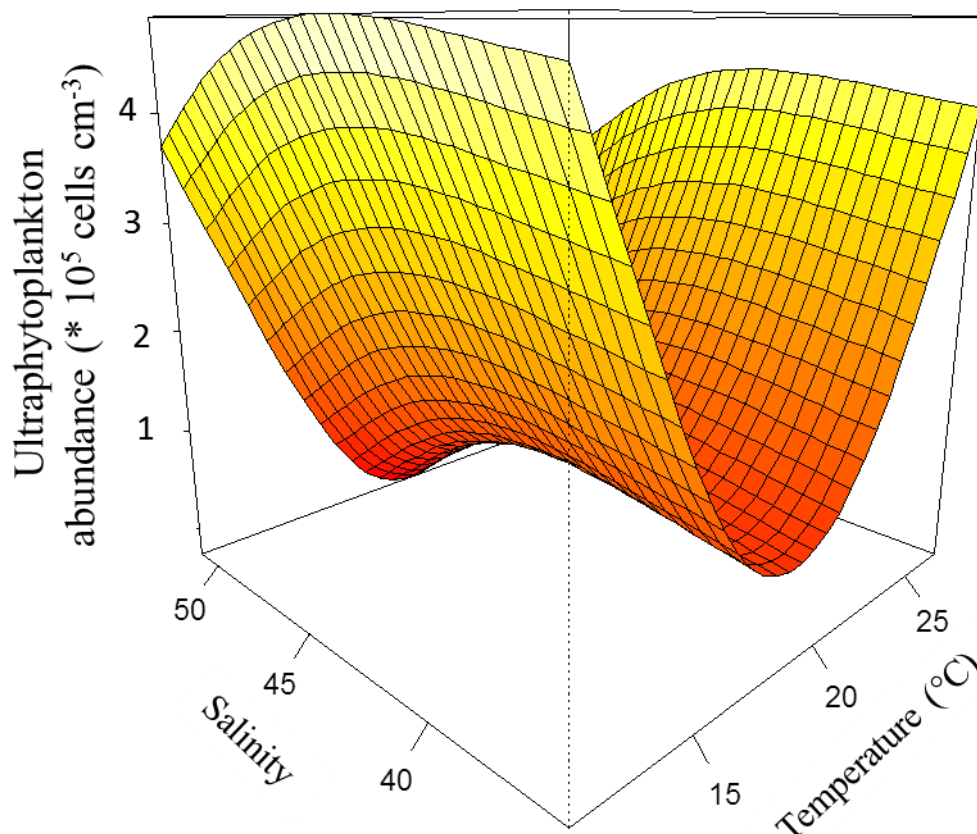


Figure 36. 3D plot showing the nonlinear relationship between ultraphytoplankton abundance and both temperature and salinity. The orange map represents the estimations of the GAM.

Tableau 26. Summary of the GAM statistics that depict the relationship between ultraphytoplankton abundance and both temperature and salinity. The effective degree of freedom can be considered as indicative of the degree of nonlinearity of the relationship between ultraphytoplankton abundance and either temperature or salinity.

Covariates	Effective degrees of freedom	F	<i>p values</i>
Temperature	2.21	15.53	< 0.001
Salinity	4.46	4.41	0.01

Microplankton

During the four sampling seasons, microphytoplankton community consisted of only three groups identified as Dinophyceae, Bacillariophyceae and Cyanobacteriae. Microphytoplankton abundance ranged from 80 to 31000 cells dm^{-3} . Two peaks of microphytoplankton were detected in spring (mean \pm sd = $14.8 \cdot 10^3 \pm 9.7 \cdot 10^3$ cells dm^{-3}) and winter (mean \pm sd = $6.56 \cdot 10^3 \pm 7.23 \cdot 10^3$ cells dm^{-3}) mainly assigned to dinoflagellates. Minimum concentrations were detected in summer (mean \pm sd = 169 ± 93 cells dm^{-3}) (Table 24).

For ciliates, total abundance ranged from 0 to 600 cells dm^{-3} with maximum concentrations in spring (mean \pm sd = 376 ± 183 cells dm^{-3}) and winter (mean \pm sd = 160 ± 143 cells dm^{-3}), and minimum values in summer (mean \pm sd = 32 ± 30 cells dm^{-3}) (Table 24).

The different relationships between the diverse communities of microplankton and ultraplankton are summarised in Table 26. Similar trends were observed for microphytoplankton and ciliates ($r = 0.66$; $p = 0.002$). Interestingly, significant negative correlations were found between ultraplankton and microplankton, suggesting ultraplankton vanishing in the presence of microplankton and vice versa. This was very pronounced in the case of microphytoplankton and both picoeukaryotes ($r = -0.59$; $p = 0.006$) and HNA ($r = -0.39$; $p = 0.05$) and between ciliates and cryptophytes ($r = -0.55$; $p = 0.012$), HNA ($r = -0.6$; $p = 0.005$) and LNA ($r = -0.47$; $p = 0.03$) (Table 27).

Tableau 27. The most significant correlations assessed between ultra and microplankton communities.

	HNA	LNA	Nano-eukaryotes	Pico-eukaryotes	Cryptophytes	<i>Synechococcus</i>	<i>Prochlorococcus</i>	Micro-phytoplankton	Ciliates
HNA	***								
LNA	-	***							
Nano-eukaryotes	-	-	***						
Pico-eukaryotes	r = 0.62; p = 0.004	-	-	***					
Cryptophytes	-	-	-	-	***				
<i>Synechococcus</i>	-	r = 0.51; p = 0.022	-	-	-	***			
<i>Prochlorococcus</i>	-	-	-	-	-	-	***		
Microphytoplankton	r = -0.39; p = 0.05	-	-	r = -0.59; p = 0.006	-	-	-	***	
Ciliates	r = -0.6; p = 0.005	r = -0.47; p = 0.03	-	-	r = -0.55; p = 0.012	-	-	r = 0.66; p = 0.002	***

3.4. Discussion

The seawater temperature in GML (11-26.9 °C) was typical of that in Mediterranean coastal waters. In contrast, salinity reached values (up to 51) usually found in solar salterns. The freshwater inflows are somehow nonexistent in summer which, combined to high evaporation rates and weak marine inflows through the narrow connection to the sea, leads to a substantial rise in salinity within the lagoon (Romdhane, 1985; Beyrem, 2002; Thompson *et al.*, 2009). An additional peculiarity was the fact that nutrients were never exhausted (Table 21, Chap IV. 2), an expression of the lagoon eutrophication with, for instance, a remarkable increase in total phosphorus and dissolved inorganic nitrogen concentrations in autumn and winter linked to soil washing and fresh water inflow (SCET-ERI, 2000; Ramdani *et al.*, 2004; Dhib *et al.*, 2013a). The significant temporal variability of seawater temperature, salinity and nutrient concentration in GML (ANOVA; $p < 0.01$) contrasted with their non-significant spatial variability (ANOVA; $p > 0.05$). Despite its large surface, one of the largest among Tunisian lagoons, GML seemed to behave as buffering spatial variations.

The same pattern applied to HP and ultra-phytoplankton abundances with non-significantly different distributions among stations (RDA) contrasting with their high seasonal variations (RDA). This would be constraint by environmental conditions that mainly varied with time rather than with space.

Heterotrophic prokaryotes

Outbreaks of HP abundance ($> 9 \cdot 10^6$ cells cm^{-3} , Table 24) were observed in the lagoon, similar to those recorded in other eutrophic bays and lagoons (Andrade *et al.*, 2003; Torr eton *et al.*, 2007), but much higher than those reported for oligotrophic oceans and seas (Andrade *et al.*, 2003; Martin *et al.*, 2005; Martin *et al.*, 2008).

Despite the close relative abundances of HNA and LNA (57.47 % and 42.52 % respectively), the comparison of the relative nucleic acid contents indicated a different temporal variability regarding the percentage distribution of each bacterial group (RDA, Figure 30). Indeed, the relative abundance of LNA was more stable than that of HNA, and represented about 35 % (autumn), 20 % (winter), 20 % (spring) and 24 % (summer). From 35 % in autumn, HNA relative abundance decreased drastically to only 6 % in winter and spring and then peaked to 57 % in summer. This suggests that HNA were the dynamic members of the GML bacterial assemblage and that LNA were inactive or dormant members as no clear seasonal pattern was observed for them. Indeed, HNA were shown (i) to have a higher growth rate or higher activity than LNA (Li *et al.*, 1995; Jellett *et al.*, 1996; Gasol *et al.*, 1999; Lebaron *et al.*, 2001; Longnecker *et al.*, 2006; Lemonnier *et al.*, 2010), (ii) to be responsible for almost all of the

HP activity (Gasol and del Giorgio, 2000; Lebaron *et al.*, 2001), and (iii) more prone to protozoan predation (Gasol *et al.*, 1999; Corzo *et al.*, 2005). This hypothesis would also be supported by a HNA diversity (2 subgroups in winter and spring and 3 subgroups in summer) higher than that of LNA (2 subgroups only in winter and spring).

The ratio between HNA and total HP abundances ranged from 27 to 74 % and was inside the ranges reported by Li *et al.* (1995) for the Mediterranean and North Atlantic (between 10 % and 90 %) and those from Jellett *et al.* (1996) for the Bedford Basin, Canada (between 19 % to 80 %).

High seawater temperature seemed to enhance HP abundance with maximum concentrations recorded above 20 °C (GAM, Figure 31). Indeed, temperature is one of the most important factors controlling bacterial abundance and production (e.g. Shiah and Ducklow, 1994; Kirchman and Rich, 1997; Pomeroy and Wiebe, 2001; Piontek *et al.*, 2014). According to the metabolic theory of ecology, the rate of chemical reactions usually increases with temperature (Stanier *et al.*, 1977; Gillooly *et al.*, 2001, Brown *et al.*, 2004). At low temperature, bacterial growth rate is generally considered to be limited (Pomeroy *et al.*, 1991; White *et al.*, 1991; Wiebe *et al.*, 1992; Kirchman *et al.*, 1993; Russell, 1993; Vrede, 2005).

GAM analyses pointed to a drop of HP abundances in the context of orthophosphate between 0.15 and 0.20 $\mu\text{mol l}^{-1}$ instead of normal HP proliferation outside this range. This range of orthophosphate was mainly detected in winter (Table 21, Chap IV. 2) in the presence of highly abundant microphytolankton, an efficient competitor for nutrients (Thingstad *et al.*, 1993; Caron *et al.*, 2000; Joint *et al.*, 2002). This is consistent with several investigations showing that HP abundance could be limited by the availability of inorganic nutrients (Rejas *et al.*, 2005; Mills *et al.*, 2008; Carlson *et al.*, 2012). However, this effect of inorganic nutrient availability might not be a direct effect since lower orthophosphate concentrations (Table 21, Chap IV. 2) in autumn and summer were associated with higher HP abundances (Table 24), in agreement with the GAM analysis (Figure 31).

This could be explained in relation to water temperature which acted in simultaneous with orthophosphate and thus the decrease of temperature in winter may decrease the assimilation of this nutrient which is already present in low concentrations in the field (Pomeroy and Wiebe, 2000).

Consistently, the low orthophosphate concentrations observed in the other seasons did not affect HP proliferation that was stimulated by higher seawater temperatures (average water temperatures were 17.66, 17 and 26.22 for autumn, spring and summer respectively). It should not be concluded that low winter temperature would lead to the HP disappearance

which is not supported by observations (GAM, Figure 31). According to Pomeroy and Wiebe (2001), substrate concentrations and temperature interact at all temperatures and substrate concentrations, and their interactive effects can differ with temperature regime. Verde (2005) concluded that, when studying HP, it is important to consider inorganic nutrients, organic substrates, and temperature simultaneously.

In spring, the decrease of HP abundance may be explained by the maximum concentrations recorded for microphytoplankton (especially dinoflagellates) and ciliates which known as potential feeders of bacterioplankton (Larsen and Sournia, 1991; Simek *et al.*, 1998; Jürgens and Massana, 2008; Karayanni *et al.*, 2008). The negative significant correlations that were established between HNA and LNA, and microphytoplankton and ciliates (Table 27) are consistent with the capacity of microphytoplankton and ciliates to feed on bacterioplankton (Larsen and Sournia, 1991; Simek *et al.*, 1998; Jürgens and Massana, 2008; Karayanni *et al.*, 2008). These correlations are also in agreement with our previous works in GML showing that spring was the most prominent period for maximum proliferation of harmful mixotrophic dinoflagellates (Dhib *et al.*, 2015) and a wide range of ciliates belonging to different size classes with different feeding modes (Dhib *et al.* 2013b).

Ultraphytoplankton

Nanophytoplankton (nanoeukaryotes + cryptophytes-like cells \approx 92 %) was outnumbering picophytoplankton (picoeukaryotes + *Synechococcus*-like cells + *Prochlorococcus*-like cells \approx 6 %) in GML. This was consistent with the eutrophic status of GML. Indeed, the abundance ratio of nanophytoplankton over picoeukaryotes, a trophic index (Rnp) established by Denis *et al.* (2003) in the western Mediterranean Sea (2003), was always >1 (3.56, 666.07, 14.03, 32.77 in autumn, winter, spring and summer respectively). Accordingly, the GML trophic status was markedly eutrophic in winter and to a lesser extent in spring and summer, and rather mesotrophic in autumn where the average abundances of nanophytoplankton and picoeukaryotes were within the same order of magnitude (37403 and 10515 cells cm^{-3} respectively). Within picophytoplankton, picoeukaryote cells were well represented in all seasons (more particularly in autumn; RDA, Figure 35) whereas *Synechococcus*-like and *Prochlorococcus*-like cells were only observed at a single season, autumn and winter respectively. Except in autumn, nanoeukaryotes were largely more abundant than cryptophytes-like cells. The picophytoplankton abundances (picoeukaryotes + *Synechococcus*-like cells) detected in GML were close to those reported by Vaquer *et al.* (1996) in the Thau lagoon (France) and to those found in a shallow Mediterranean bay (Charles *et al.*, 2005) and other coastal seas (Agawin *et al.*, 2003; Worden *et al.*, 2004; Liu *et*

al., 2007; Calvo-Díaz *et al.*, 2008). In open ocean oligotrophic ecosystems, the photosynthetic prokaryotes, represented primarily by genus *Synechococcus* and genus *Prochlorococcus* were shown to be the major contributors to biomass and productivity (Partensky *et al.*, 1999). The present results show that it is not the case in the GML ecosystem, even in the absence of productivity measurement.

The use of flow cytometry was critical to detect the sporadic presence of *Prochlorococcus* - like cells (average abundance = 495 cells cm⁻³) in GML. The exclusive appearance of *Synechococcus*-like and *Prochlorococcus*-like cells in autumn and winter could be linked to the inflow of marine and fresh water during this period of the year. Indeed, for instance, the maximum abundance (8.26 10⁴ cells cm⁻³) of *Synechococcus*-like cells was recorded at station 2 in front of “El Boughaz” pass, the only connection with the Mediterranean Sea. Furthermore, we detected four sub-populations of *Synechococcus*-like cells which may comprise some riverine species. Indeed, many authors showed that lots of picocyanobacteria present in the coastal waters could be of riverine origin (Waleron *et al.*, 2007; Schloss *et al.*, 2008; Mitbavkar and Anil, 2011). Further work is needed to establish the taxonomic identification of the numerous flow cytometry sub-groups that highlight the originality of the GML ecosystem.

Two optimal ranges of temperature were revealed for ultraphytoplankton community with maximum concentrations at low and high temperatures. Except for *Prochlorococcus*-like cells and *Synechococcus*-like cells which appeared in one season, all the ultraphytoplankton groups exhibit a large tolerance to water temperature displaying high abundances both in high and less water temperatures. The drop of ultraphytoplankton abundances in medium water temperatures (GAM; Figure 36) coincided with that recorded in spring season (RDA; Figure 35). This may be in close relation to the maximum concentrations of microplankton during this period (Table 24). Indeed, negative significant correlations were detected between picoeukaryotes and microphytoplankton and between cryptophytes-like cells and ciliates (Table 27) and could be an ultimate result of grazing pressure of ciliates over nanophytoplankton (Johansson *et al.*, 2004; Loder *et al.*, 2011) and of feeding competition for nutrients between microphytoplankton and ultraphytoplankton.

Salinity seems to enhance ultraphytoplankton abundance at the range of 40-45 and appears with no imperative influence outside this range. This range was detected exclusively in summer concomitantly with the greatest ultraphytoplankton concentration detected in this study (6.18 10⁵ cells cm⁻³). A positive correlation of ultraphytoplankton with salinity was also reported from other works (e.g. Robineau *et al.*, 1999; Jyothibabu *et al.*, 2013).

The non-apparent correlation of ultraphytoplankton with any nutrient may be due to the complex dynamic of the formers in response to the perturbation of N/P ratio (Klausmeier *et al.*, 2004) especially in peculiar ecosystems such as lagoons.

3.5. Conclusion

This study provided evidence for (1) HP abundance peaks and dominance of nanophytoplankton over picophytoplankton reflecting the eutrophic conditions in GML waters, (2) a large ultraplankton diversity with 5 HP and 12 ultraphytoplankton sub-groups, highlighting the originality of the site, (3) a clear seasonal pattern of ultraplankton community with HP proliferation in autumn-summer, and winter-summer ultraphytoplankton proliferation, (4) apparent link between HP abundance and water temperature and orthophosphate concentration, (5) water temperature and salinity variations as the main factors influencing ultraphytoplankton development and (6) potential link between ultraplankton abundance decrease in spring season and the grazing pressure exerted by microplankton communities (microphytoplankton + ciliates).

Acknowledgements

This study is part of Amel DHIB PhD research work. We are grateful to Serge Nitsche, head of the SEM facility at the Centre Interdisciplinaire de Nanoscience de Marseille (CINaM) for providing access to SEM and his helpful assistance.

Chapitre V. Distribution saisonnière des ciliés et relation avec les communautés microlagales

Article 3. Factors driving the seasonal distribution of ciliate assemblages in the eutrophicated Mediterranean Ghar El Melh Lagoon, Tunisia

Article publié dans le journal 'Marine pollution bulletin' (IF = 2.99)

Marine Pollution Bulletin 74 (2013) 383–395



Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul



Factors driving the seasonal distribution of planktonic and epiphytic ciliates in a eutrophicated Mediterranean Lagoon

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ARTICLE INFO

Keywords:

Planktonic ciliates
Epiphytic ciliates
Microalgae
Physico-chemical parameters
Dynamics
Ghar El Melh Lagoon

ABSTRACT

We studied the distribution of planktonic and epiphytic ciliates coupled with environmental factors and microalgae abundance at five stations in Ghar El Melh Lagoon (Tunisia). Planktonic ciliates were monitored for a year and epiphytic ciliates were sampled during summer 2011 in concordance with the proliferation of the seagrass *Ruppia cirrhosa*. Ciliate assemblage was largely dominated by Spirotrichea followed respectively by Tintinnida and Strombidiida. No significant difference was found in the distribution of ciliate species among the stations. Redundancy analysis indicates that abiotic factors (temperature and nutrients) have a significant effect on the dynamics of certain ciliates. For epiphytic ciliates, 4 species were identified: *Tintinnopsis campanula*, *Aspidisca* sp., *Strombidium acutum* and *Amphorides amphora*. Based on PERMANOVA analyses, ciliates exhibit significant correlations among months and stations. According to ACP, epiphyte distribution follows roughly those of *R. cirrhosa* and pH. Significant correlations were found between harmful dinoflagellates and both planktonic and epiphytic ciliates.

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Résumé

Dans ce chapitre, il s'agit de l'étude des ciliés planctoniques et épiphytes en relation avec les proliférations microalgales dans la lagune de Ghar el Melh. Le prélèvement bimensuel des ciliés et des microalgues planctoniques a été effectué pendant une année (de janvier 2011 à janvier 2012) et le prélèvement des ciliés et des microalgues épiphytes a été réalisé pendant les 3 mois d'été de l'année 2011, sur la macrophyte « *Ruppia cirrhosa* », espèce dominante du milieu.

Pour les ciliés planctoniques, on a identifié 28 espèces qui se répartissent sur 5 classes. Les ciliés ont été largement représentés par le groupe de Spirotriches (80.66 %) dont les choreotrichidés représentent 35.62 % suivi par celui des tintinnides (27.54 %) et les strombidiidés (13.22 %). Selon l'Analyse de Redondance, l'abondance des ciliés est étroitement liée aux différentes saisons, avec des concentrations maximales enregistrées au printemps, pour 12 espèces de ciliés ayant différentes classes de taille et caractérisés par différents modes de nutrition. L'Analyse de Redondance a révélé aussi l'implication de la température et des nutriments sur les variations des espèces dominantes de ciliés : *Leegaardiella sol*, *Strombidium conicum*, *Metacylis mediterranea*, *Paramecium* sp. et *Euplotes charon*.

Concernant les ciliés épiphytes, on a identifié 4 espèces : *Tintinnopsis campanula*, *Aspidisca* sp., *Strombidium acutum* et *Amphorides amphora*. Selon les analyses de PERMANOVA, l'abondance des ciliés épiphytes a varié selon les mois et les stations ($p = 0.001$). En se basant sur les analyses de l'ACP, la distribution des épiphytes est étroitement associée au développement de la macrophyte *R. cirrhosa* et du pH.

A l'issue de cette étude, il ressort des corrélations hautement significatives entre les dinoflagellés nuisibles et les ciliés. Parmi les corrélations les plus importantes, on retrouve celle du cilié *S. conicum* et le dinoflagellé nuisible *Prorocentrum micans* et celle du cilié *Aspidisca* sp. et le dinoflagellé toxique *Prorocentrum lima*.

Abstract

We studied the distribution of planktonic and epiphytic ciliates coupled with environmental factors and microalgae abundance at 5 stations in Ghar El Melh Lagoon (GML, Tunisia, southern Mediterranean Sea). Planktonic ciliates were monitored for a year (January 2011-January 2012) and epiphytic ciliates were sampled only during summer 2011 in concordance with the colonization of the site by the seagrass *Ruppia cirrhosa*. For the planktonic ciliates, a total of 28 species belonging to 5 taxonomic classes were identified. Ciliate assemblage was largely dominated by Spirotrichea (80.66 % of total abundance) of which Choreotrichida represented 35.62 % of total abundance followed respectively by Tintinnida (27.54 % of total abundance) and Strombidiida (13.22 % of total abundance). No significant difference was found in the distribution of ciliate species among the stations, though seasonal variability was observed, mainly related to maximal abundances recorded in spring for 12 ciliates of different size classes and with different feeding modes. Redundancy analysis indicates that abiotic factors (temperature and nutrients) have a significant effect on the dynamics of *Leegaardiella sol*, *Strombidium conicum*, *Metacylis mediterranea*, *Paramecium* sp. and *Euplotes charon*. For epiphytic ciliates, 4 species were identified: *Tintinnopsis campanula*, *Aspidisca* sp., *Strombidium acutum* and *Amphorides amphora*. Based on PERMANOVA analyses, ciliates exhibit significant correlations among months and stations. According to ACP, epiphyte distribution follows roughly those of *R. cirrhosa* and pH. Significant correlations were found between harmful dinoflagellates and both planktonic and epiphytic ciliates. Further grazing essays, for example between *S. conicum* and the harmful *P. micans* or between *Aspidica* sp. and the toxic *P. lima*, might improve our understanding of the harmful dinoflagellate blooms in this lagoon.

Keywords: planktonic ciliates, epiphytic ciliates, microalgae, physico-chemical parameters, dynamics, Ghar El Melh Lagoon.

1. Introduction

Coastal lagoons are considered to be distinct systems rather than adjoining ones (Knoppers, 1994). As interfaces between land and sea, they exhibit high primary and secondary productions that promote the development of extensive fisheries and aquaculture (Kjerfve, 1994; Chessa *et al.*, 2005; Pérez-Ruzafa *et al.*, 2007). As semi-enclosed systems, coastal lagoons are strongly influenced by freshwater input (Valiela *et al.*, 1997; De Wit *et al.*, 2005) and are usually impacted by agricultural, industrial and tourism activities (Cloern, 2001; Verlaque, 2001; Lloret *et al.*, 2008). These unique features allow lagoon waters to acquire significantly different characteristics compared to the nearby seawater, which leads to greater diversity in the biological communities in these ecosystems. Despite their economic and environmental importance, lagoons remain insufficiently studied (Alvarez-Borrego, 1994; Danovaro and Pusceddu, 2007) and little is known about the factors driving ciliate dynamics in their ecosystems. Ciliates are one of the major functional groups in the aquatic food web (Landry and Calbet, 2004; Pomeroy *et al.*, 2007; Fenchel, 2008; Sherr and Sherr, 2008) with numerous works reporting ciliate feeding on bacterioplankton (Simek *et al.*, 1998; Karayanni *et al.*, 2008) and phytoplankton (Gismervik *et al.*, 1996; Loder *et al.*, 2011), making them a likely link in the transfer of energy from microbial components to higher trophic levels (Azam *et al.*, 1983, Sherr *et al.*, 1986). Furthermore, ciliates are valuable bioindicators of water quality considering their specific eco-physiological properties as rapid sensors of variation in environmental changes (Aleya *et al.*, 1992; Foissner and Berger, 1996; Jiang, 2006).

It is noteworthy that in the Mediterranean, the majority of studies on the ciliate community structure have dealt with coastal and/or oceanic plankton communities (Admiraal and Venekamp, 1986; Cariou *et al.*, 1999, Dolan, 2000; Dolan *et al.*, 2002; Krsinic and Grbec, 2006) while lagoons have been neglected. Yet, the nutrient dynamics at the pelagic/benthic interface in the lagoons are likely to be complex with regenerated nutrients released from the sediment when disturbed. In Tunisia, most studies of ciliates have been reported from coastal and open sea ecosystems (Hannachi *et al.*, 2009, 2011; Kchaou *et al.*, 2009; Drira *et al.*, 2009; Rekik *et al.*, 2012), or solar salterns (Elloumi *et al.*, 2006, 2009) whereas, studies in lagoons despite their abundance in Tunisia and their many interesting characteristics, are very limited (Sakka *et al.*, 2008). This is particularly true for the shallow Ghar El Melh Lagoon (GML) which: (i) has limited connection with the sea and is now under stress from both natural and anthropogenic pressures such as pollution, rising sea level and silting, and also has episodes of harmful dinoflagellate blooms (Romdhane *et al.*, 1998, Turki *et al.*, 2007, Dhib *et al.*, 2013a), (ii) is a well-known breeding site for birds (grebes, herons, sterna, flamingos, gulls...)

(Kraiem *et al.*, 2009), (iii) is permanently connected to Sabkhet El Ouafi; sabkha is an Arabic term for a coastal and inland saline mud flat built up by the deposition of silt, clay and sand in shallow, sometimes extensive, depressions (Warren, 2006), and (iv) has a benthic vegetation dominated by a *Ruppia cirrhosa* meadow (Shili *et al.*, 2002) which spreads spectacularly in summer and whose leaves overwhelm the surface water in different parts of the lagoon; we hypothesized that these leaves may harbour ciliates whose structure and abundance should be explored.

Altogether, these conditions prompted us to explore over the period of a year the species composition abundance, and biomass of planktonic ciliates in GML waters in relation to physical and chemical factors as well as phytoplankton abundance. In addition, in order to obtain a good overview of the lagoon's entire ciliate community, epiphytic ciliates of *R. cirrhosa* were investigated in summer due to the meadow's massive proliferation.

We hypothesized that ciliates should exhibit interspecific differences in relation to both environmental variability and potential phytoplankton prey.

2. Materials and methods

Study area

Ghar El Melh Lagoon is a RAMSAR site (RAMSAR, 2007), located in the southern Mediterranean Sea on the north-eastern coast of Tunisia (37°06'-37°10' N and 10°08'-10°15' E) (Figure 19, Chap IV.1). GML has an area of about 3000 ha including 2 small sub-lagoons, namely Sabkhet El Ouafi and Sabkhet Sidi Ali El Mekki. The main lagoon is permanently connected with Sabkhet El Ouafi but isolated from Sabkhet Sidi Ali El Mekki by embankments. It is connected to the Mediterranean Sea via a permanent channel called El Boughaz that passes through the coastal sand bars. The lagoon exhibits different levels of salinity with the highest recorded in stagnant areas within the lagoon. Freshwater inflows are seasonal, limited in summer and high in winter, sometimes with the occurrence of exceptional floods creating a connexion between the lagoon and the Mejerda River. Benthic vegetation currently consists of *R. cirrhosa* and *Cladophora* (Shili *et al.*, 2002). Birds including grebes, herons, sterna, flamingos and gulls are frequent in the shallow south-eastern part of GML. The small fishery in GML comprises eel, mullet, sea-bass and sole (Kraiem *et al.*, 2009).

Sampling

Five stations (S1 to S5) were chosen to cope with the different environmental conditions found within GML (Figure 19, Chap IV.1). S1 is located in the north-eastern part of the lagoon, a shallow area influenced by a supply of freshwater from 'El Ayoune', with a mat of

limnetic plants (reeds) around the edges. S2 faces the El Boughaz Channel which is a permanent 70-m wide connection with the Gulf of Tunis. The deepest area of GML, it is influenced by strong hydrodynamics despite continuous silting of the channel. S3, located in the south-east of the lagoon in the middle of Sabkhet El Ouafi, is a semi-closed zone and one of the most stagnant areas. S4 in the south-west of GML is affected by heavy industrial discharge (from 43 industrial units) and is drained by tributaries mainly in winter. S5, located in the northern part of the lagoon, is affected by agricultural and urban discharge. Leachate from rural zones, rainwater and the wastewater from the city of Ghar El Melh arrive directly into this part of the lagoon.

Samples for planktonic ciliate and microalgae identification and enumeration were collected using a polyvinylchloride (PVC) tube twice a month, from January 2011 to January 2012 at the five sampling sites (N = 110). Additionally, three replicates of epiphytic ciliate and microalgae of *R. cirrhosa* were collected (N = 45) at each station during the summer (early June, mid-July and late August). To detach the micro-communities, 100 g of leaf samples were placed in plastic bottles and the epiphytes then separated by vigorous shaking and washing before being filtered (200 ml of seawater) through a 0.2 µm pore-size membrane (Millipore). The obtained solution was then passed through 250 µm mesh sieves to remove large particles and was then fixed with formaldehyde (5 %). Concomitantly with epiphyte sampling, shoot density of *R. cirrhosa* was measured *in situ* using a quadrat (40 cm x 40 cm) that was randomly placed over the shoots before carefully collecting them. At each station, three replicate quadrats were sampled. Meadow density (shoot m⁻²) was calculated as the number of shoots bearing leaves in each quadrat (Ott, 1980).

Environmental variables were measured in the field concomitantly with ciliate and phytoplankton sampling. A WTW multiparameter was used for water temperature (°C) and salinity. Transparency was measured using a Secchi disk. Water samples were collected in 1000-ml polypropylene bottles at 30 to 50 cm depth. In the laboratory, nutrients (ammonium, nitrite, nitrate, phosphate, silicate) were analysed with a BRAN and LUEBBE type 3 autoanalyser and concentrations were determined colourimetrically using a UV-visible (JENWAY 6705) spectrophotometer (APHA, 1992).

Ciliate and microalgae enumeration

Sub-samples (25 ml) of planktonic communities were counted under an inverted microscope after fixation with lugol solution (final concentration 1% v/v) and settling for 48 hours using the Utermöhl method (1958).

Sub-samples (1 ml) of epiphytic communities (Turki, 2005) were also enumerated according

to the Utermöhl method by means of an inverted microscope.

Ciliate identification was performed using the keys of Corliss (1961), Petz (1999) and Struder-Kypke and Montagnes (2002). Tintinnids were identified using lorica morphology and species description according to Kofoid and Campbell (1929, 1939) and Balech (1959). Naked ciliates were identified based on the methods of Lynn and Small (1997), Petz (1999), Alder (1999) and Strüder-Kypke and Montagnes (2002). Cell numbers were expressed as cells l⁻¹. Mean biovolume of each ciliate taxon was estimated from length and width measurements of more than 20 individuals for the abundant taxa, and converted to carbon biomass with the conversion factor proposed by Putt and Stoecker (1989). The level of community structure was assessed with Shannon & Weaver's (1949) H' diversity index.

$$H' = - \sum ni / N * \log_2 ni / N$$

ni/N: is the frequency of species i in the sample

N: number of species in the community

Statistical analysis

For the planktonic community, we used one-way variance analysis (ANOVA) to assess the variability of parameters among stations and months. Potential relationships between variables were tested by Spearman's correlation coefficient. Redundancy analysis (RDA) was performed to define the structuring effects of sampling sites and seasons, along with environmental conditions on ciliate species abundances. To prevent any disproportionate influence of rare species in the subsequent analysis, the only species considered were those having an abundance > 2 % of total ciliate abundance. Ciliate abundances were normalised prior to analysis. Statistical analysis was produced by R 2.15.0 (R Core Team development, 2012) with "Vegan" (Oksanen, 2011) and "Pgirmess" (Giraudoux, 2012) packages.

The data recorded for epiphytic ciliates were subjected to i) permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2005) to analyse the differences in the community composition between stations and months, and ii) principal component analysis (PCA) (Chessel and Doledec, 1989) to assess the differences among the stations of sampled epiphytes and physicochemical variables.

3. Results

Environmental conditions

The physico-chemical characteristics of the study are summarised in Figure 37 and Table 28. The highest temperature (27.6 °C) was recorded in June 2011 at station S5, while the lowest (11.1 °C) in January 2012 at S4. Mean water temperature increased in spring and reached its

maximum in summer. Temperature varied significantly from season to season (ANOVA, $F = 101.27$, $p < 0.001$), but not among sampling sites (ANOVA, $F = 0.08$, $p = 0.98$). Because of the shallowness of the lagoon ($< 2\text{m}$) thermal stratification did not occur. Water salinity varied from 51.2 in July at S4 to 26.6 in December at S3. Salinity peaked in summer due to the combined effect of high temperature, inducing water evaporation and the low inflow of freshwater to the lagoon. It varied significantly according to season (ANOVA, $F = 43.1$, $p < 0.001$) and station (ANOVA, $F = 6.07$, $p < 0.001$), and its seasonal changes roughly followed those of temperature ($r = 0.81$, $n = 110$, $p = 0$). The pH ranged from 7.2 in winter at S1 to 9.11 in summer at S5 where *R. cirrhosa* was massively present. It varied significantly according to season (ANOVA, $F = 3.8$, $p = 0.01$) and station (ANOVA, $F = 8.93$, $p < 0.001$). Water transparency was high in spring ($Z_s = 1.9\text{ m}$) and low in autumn, especially in the turbid area of the lagoon such as at S5 ($Z_s = 43\text{ cm}$) which is affected by agricultural and urban discharge, and at S2 ($Z_s = 60\text{ cm}$) opposite the El Boughaz Channel, the only access for the small fishing boats. Transparency did not differ significantly between seasons (ANOVA, $F = 1.47$, $p = 0.22$), but experienced significant variations among the stations (ANOVA, $F = 5.51$, $p < 0.001$).

Nutrients varied significantly among seasons but not among sampling stations (Table 29). Most of them decreased in spring and showed a remarkable increase in autumn and winter (Figure 24) due to dilution by floods. Total phosphorus (T-P) concentrations were high ($0.74\text{-}6.93\ \mu\text{mol l}^{-1}$). Orthophosphate concentrations were lower ($0.05\text{-}0.74\ \mu\text{mol l}^{-1}$), representing only 8.1 % of total phosphorus. Total nitrogen (T-N) concentrations showed a regular trend (mean \pm s.d. = 19.27 ± 2.97). Nitrogen appeared mainly in its dissolved organic form (68.5 %) with the dissolved inorganic form (DIN: $\text{NO}_2^- + \text{NO}_3^- + \text{NH}_4^+$) representing only 31.5 % of the total. Ammonium concentrations accounted for 53.79 % of total DIN followed by nitrate 41.92 % and nitrite 4.28 %. DIN values were higher ($0.83\text{-}22.38\ \mu\text{mol l}^{-1}$). The nitrate/ammonium ratio was low and peaked in winter with a maximum of 360.62 in February at S5. Total nitrogen and phosphorus show similar trends ($r = 0.32$, $n = 110$, $p = 0.001$). However, DIN/DIP ratio was generally greater (mean \pm s.d. = 27.63 ± 23.32) than the Redfield ratio (16) suggesting potential P limitation except in summer (mean \pm s.d. = 12.55 ± 6.94). Silicate concentrations ranged from $0.38\ \mu\text{mol l}^{-1}$ (winter) to $21.2\ \mu\text{mol l}^{-1}$ (spring).

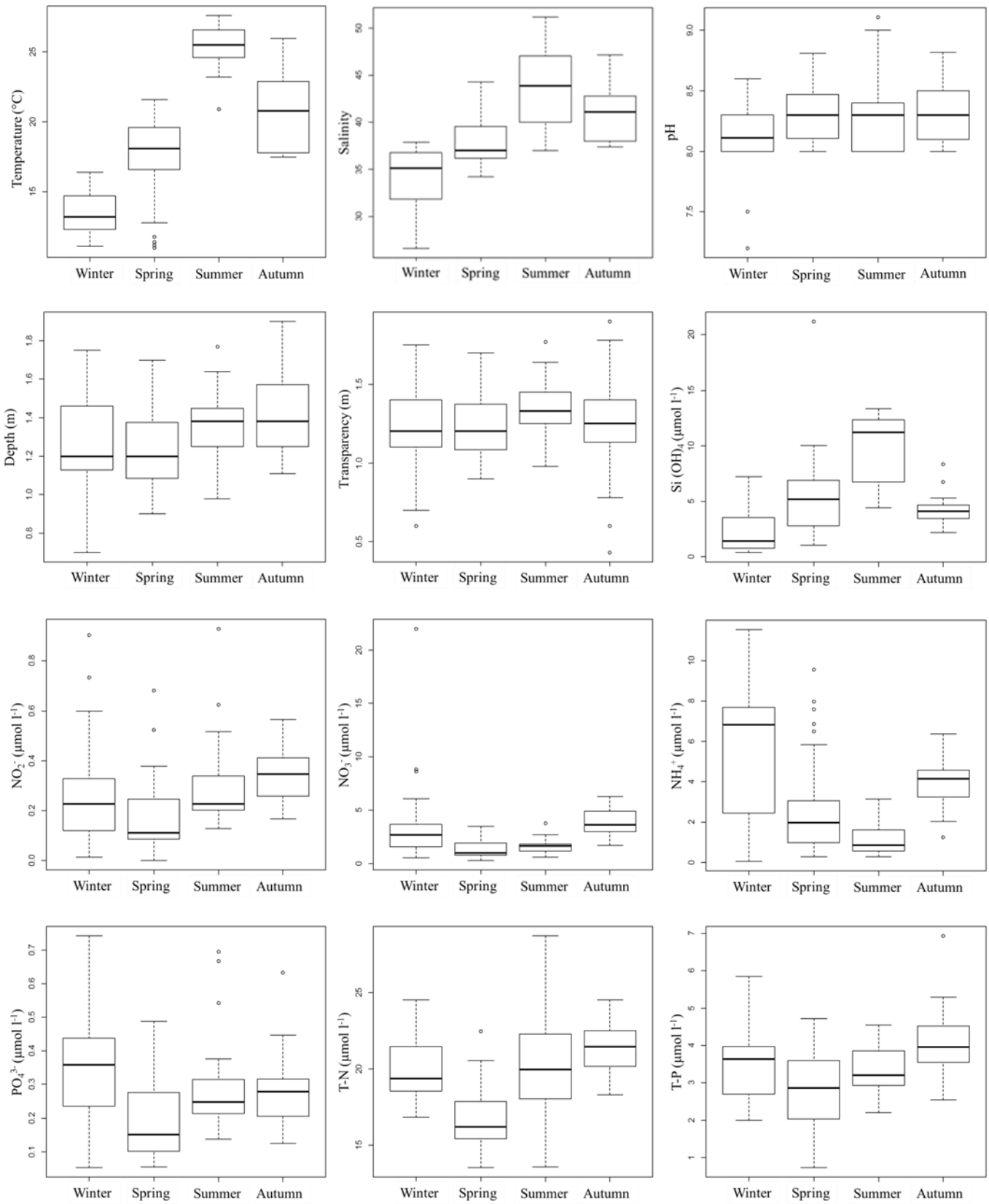


Figure 37. Boxplots depicting the seasonal variations of abiotic factors in Ghar El Melh lagoon.

Tableau 28. Min (minimum), max (maximum) and mean \pm SD of physico-chemical and biological parameters in Ghar El Melh Lagoon.

Physical parameters	Min	Max	Mean \pm SD
Temperature ($^{\circ}$ C)	11.1	27.6	19.18 \pm 4.81
Salinity	26.6	51.2	39.04 \pm 4.71
pH	7.2	9.11	8.26 \pm 0.27
Transparency (m)	0.43	1.9	1.26 \pm 0.26
Depth (m)	0.7	1.9	1.32 \pm 0.22
Chemical parameters			
Si(OH) ₄ (μ mol l ⁻¹)	0.386	21.204	5.43 \pm 3.77
NO ₂ ⁻ (μ mol l ⁻¹)	0.001	0.929	0.26 \pm 0.17
NO ₃ ⁻ (μ mol l ⁻¹)	0.297	21.998	2.54 \pm 2.50
NH ₄ ⁺ (μ mol l ⁻¹)	0.055	11.533	3.26 \pm 2.71
T-N (μ mol l ⁻¹)	13.512	28.734	19.27 \pm 2.97
PO ₄ ³⁻ (μ mol l ⁻¹)	0.053	0.742	0.27 \pm 0.14
T-P (μ mol l ⁻¹)	0.736	6.928	3.34 \pm 1.05
DIN/DIP	2.11	160.96	27.63 \pm 23.32
NO ₃ ⁻ /NH ₄ ⁺	0.11	360.62	4.68 \pm 34.29
Biological parameters			
Total ciliate density (x10 ³ cells l ⁻¹)	0	5.35	0.38 \pm 0.68
Total ciliate biomass (μ g C l ⁻¹)	0	42.61	1.27 \pm 4.89
Choreotrichida density (x10 ³ cells l ⁻¹)	0	1.64	0.09 \pm 0.25
Choreotrichida biomass (μ g C l ⁻¹)	0	2.87	0.19 \pm 0.49
Tintinnida density (x10 ³ cells l ⁻¹)	0	1.48	0.07 \pm 0.17
Tintinnida biomass (μ g C l ⁻¹)	0	3.91	0.22 \pm 0.56
Strombidiida density (x10 ³ cells l ⁻¹)	0	2.6	0.05 \pm 0.26
Strombidiida biomass (μ g C l ⁻¹)	0	6.43	0.14 \pm 0.66
Other ciliate density (x10 ³ cells l ⁻¹)	0	5.35	0.08 \pm 0.52
Other ciliate biomass (μ g C l ⁻¹)	0	42.61	0.71 \pm 0.47

Tableau 29. Summary of ANOVA statistics depicting significance of sampling sites (spatial effect) and seasons (temporal effect) on nutrient concentrations.

	Sampling seasons		Sampling sites	
	F	<i>p</i>	F	<i>p</i>
NO ₂ ⁻	5.52	<0.001***	0.42	0.79
NO ₃ ⁻	10.73	<0.001***	1.62	0.17
NH ₄ ⁺	16.11	<0.001***	0.14	0.96
T-N	22.11	<0.001***	0.90	0.46
PO ₄ ³⁻	6.66	<0.001***	1.06	0.37
T-P	8.43	<0.001***	0.92	0.44
Si(OH) ₄	34.51	<0.001***	1.68	0.15
DIN/DIP	6.98	<0.001***	1.22	0.30

Plankton communities

- *Ciliate community*

Species composition

We found 28 planktonic taxa among which 25 were identified to the species level and 3 to the genus level (Table 30). The different species belong to 5 classes (Spirotrichea, Litostomatea, Karyorelictea, Oligohymenophorea and Ciliatea) and 9 orders, namely Tintinnida, Choreotrichida, Strombidiida, Euplotida, Haptorida, Loxodida, Philasterida, Sessilida and Peniculida. The ciliate assemblage was largely dominated by Spirotrichea (80.66 % of total abundance, 78.80 % of total biomass) with Choreotrichida representing 35.62 % of total abundance and 35.81 % of total biomass, followed respectively by Tintinnida (27.54 % of total abundance, 26.43 % of total biomass) and Strombidiida (13.22 % of total abundance, 15.4 % of total biomass). Loricated ciliates were dominant in terms of diversity with 15 identified taxa, followed by Choreotrichida (4 species) and Strombidiida (3 species). Ciliate community diversity estimated by the Shannon index varied from 1 to 4.87 bits cell⁻¹ (mean ± s.d. = 1.78 ± 0.98 bits cell⁻¹). The maximum was recorded in December at S5, associated with the presence of *Leegaardiella sol*, *Strombidium conicum*, *Strobilidium spiralis*, *Tintinnopsis baltica*, *Codonella aspera* and *Aspidisca* sp. The Shannon index record is remarkably stable both seasonally (ANOVA, F = 0.65, p = 0.58) and among stations (ANOVA, F = 0.39, p = 0.81).

Tableau 30. List of planktonic ciliate found in Ghar El Melh Lagoon (- not detected; + 40-1000 cells l⁻¹; *** > 1000 cells l⁻¹).

Planktonic ciliates			Mean length (µm)	Mean biovolume (x10 ³ µm ³)	S1	S2	S3	S4	S5	Winter	Spring	Summer	Autumn		
Spirotrichea	Oligotrichia	Strombidiida													
		<i>Strombidium conicum</i>	65.4	13.03	+	+	+	+	***	+	***	-	+		
		<i>Strombidium acutum</i>	55	43.53	+	+	+	+	-	-	+	+	+		
			<i>Strombidium chlorophilum</i>	40	9.42	-	-	-	+	-	+	-	-		
		Choreotrichia	Choreotrichida												
			<i>Strobilidium neptuni</i>	20	2.09	+	+	+	+	+	+	-	+	+	
			<i>Strobilidium spiralis</i>	48	28.93	+	+	+	+	+	+	+	+	+	
			<i>Leegaardiella sol</i>	30	7.06	***	+	+	***	***	+	***	***	+	
			<i>Lohmanniella oviformis</i>	27	4.06	+	+	+	+	-	+	+	+	+	
			Tintinnida												
			<i>Tintinnopsis baltica</i>	50	5.88	+	+	+	+	+	+	+	+	+	+
			<i>Tintinnopsis beroidea</i>	70	8.24	+	+	-	+	+	+	-	-	-	+
			<i>Tintinnopsis tocaninensis</i>	80	6.02	-	-	+	+	-	-	-	-	+	-
			<i>Tintinnopsis compressa</i>	60	3.14	-	-	-	-	+	-	-	-	-	+
		<i>Tintinnopsis radix</i>	160	74.18	+	+	+	-	+	-	-	-	-	+	
		<i>Eutintinnus tubulosus</i>	120	39.25	-	+	-	-	+	-	+	-	-	-	
		<i>Eutintinnus macilentus</i>	140	16.48	-	+	+	-	-	-	-	-	+	+	
		<i>Codonella aspera</i>	50	4.08	+	+	+	+	+	-	+	+	+	+	
		<i>Favella serrata</i>	180	94.20	-	+	-	-	-	-	+	-	-	-	
		<i>Favella azorica</i>	108	50.86	+	-	+	-	-	+	+	+	+	-	
		<i>Petalotriccha ampula</i>	60	15.89	-	-	+	+	-	-	+	+	+	-	
		<i>Amphorides amphora</i>	120	19.23	-	+	-	-	-	-	-	-	+	-	
		<i>Codonellopsis morchella</i>	60	7.06	-	+	-	-	-	-	-	-	-	+	
	<i>Amphorellopsis acuta</i>	100	11.77	-	-	+	-	-	-	-	-	-	+		
	<i>Metacylis mediterranea</i>	60	14.57	+	+	+	***	+	-	***	+	+	+		
	Hypotrichia	Euplodida													
		<i>Euplotes charon</i>	30	4.90	***	-	-	+	+	-	-	***	-		
Litostomatea		<i>Mesodinium rubrum</i>	23	2.17	+	-	+	+	+	+	+	+	+		
Karyorelictea		<i>Aspidisca</i> sp.	60	14.13	-	-	+	-	+	+	-	+	-		
Ciliata		<i>Paramecium</i> sp.	100	41.86	+	+	-	+	***	+	-	+	***		
Oligohymenophorea		<i>Uronema marinum</i>	36	5.88	+	+	+	-	+	-	+	+	+		
		<i>Vorticella</i> sp.	210	87.92	+	-	-	-	-	-	+	-	-		

Size structure

The ciliate community ranged within three size fractions: small (20-30 μm), medium-sized (30-60 μm) and large ciliates (> 60 μm), belonging to distinct taxonomic groups and showing different ecological preferences as well. The three size fractions were well represented in GML waters with, however, the dominance of ciliates larger than 30 μm (61.2 %). Small ciliates (5 species) were comprised of different choreotrichs such as *L. sol*, *Strobilidium neptuni* and *Lohmanniella oviformis*, the hypotrich *Euplotes charon* and the autotrophic cyclotrich *Mesodinium rubrum*. Medium-sized ciliates (11 species) were represented essentially by different oligotrichs (*Strombidium chlorophilum*, *Strombidium acutum*) and choreotrichs (e.g. genera *Strobilidium*, *Codonella*, *Codonellopsis*, *Metacylis*), although other taxa were also observed in the samples such as *U. marinum* and the benthic *Aspidisca* sp. Large ciliates (12 species) comprised of tintinnids, namely the genera *Eutintinnus*, *Tintinnopsis*, *Favella*, *Amphorides* and *Amphorellopsis* and *Favella serrata*, showed the maximum size mainly attributed to its large lorica (length = 180 μm). We also found within this group the mixotrophic ciliate *S. conicum*, the benthic species *Vorticella* sp. and *Paramecium* sp. The mean length of the different ciliates is summarised in Table 3.

The highest contribution of each size class to total ciliate abundance was recorded in spring with 37.41 %, 53.53 % and 34.8 % for small, medium and large ciliates respectively (Figure 38). All size fractions were found at the five sampling stations with dominance of large ciliates at S5 and small ones at S1 (Figure 39).

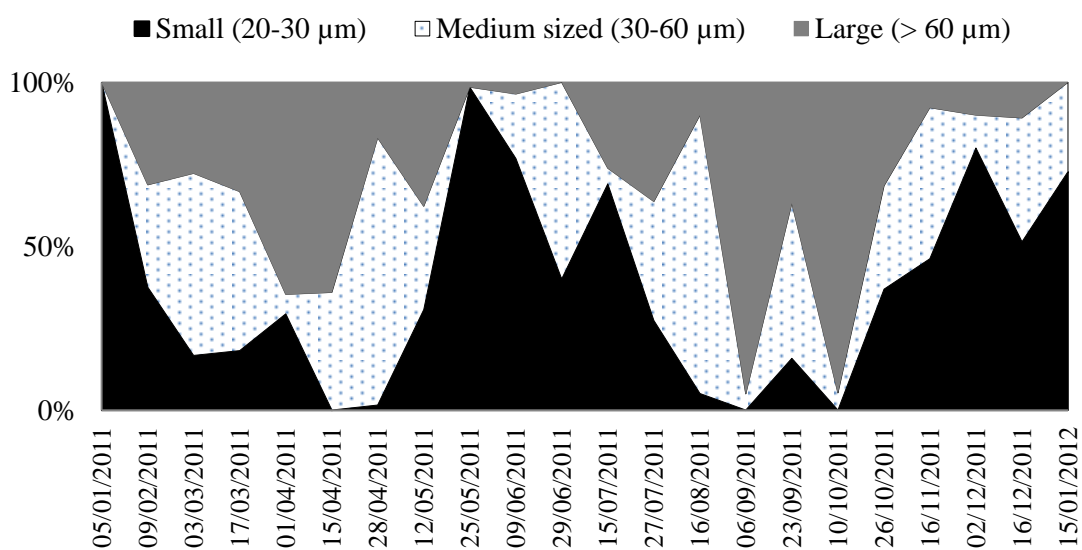


Figure 38. Frequency of different size class of ciliates during the period study.

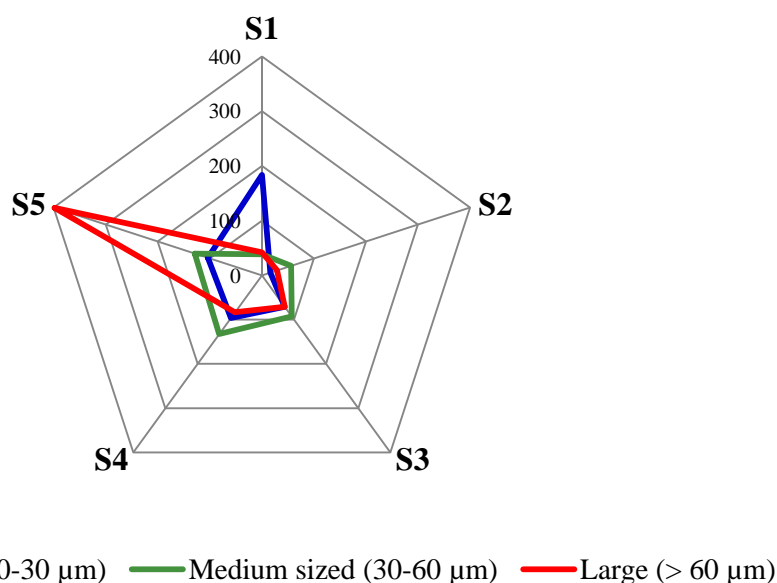


Figure 39. Mean abundances (cells l⁻¹) of different size class of ciliates in the five sampled stations.

Abundance and biomass

Total ciliate abundances ranged from 0 to $5.35 \cdot 10^3$ cells l⁻¹ (mean \pm s.d. = $3.8 \cdot 10^2 \pm 6.8 \cdot 10^2$ cells l⁻¹), showing a remarkable increase in spring (mean \pm s.d. = $4.76 \cdot 10^2$ cells l⁻¹ \pm $2.13 \cdot 10^2$ cells l⁻¹) ascribed to the high densities of different ciliate species including *S. conicum* (maximal abundance = $2.6 \cdot 10^3$ cells l⁻¹ at S5), *L. sol* (maximal abundance = $1.64 \cdot 10^3$ at S1) *Metacylis mediterranea* (maximal abundance = 10^3 cells l⁻¹ at S4), *C. aspera* (maximal abundance = 720 cells l⁻¹ at S5), *S. spiralis* (maximal abundance = 520 cells l⁻¹ at S5), *Uronema marinum* (maximal abundance = 320 cells l⁻¹ at S2), *M. rubrum* (maximal abundance = 200 cells l⁻¹ at S5), *T. baltica* (maximal abundance = 200 cells l⁻¹ at S3), *Eutintinnus tubulosus* (maximal abundance = 120 cells l⁻¹ at S2), *Petalotriccha ampula* (maximal abundance = 120 cells l⁻¹ at S3) and a single apparition of *S. chlorophilum* (maximal abundance = 40 cells l⁻¹ at S4) and *Vorticella* sp. (maximal abundance = 40 cells l⁻¹ at S1) respectively. Another peak of ciliate abundance occurred in September associated with *Paramecium* sp. (maximal abundance = $5.36 \cdot 10^3$ cells l⁻¹ at S5).

Total ciliate biomass varied from 0 to $42.61 \mu\text{g C l}^{-1}$ (mean \pm s.d. = $1.27 \pm 4.89 \mu\text{g C l}^{-1}$). Overall, ciliate abundances and biomass showed similar trends ($r = 0.9$, $n = 110$, $p = 0$). Choreotrichida, Tintinnida, and Strombidiida exhibited their highest abundance and biomass in spring while the remaining groups reach theirs in autumn, mainly associated with *Paramecium* sp. (Figure 40).

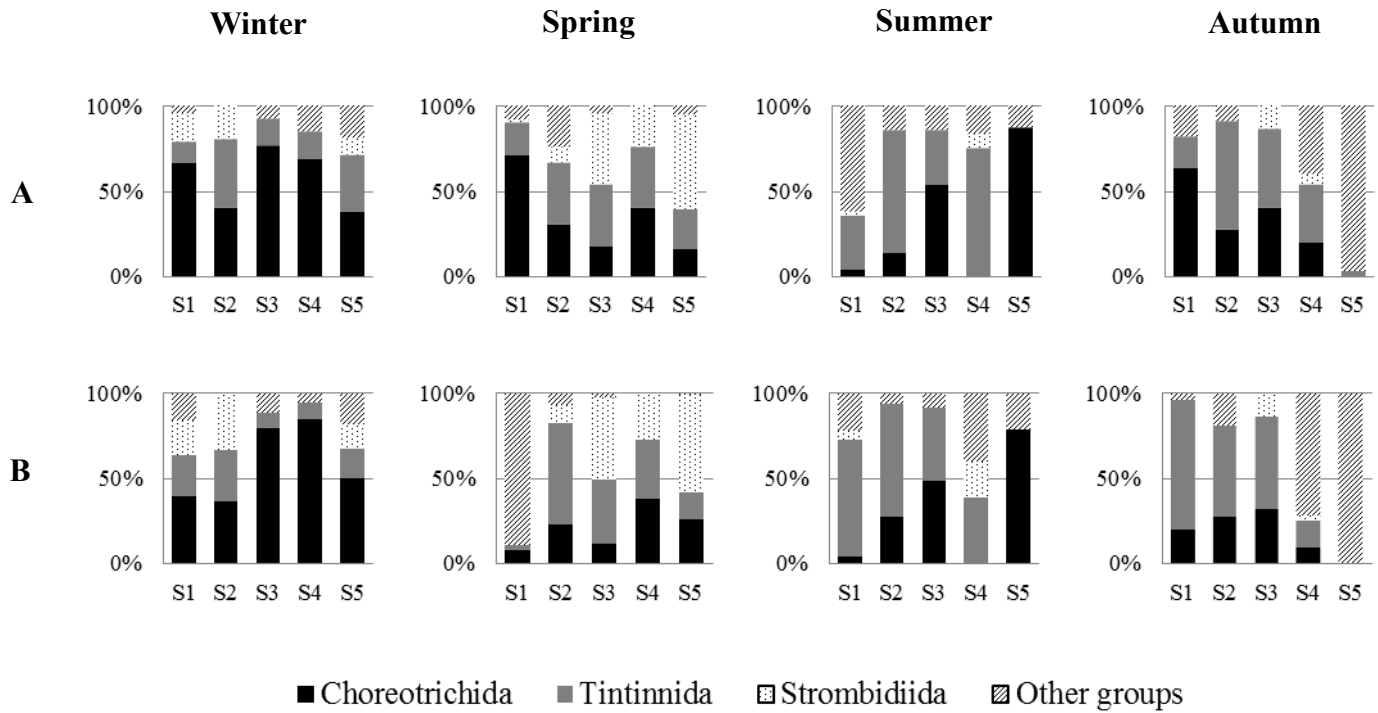


Figure 40. Relative contribution of ciliate groups to total ciliate abundances (A) and biomass (B) during the four sampled seasons.

Dynamics of dominant ciliates

Only 12 ciliates made a considerable contribution to total ciliate abundance (88.92 %) (Figure 41). No significant difference in ciliate abundances was found among sampling sites (RDA, $F = 0.65$, $p = 0.95$), however a significant change was seen according to season (RDA, $F = 2.66$, $p < 0.001$). This temporal variability explains 7.62 % of changes in ciliate abundances with RDA axes 1 and 2 both supporting a significant effect on this variability ($p < 0.05$; Figure 42). Spring has a negative RDA score indicative of close relationships with *S. conicum*. The tintinnids *C. aspera* and *M. mediterranea* also seem to be associated with spring. The winter was associated with *L. sol*, *S. neptuni*, *T. baltica* and *M. rubrum* development. Summer and autumn showed close RDA scores attributed to *Paramecium* sp.

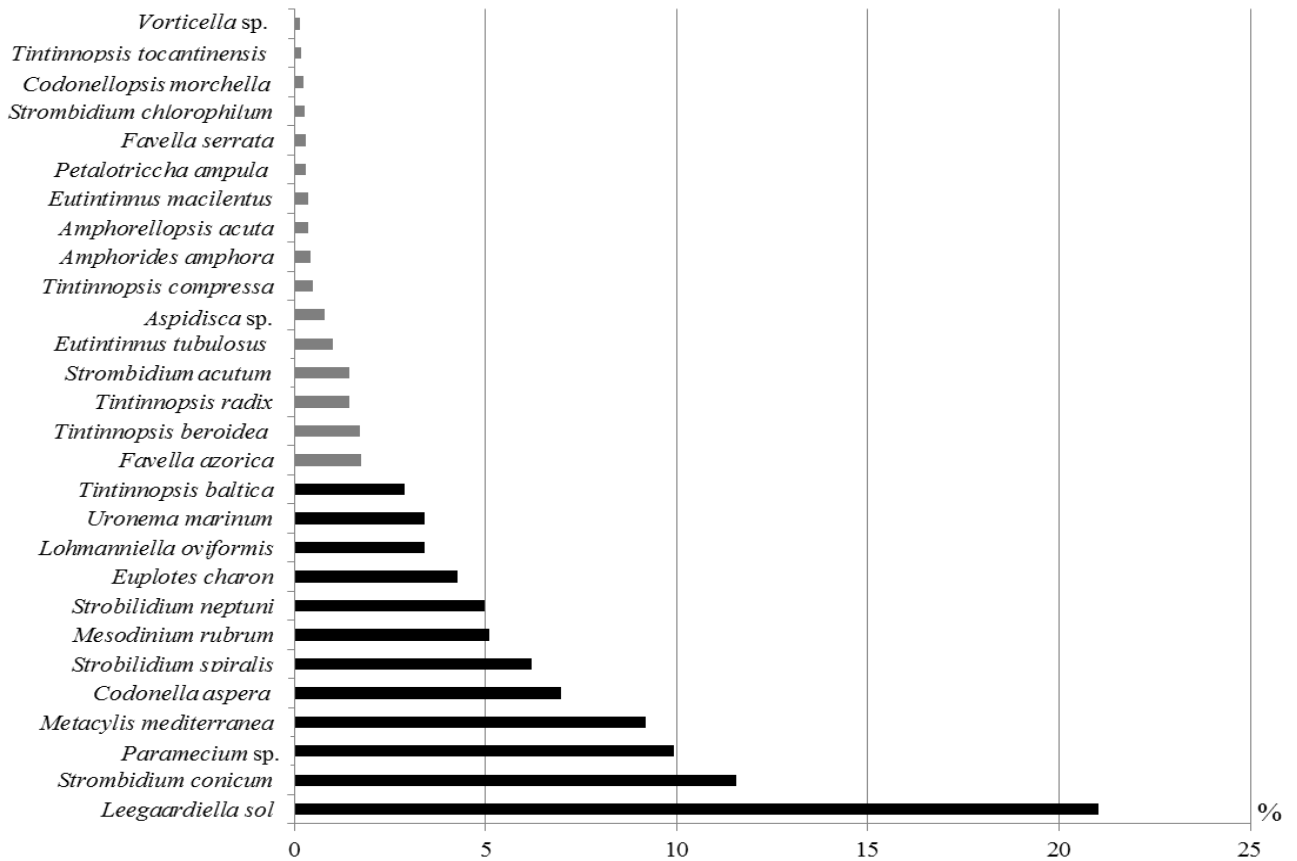


Figure 41. Relative contribution of different species to ciliate community. Black histograms refers to dominant species (> 2 %).

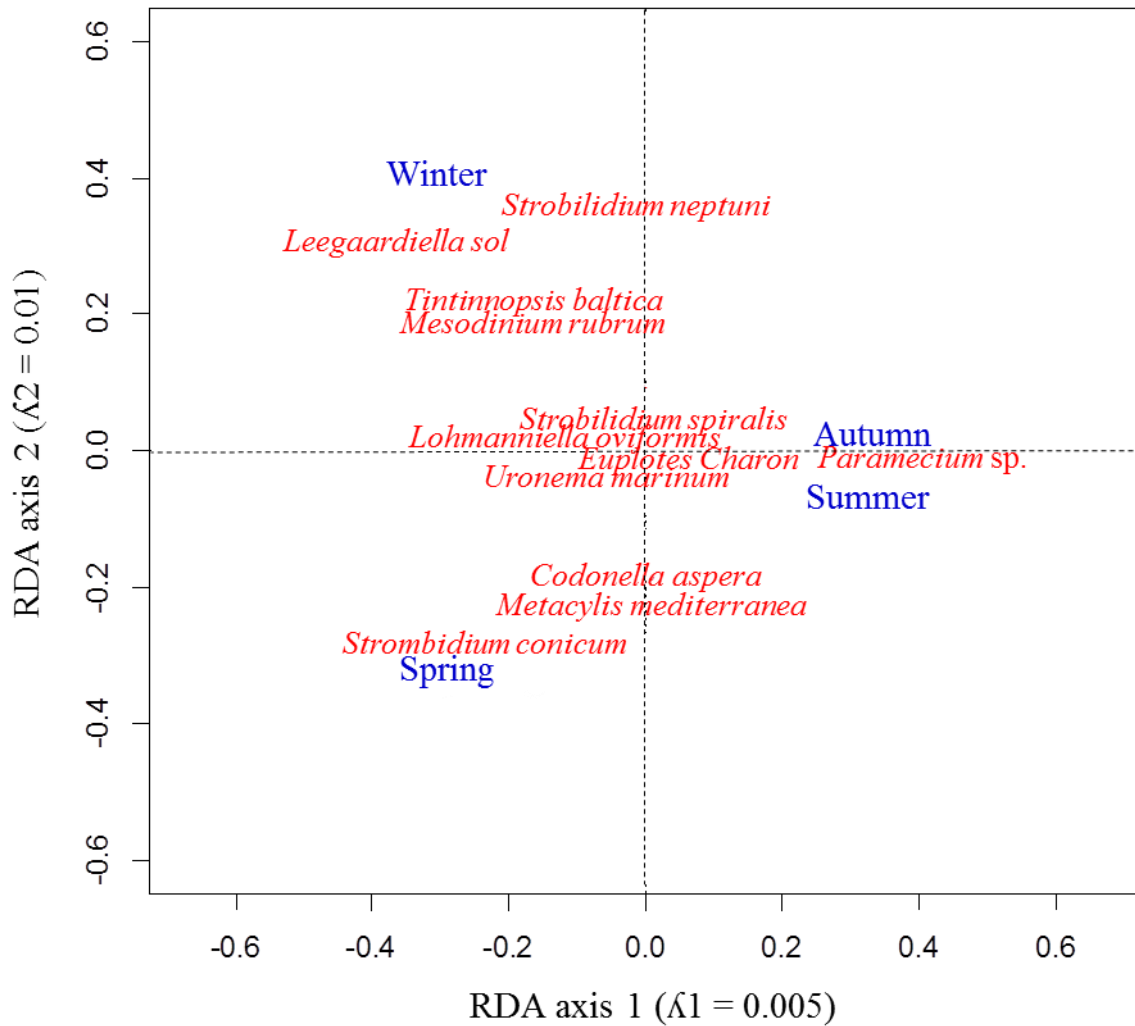


Figure 42. RDA TriPlot depicting the association between ciliate dominant species and sampling season in Ghar El Melh Lagoon. Eigenvalues of the first two axes are indicated by λ_1 and λ_2 .

Significant correlations were found between ciliate abundances and physico-chemical variables such as T-P (RDA, $F = 4.15$, $p < 0.001$), temperature (RDA, $F = 2.89$, $p = 0.002$), T-N (RDA, $F = 2.54$, $p = 0.006$), nitrite (RDA, $F = 2.30$, $p = 0.01$) and orthophosphate (RDA, $F = 2.28$, $p = 0.01$). Physico-chemical fluctuations explain 11.99 % of changes in ciliate abundances. RDA axes 1 and 2 both support a significant effect on the variability of ciliate abundance ($p < 0.05$; Figure 43). Along RDA axis 1, the dominant species *L. sol* stands out with a negative score and is thus associated with low temperature. *S. conicum* shows negative RDA axis 1 and 2 scores correlating with low concentrations of total phosphorus, total nitrogen, orthophosphate and nitrite. *M. mediterranea* and *E. charon* have a positive RDA

axis 1 and a negative RDA axis 2 score indicative of close relationships with high temperature. *Paramecium* sp. stands out clearly with a positive score along RDA axis 1 correlating with a high concentration in total phosphorus and total nitrogen. *S. neptuni* was strongly associated with enhanced concentrations of nitrite and orthophosphate. The remaining ciliates did not seem to exhibit a specific feature according to environmental variability at the stations. Similarly, the remaining abiotic factors monitored in this study did not correlate with any of the observed ciliates. Salinity, which is a characteristic feature of GML waters, reached high levels in summer (maximal salinity = 51.2 at S4), but was related neither to the abundance of each ciliate nor to total ciliate abundance (Spearman's test, $p = 0.1$).

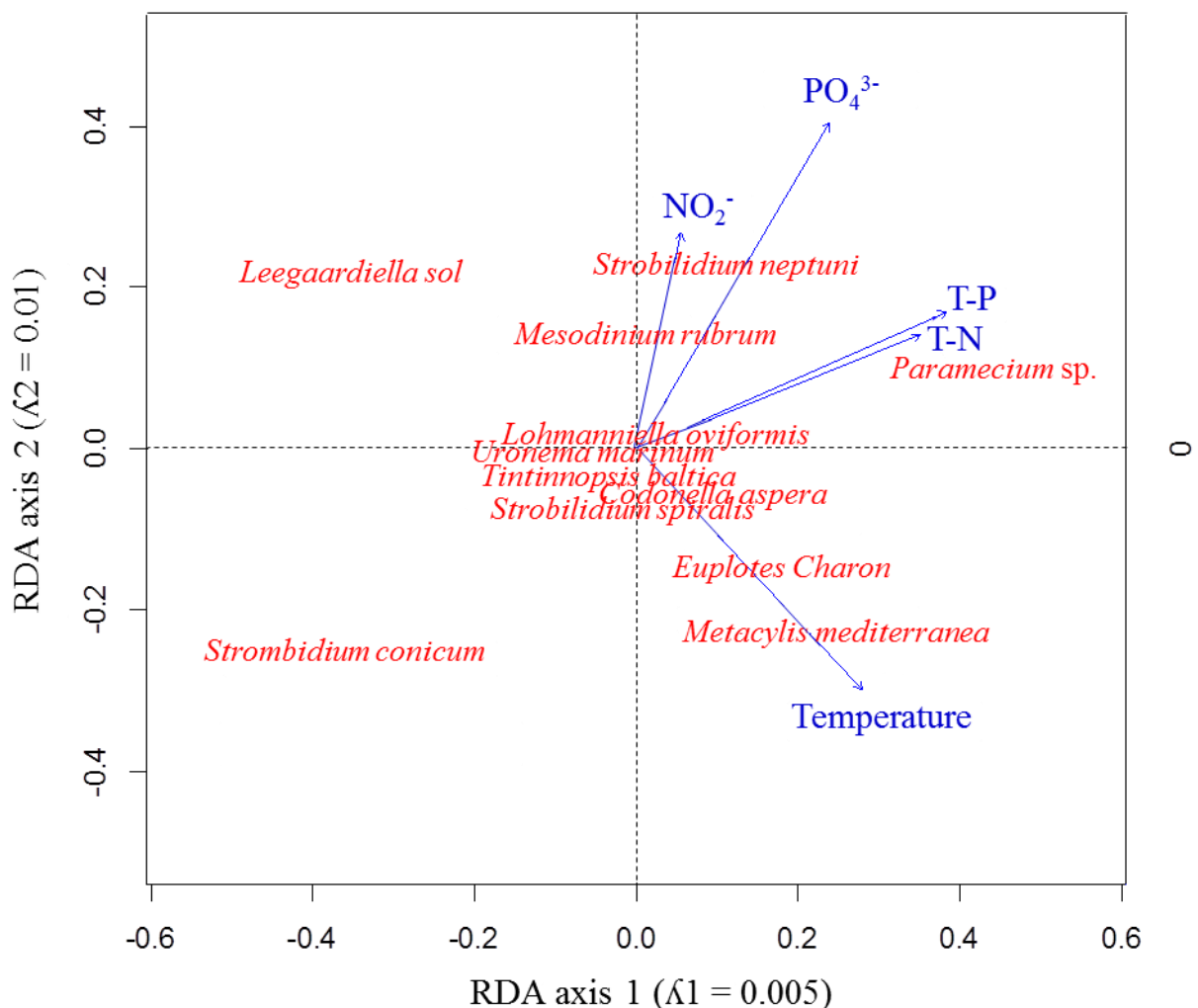


Figure 43. RDA TriPlot depicting the association between ciliate dominant species and environmental factors in Ghar El Melh Lagoon. Eigenvalues of the first two axes are indicated by λ_1 and λ_2 .

- *Phytoplankton community*

Phytoplankton groups consisted of Dinophyceae (32 species), Bacillariophyceae (22 species), Cyanobacteriae (5 species), Chlorophyceae (one species) and Euglenophyceae (one species). Dinoflagellates (54.88 % of total abundance) and diatoms (38.86 % of total abundance) were the dominant groups. Two peaks of dinoflagellate abundance were recorded in spring (3.68 10^5 cells l^{-1} at S5) and early summer (3.95 10^5 cells l^{-1} at S4) associated exclusively with *Prorocentrum micans*. Two additional high concentrations were recorded, the first in summer associated exclusively with *Scrippsiella trochoidea*, the latter during the winter season and mainly ascribed to the proliferation of *P. micans*, *S. trochoidea*, *Prorocentrum triestinum* and *Dinophysis sacculus*. Two peaks of diatom abundance were detected in summer with 4.4 10^5 cells l^{-1} and 3.90 10^5 cells l^{-1} respectively, both at S5 associated with the development of *Navicula duerrenbergiana*, *Navicula phyllepta*, *Cylindrotheca closterium* and *Nitzschia linearis*.

The correlations found between the dominant species of both ciliate and phytoplankton are given in Table 31. Similar trends were seen between phytoplankton and ciliate groups (Figure 44). Total phytoplankton were related to Strombidiida ($r = 0.24$, $n = 110$, $p = 0.01$) and dinoflagellates with total ciliates ($r = 0.33$, $n = 110$, $p = 0$), Strombidiida ($r = 0.34$, $n = 110$, $p = 0$) and Choreotrichida ($r = 0.26$, $n = 110$, $p = 0.006$).

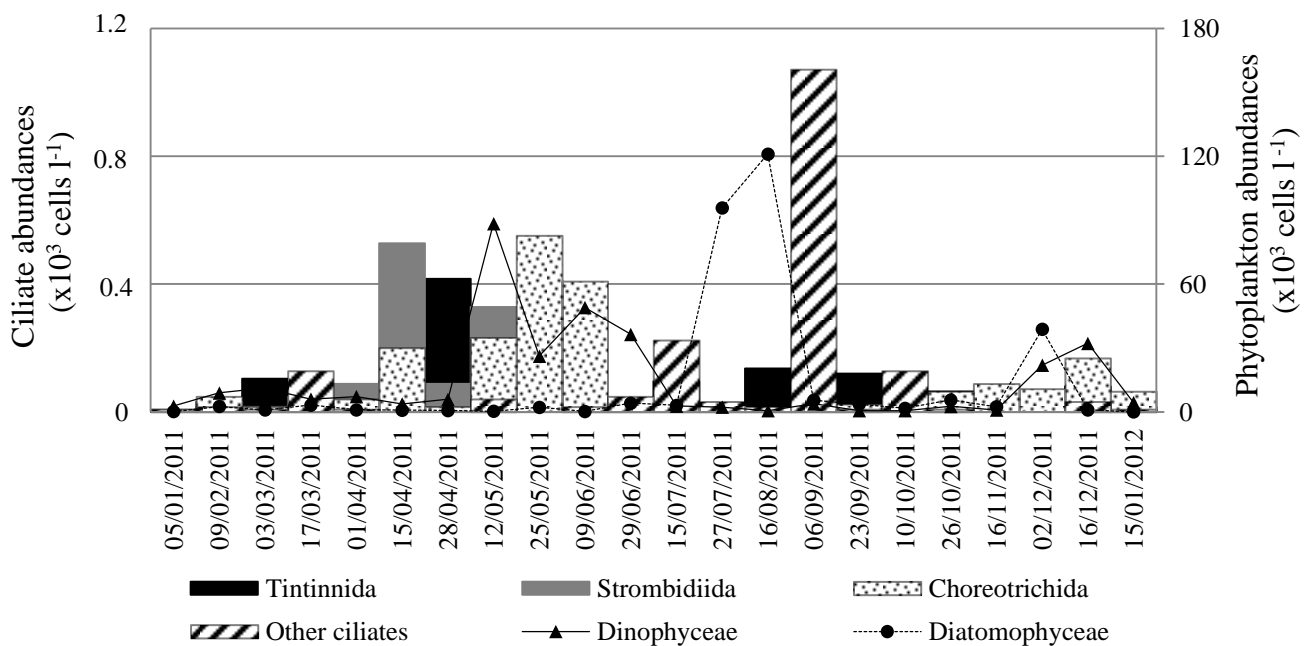


Figure 44. Seasonal distribution of the major groups of planktonic ciliate and phytoplankton.

Tableau 31. Correlation matrix (Spearman test) for biological variables (planktonic ciliates/phytoplankton) under study in Ghar El Melh Lagoon (number of analysed samples = 110).

	<i>Prorocentrum micans</i>	<i>Scrippsiella trochoidea</i>	<i>Dinophysis sacculus</i>	<i>Prorocentrum triestinum</i>	<i>Navicula duerrenbergiana</i>	<i>Navicula phyllepta</i>	<i>Nitzschia linearis</i>
<i>Strombidium conicum</i>	r = 0.39; p = 0	r = 0.37; p = 0	-	-	-	-	r = 0.25; p = 0.007
<i>Strobilidium spiralis</i>	-	-	r = 0.19; p = 0.05	r = 0.23; p = 0.015	-	r = 0.28; p = 0.003	-
<i>Leegaardiella sol</i>	r = 0.29; p = 0.002	r = 0.32; p = 0.001	r = 0.24; p = 0.012	-	r = -0.2; p = 0.032	r = -0.23; p = 0.015	-
<i>Lohmanniella oviformis</i>	-	r = 0.24; p = 0.012	r = 0.23; p = 0.014	-	-	-	-
<i>Codonella aspera</i>	r = 0.21; p = 0.027	r = 0.32; p = 0.001	-	-	-	-	-
<i>Metacylis mediterranea</i>	-	-	-	-	r = 0.21; p = 0.024	-	-
<i>Mesodinium rubrum</i>	-	r = 0.22; p = 0.024	-	-	-	-	-
<i>Paramecium sp.</i>	r = -0.23; p = 0.018	-	-	-	r = 0.4; p = 0	-	-
<i>Euplotes charon</i>	-	-	-	-	r = 0.4; p = 0	r = 0.2; p = 0.033	-

Ciliates and microalgae on leaves of *R. cirrhosa*

- *Ciliate community*

Epiphytic ciliates of *R. cirrhosa* were *Tintinnopsis campanula*, *Aspidisca* sp., *Strombidium acutum* and *Amphorides amphora*. Except for the first species, the three latter taxa were found within the planktonic community as well (Table 32). *T. campanula* exhibited a big lorica reaching up to 170 μm . Ciliate abundance ranged from 0 to $9.27 \cdot 10^3$ cells 100 g^{-1} FW (mean \pm s.d. = $7.47 \cdot 10^2 \pm 1.9 \cdot 10^3$ cells 100 g^{-1} FW) showing remarkable development in June (mean \pm s.d. = $1.94 \cdot 10^3$ cells 100 g^{-1} FW $\pm 2.99 \cdot 10^3$ cells 100 g^{-1} FW), attributed to the maximum concentrations recorded for *T. campanula* (maximal abundance = $7.64 \cdot 10^3$ cells 100 g^{-1} FW at S5) and *Aspidisca* sp. (maximal abundance = $1.82 \cdot 10^3$ cells 100 g^{-1} FW at S2). According to PERMANOVA analysis, ciliate abundance exhibits a significant correlation to both station and month (Table 33). The highest abundance of ciliates was registered in June at S5, while the lowest was recorded the same month at stations S1 and S3, in July at S1 and S5 and in August at S1, S2, S3 and S5 (Figure 45). No significant difference between July and August was detected (Table 33).

The relationship between epiphytic ciliates and environmental variables is illustrated in the ACP (Figure 46) whose two component axes explain 50 % of total variance. The first component axis explains 30.89 % of total variability while the second explains 20.69 % of total variance. Ciliate abundance follows roughly that of *R. cirrhosa* ($r = 0.66$; $p < 0.01$) and clearly stands out with its pH values, especially in June ($r = 0.584$, $p < 0.05$). However, no correlations were found with the remaining abiotic factors.

Tableau 32. List of epiphytic ciliates found in Ghar El Melh Lagoon (- absence; + presence).

Epiphytic ciliates			Mean length (μm)	Min/Max (cells 100 g ⁻¹ FW)	S1	S2	S3	S4	S5	June	July	August	
Spirotrichea	Oligotrichia	Strombidiida											
		<i>Strombidium acutum</i>	60	0-800	-	-	-	+	-	-	+	+	
	Choreotrichia	Tintinnida											
		<i>Tintinnopsis campanula</i>	170	0-7636	-	-	-	-	+	+	-	-	
		<i>Amphorides amphora</i>	125	0-800	-	-	-	+	-	-	+	-	
Karyorelictea		<i>Aspidisca</i> sp.	65	0-1824	-	+	+	+	+	+	+	+	

Tableau 33. PERMANOVA results for ciliate abundance on *Ruppia cirrhosa* leaves. Bold numbers indicate significant effects. Mo = Month, Si = site.

Source of variation	<i>df</i>	<i>MS</i>	<i>F</i>	<i>Perm</i>
Month	2	12767.52	28.53	0.001
Site	4	7055.24	15.76	0.001
Month * Site	8	5168.25	11.55	0.001
Residual	30	447.41		
Cochran's C-test		C = 0.981		
Transformation		Ln (x+1)		
SNK test		June > August = July		

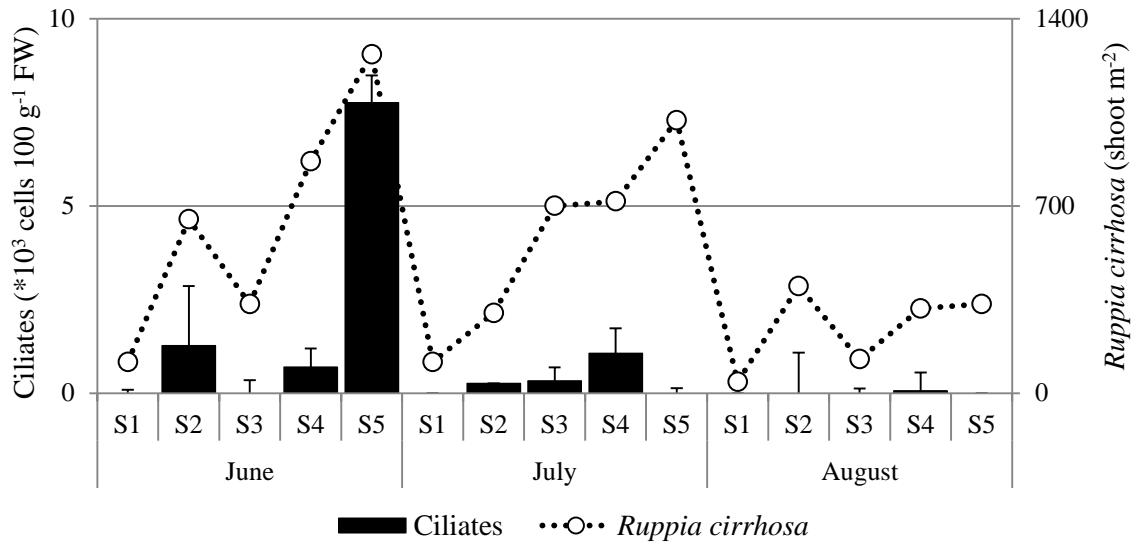


Figure 45. The mean abundance of epiphytic ciliates (cells 100 g⁻¹ FW) and *Ruppia cirrhosa* (shoot m⁻²) in the five stations and for each month.

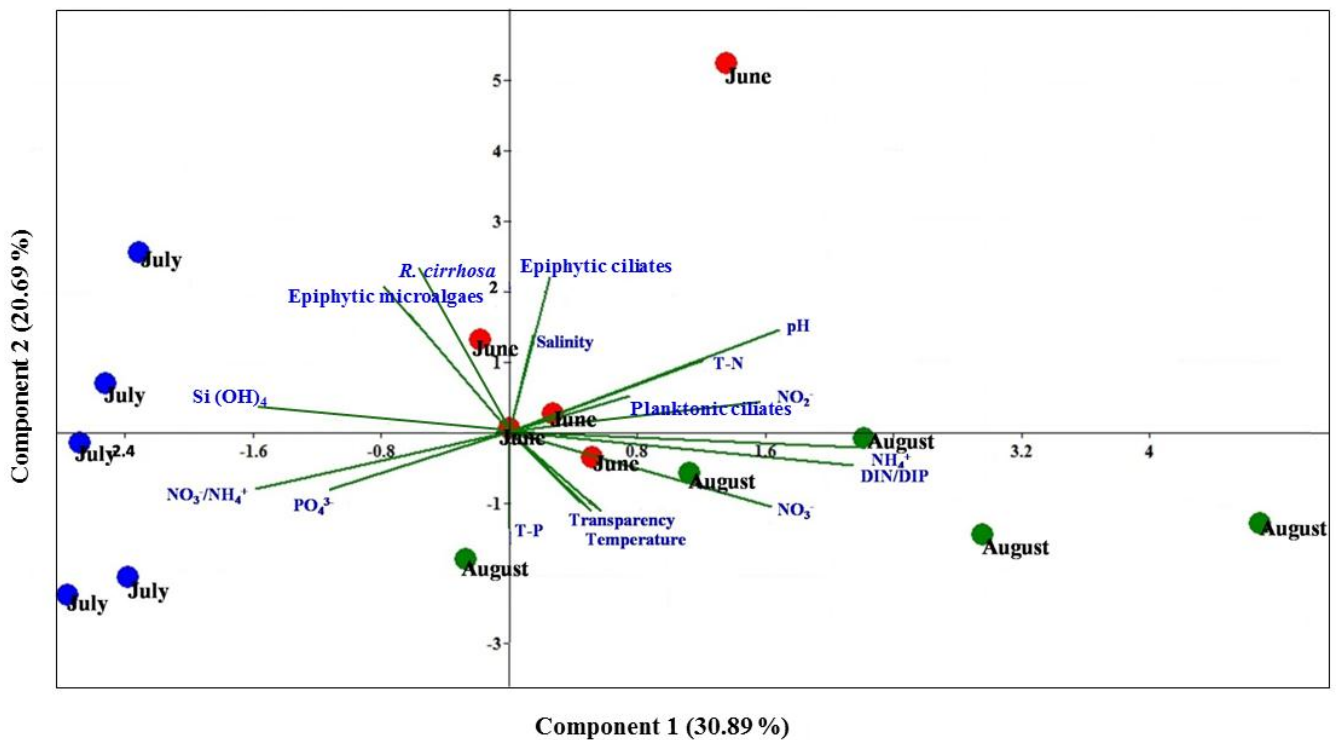


Figure 46. Principal Component Analysis (PCA) of the epiphytes communities in relation to abiotic factors and *Ruppia cirrhosa* density.

- *Microalgae community*

Algal epiphytes were comprised of Dinophyceae (4 species), Bacillariophyceae (11 species) and Cyanobacteriae (5 species). Two peaks of dinoflagellate abundance were recorded in June ($6.05 \cdot 10^5$ cells 100 g^{-1} FW at S5) and July ($3.72 \cdot 10^5$ cells 100 g^{-1} FW at S5), mainly associated with *Prorocentrum lima* proliferations. One peak of diatoms was detected in July, reaching $1.1 \cdot 10^6$ cells 100 g^{-1} FW at S4 and characterised by the development of *Navicula phyllepta*, *Thalassionema nitzschioides* and *Licmophora gracilis*. According to PCA, algal epiphytes exhibit a significant correlation with epiphytic ciliates ($r = 0.73$; $p < 0.01$) (Figure 46). At the species level, the ciliate *Aspidisca* sp. was positively correlated with *P. lima* ($r = 0.71$; $p < 0.01$) and *T. nitzschioides* ($r = 0.74$; $p < 0.01$).

4. Discussion

Ciliate assemblages in Ghar El Melh Lagoon show some similarities as well as singularities compared to other aquatic systems. During this study, planktonic ciliate composition was largely dominated by Spirotrichea species (80.66 % of total abundance; 78.80 % of total biomass) which is a common feature in many aquatic ecosystems (Dolan, 1991; Laybourn-Parry, 1992; Muylaert *et al.*, 2000; Balkis, 2004; Nogueira *et al.*, 2005; Mironova *et al.*, 2009). Naked ciliates lead the ciliate community (72.47 % of total abundance; 72.5 % of total biomass), as has been reported from most environments where loricate ciliates form a minority component (Dolan, 2000; McManus and Santoferrara, 2012; Montagnes, 2012; Dolan *et al.*, 2013). Nevertheless, while naked ciliates prevailed in the planktonic ciliate composition, tintinnids dominated in terms of species number (15 taxa) and took second place in terms of abundance, accounting for 43.6 %, 34.15 % and 27.54 % of total choreotrichs, spirotrichs and total ciliate abundances respectively. In addition, epiphytic tintinnids represented by *T. campanula* dominated the ciliate community during summer (57.09 % of total abundance), with a high density exceeding $7.5 \cdot 10^3$ cells per 100 g of *R. cirrhosa* leaves. Only *T. campanula* was exclusively found as an epiphytic ciliate, with a sudden peak in June that may be a response to food availability on *R. cirrhosa* leaves. It is well known that tintinnids form patchy distributions in relation to their food resources (e.g. Stoecker *et al.*, 1984; Christaki *et al.*, 2008). We did not find *T. campanula* in the July and August samples, which may be due to the predatory activity of the copepods known to control the ciliate abundance in summer (Dolan and Gallegos, 2001; Katechakis *et al.*, 2004). Our assumption may also be supported by the highest copepod abundance (up to $4.95 \cdot 10^4$ ind m^{-3}), coming from the El Boughaz Channel and recorded in summer from samples taken in parallel to those

of phytoplankton and ciliates. This quantitative importance of tintinnids may be due to the eutrophic conditions of GML waters (prominent concentrations of total phosphorus and dissolved inorganic nitrogen) to which tintinnids can adapt efficiently (Cordeiro *et al.*, 1997; Krsinic, 2010; Bojanic *et al.*, 2012). The karyorelictea group represented by *Aspidisca* sp. assumes an important place within the epiphytic ciliates (34.18 % of total abundance). Despite its low presence in the water column (maximal abundance = 80 cells l⁻¹), this taxa reached high concentrations on *R. cirrhosa* leaves, above 1.5 10³ cells 100 g⁻¹. The three epiphytic ciliate species *Aspidisca* sp., *A. amphora* and *S. acutum* were also found in our plankton samples, a feature often noted in coastal and shallow waters due to a strong wind impact facilitating the intensive mixing of water masses (Klinkenberg and Schumann, 1994; Telesh, 2004; Telesh *et al.*, 2008).

In this study, Planktonic ciliate diversity was always high with a maximum of 4.87 bits cell⁻¹, suggesting that ciliates in coastal lagoons occupy a wide range of ecological niches, greater than in other planktonic ecosystems. Our results indicate that abundances of dominant species do not overlap, and, therefore, diversity could consist of a rich assemblage of rare species which should not be negligible taxa in a survey. Indeed, rare species can change in abundance rapidly and many of the non-dominant species may become dominant (Orsi *et al.*, 2012; Dolan *et al.*, 2013). This has been well established by Dolan *et al.* (2013) for the tintinnid group which is the more diversified population in our case (15 plankton + 1 epiphyte) and contains many rare species. Contrary to planktonic ciliates, epiphytic taxa exhibit a low species richness (4) and high abundance, a characteristic feature of *Ruppia* plants which usually inhabit an abundant but species-poor fauna (Verhoeven, 1980). Ciliate abundance was low for planktonic ciliates, typical in nutrient-limited open waters (van Beusekom *et al.*, 2007), and high for the epiphytic form. However, in the shallow and eutrophicated GML, ciliates may be present in low numbers in the water column but highly abundant on *Ruppia* leaves. Abundance and biomass of planktonic ciliates show similar trends, mainly attributed to the peak in different size classes of ciliates in spring, with a peak of large ciliates in early autumn (Figure 38). Epiphytic ciliates recorded during the summer were characterised by their large size (60 µm - 170 µm), a typical trait of benthic communities (Klinkenberg and Schumann, 1994).

Based on redundancy analysis, three distinct associations of planktonic ciliate species were identified: a spring association involving *S. conicum*, *M. mediterranea* and *Codonella* sp., a winter assemblage concerning the small ciliates *L. sol*, *S. neptuni*, *M. rubrum* and the tintinnid *T. baltica*, and an early autumn segregation comprised only of *Paramecium* sp. We think that

spring was the most prominent period for the distribution of planktonic ciliates in Ghar El Melh Lagoon as shown by the maximal abundance recorded in spring for a wide range of ciliates (12 taxa) belonging to different size classes with different feeding modes. The presence of ciliates as a major group in spring has been reported in several studies (Montagnes *et al.*, 1988; Brussaard *et al.*, 1995; Johansson *et al.*, 2004; Loder *et al.*, 2011). Epiphytic ciliates were quantitatively important only in summer as they coincided with the strong presence of *R. cirrhosa* which is an adequate habitat for these species. Indeed, both temporal and spatial fluctuations of epiphytic ciliates follow roughly those of *R. cirrhosa* ($r = 0.66$; $p < 0.01$; Figure 46), with maximal abundances found in June and July, especially at stations 2, 4 and 5, associated with massive beds of *Ruppia* seagrass, and minimal ciliate abundances found in August at the majority of stations at the start of seagrass mat decomposition (Figure 45). This is also confirmed by the correlation found between epiphytic ciliates and pH while no correlation was found between planktonic ciliates and pH. Indeed, it is well known that seagrass contributes to increased alkalinity by photosynthesis and is typically considered to act as an ecosystem engineer by playing an important role in structuring benthic assemblages, serving to reduce physical stress and enhance food availability (Orth *et al.*, 1984; Boström *et al.*, 2006; Bos *et al.*, 2007). For instance, we always registered low concentrations of planktonic ciliates at S2 opposite the El Boughaz Channel (mean \pm s.d. = 102 ± 116.3 cells l^{-1}), which may be due to a water current impact, but for the epiphytic *Aspidisca* sp. we recorded the maximal density at this station ($1.82 \cdot 10^3$ cells 100 g^{-1} FW), illustrating the role of *R. cirrosha* as a refuge for this species. Furthermore, significant correlations were found between *Aspidisca* sp. (mean length = $60\ \mu\text{m}$) and both the toxic dinoflagellate *P. lima* (mean length = $39.81\ \mu\text{m}$) and the diatom *T. nitzshioides* (mean length = $77.25\ \mu\text{m}$), suggesting possible food availability for *Aspidisca* sp. on *Ruppia* leaves.

For planktonic ciliates, comparison of spatial temporal variation was not significant. This may be explained by the fact that environmental conditions, during the year under study, varied mainly in time rather than in space (temperature and nutrients in our case) and were probably linked to phytoplankton dynamics (spring-early summer for dinoflagellates, and summer for diatoms). We noted the prevalence of oligotrich *S. conicum* during spring following low concentrations of total phosphorus, total nitrogen, orthophosphate and nitrite. This is in line with other studies conducted in the western Mediterranean (Dolan and Marrasé, 1995; Pérez *et al.*, 2000, Balkis, 2004), in the south-western Atlantic and north-eastern Brazil (Nogueira *et al.*, 2005), relating the quantitative dominance of oligotrichs to the oligotrophic status of waters. It is of note that *Paramecium* sp. showed only one sudden peak recorded in September

at S5, coinciding with the highest total phosphorus and total nitrogen concentration ascribed to the agricultural and urban discharge in this zone at this period (SCET-ERI, 2000). This species is especially common in scum and can be considered as a bioindicator of polysaprobic water (Németh-Katona, 2010). The appearance of the small ciliate *S. neptuni* coincided with high concentrations of nitrite and orthophosphate that remained during the rainy season. The dominant planktonic ciliate *L. sol* shows important proliferation mainly in spring when the temperature is mild. It has been reported that many small ciliates seem to prefer moderate temperatures of 12-18 °C (Muller and Geller, 1993; Weisse and Montagnes, 1998). The tintinnid *M. mediterranea* was detected in spring and summer. The hypotrich *E. charon* was absent in the majority of samples but showed one peak density only in summer. Apparently the latter's proliferation is stimulated by increased water temperature and it has indeed been shown that some ciliates can survive temperatures of up to 30-34 °C (Barbanera *et al.*, 2000; Jiang and Morin, 2004). Furthermore, the species composition of ciliates is strongly affected by water temperature (Montagnes and Weisse, 2000; Aberle *et al.*, 2007) and the ciliate response to different temperature regimes has been studied in detail (Muller and Geller, 1993; Montagnes, 1996; Weisse and Montagnes, 1998; Weisse *et al.*, 2001, 2002). Salinity, which reached a maximum of 51.2, was not a segregating factor of the ciliate community since different ciliate size fractions were found at each station (Figure 39). Behind the affinity observed between planktonic ciliate fluctuations and environmental factors, we found that the majority of ciliates have significant correlations with the phytoplankton community, especially with harmful dinoflagellates (Table 31). Significant relationships between ciliate and dinoflagellate abundances have been found in previous studies (e.g. Kivi and Setälä, 1995) and in Tunisian coastal areas such as the Gulf of Gabès (Drira *et al.*, 2008, Hannachi *et al.*, 2009). The most significant correlations were found between the ciliate *S. conicum* (mean length = 65.4 µm) and both the dinoflagellates *P. micans* (mean length = 39.75 µm) and *S. trochoidea* (mean length = 18.75 µm). The *Strombidium* genus is known for its direct response and distinct succession patterns in relation to the availability of flagellate prey (Sime-Ngando *et al.*, 1999; Aberle *et al.*, 2007; Loder *et al.*, 2011). Significant relationships were also observed between the dominant ciliate *L. sol* (mean length = 30 µm) and *S. trochoidea*. Only two species of the tintinnid group have a significant correlation with microalgae proliferation, *C. aspera* (mean length = 50 µm) with dinoflagellates *P. micans*/*S. trochoidea* and *M. mediterranea* (mean length = 60 µm) with the diatom *N. duerrenbergiana* (mean length = 39 µm). Indeed, the tintinnid community found in many aquatic areas often displays broad trophic diversity (Perriss *et al.*, 1995; Dolan, 2000) and is considered a major

consumer of pico- and nano-plankton (Capriulo *et al.*, 1991; Dolan *et al.*, 2002; Fonda Umani and Beran, 2003; Karayanni *et al.*, 2008); it can even prey on other ciliates (e.g. Stoecker and Evans, 1985). Moreover, tintinnids rarely directly control the concentration or composition of their prey, as their aggregate feeding activity usually equates to clearing a maximum of 1 to 2 % per day of the surface layer waters they occupy (Dolan *et al.*, 2013). This may explain the non-correlation of the remaining planktonic and epiphytic tintinnids with the microalgae proliferation occurring in GML waters. For the remaining groups, the most significant correlation was found between *Paramecium* sp. (mean length = 100 μm), *E. charon* (mean length = 30 μm) and the diatom *N. duerrenbergiana*. The majority of ciliates prey on smaller prey (Jonsson, 1986; Tillmann, 2004), but sometimes they can also feed on prey items larger than themselves (Gifford, 1985; Johansson *et al.*, 2004; Aberle *et al.*, 2007) which is probably the case of the relationship between *E. charon* and *N. duerrenbergiana*.

In contrast, the detected size groups of ciliates may be effectively consumed by various mesozooplankton (Stoecker and Capuzzo, 1990; Schnetzer and Caron, 2005). In fact, among the samples collected concomitantly with those ciliates, we found that the copepod *Acartia clausi* was the second most abundant species (Ziadi *et al.*, 2015) and has been shown in laboratory experiments to preferentially select ciliates (Wiadnyana and Rassoulzadegan, 1989).

Considering the specificity of our study site (shallow, stagnant, large, *Ruppia* beds...) and its eutrophic conditions (prominent concentrations of nutrients), GML Lagoon is always subject to harmful dinoflagellate blooms (Romdhane *et al.*, 1998, Turki *et al.*, 2007, Dhib *et al.*, 2013a) indicating that ciliates are unable to control them through predation (Sherr and Sherr, 2009). However, ciliates may have a structuring influence which has been observed in previous studies (Riegman *et al.*, 1993; Fonda Umani *et al.*, 2005). On the other hand, dinoflagellates can feed on a wide range of prey (Jeong, 1999; Naustvoll, 2000a, b), are significant consumers of phytoplankton, especially diatoms (Sherr and Sherr, 2007), and thus are in direct feeding competition with ciliates (Hansen, 1992; Sherr and Sherr, 2007). This competition may constitute another hypothesis explaining the simultaneous presence of ciliates and dinoflagellates and the correlations recorded between them.

5. Conclusion

This study illustrates complex relationships between planktonic and epiphytic ciliates and both abiotic and biotic variables. Consideration of the body size of the ciliate community allowed us to (i) identify spring as the most favourable season for planktonic ciliates, with the

prominent presence of all size classes during this period, (ii) understand the interaction between ciliates and abiotic factors, especially for water salinity, and (iii) collect information on the adequate food size for ciliates among microalgae prey. Our study also shows the importance of investigating the epiphytic ciliates living on *R. cirrhosa* leaves which (i) are a unique habitat for the species *T. campula* which was not found in the water column, (ii) harbour ciliate concentrations exceeding those found in the plankton, (iii) provide a refuge from water current impact, for example for *Aspidisca* sp. and (iv) are probably a substrate food for *T. campula*. From the interesting correlations found between the harmful dominant dinoflagellates on the site and ciliates, grazing essays, for example between *S. conicum* and the harmful *P. micans*, and between *Aspidica* sp. and the toxic *P. lima*, may offer an opportunity to improve our understanding of the harmful dinoflagellate blooms in this lagoon.

Acknowledgments

This study was conducted by Amel DHIB as a part of her PhD research (co-directed at the University of Franche-Comté, CNRS 6249, Besançon, France, and at the Institut National des Sciences et Technologie de la Mer, Tunisia).

CONCLUSIONS GENERALES

La lagune de Ghar El Melh, constitue une étendue d'eau peu profonde où les apports polluants (rejets d'eaux usées domestiques et industrielles, rejets du système de drainage et de l'activité de la pêche) sont importants et où le renouvellement des eaux semble insuffisant pour éviter la formation de zones de confinement.

Toutes ces conditions conjuguées aux facteurs environnementaux constituent les principaux précurseurs des proliférations microalgales et surtout le développement de certaines espèces nuisibles.

L'étude des paramètres hydrologiques nous a permis de révéler que :

La température de l'eau de mer dans la lagune (11 à 26.9 °C) était typique de celle trouvée dans les eaux côtières de la Méditerranée. En revanche, la salinité atteint des valeurs exceptionnelles (jusqu'à 51.2) qu'on trouve habituellement dans les marais salants. Les apports d'eau douce sont presque inexistantes en été ce qui, combiné à une évaporation élevée et la faiblesse des entrées marines, *via* la connexion étroite à la mer, conduit à une augmentation substantielle de la salinité dans la lagune. Une particularité supplémentaire est le fait que les nutriments n'ont jamais été épuisés, une expression de l'eutrophisation de la lagune avec, par exemple, des concentrations exceptionnelles de phosphore total et d'azote inorganique dissous en automne et en hiver liés aux apports du bassin versant. La variabilité temporelle importante de la température de l'eau, de la salinité et des concentrations des éléments nutritifs dans la lagune n'est pas associée à une variabilité spatiale significative.

L'étude des populations microalgales planctoniques et épiphytes nous a permis de révéler que :

(1) Les dinoflagellés et les diatomées sont les groupes typiques de la colonne d'eau et sur les feuilles de *R. cirrhosa*. Ces deux groupes phytoplanctoniques sont les plus importants dans les côtes nord de la Tunisie et dans plusieurs régions méditerranéennes. Pour les épiphytes, les macrophytes sont généralement plus favorables aux diatomées et autres organismes.

(2) Une faible diversité des microalgues et des tendances de prolifération monospécifique avec l'envahissement de *P. micans* dans la colonne d'eau et de *P. lima* sur les feuilles de *Ruppia*. Les concentrations enregistrées pour *P. lima* dans le site d'étude ($> 6 \cdot 10^5$ cellules par

100 g de matière fraîche de *R. cirrhosa*) sont alarmantes et beaucoup plus importantes que celles enregistrées sur les feuilles des posidonies en Tunisie et dans d'autres régions du monde.

(3) Des espèces reconnues comme nuisibles et / ou toxiques étaient présentes en abondance dans la colonne d'eau et sur les feuilles de *Ruppia* (28 espèces, représentant plus de 70 %), dont 16 espèces capables de produire des toxines de type DSP, ASP, PSP et plusieurs autres symptômes toxiques alors que les autres espèces pourraient proliférer jusqu'à entraîner des événements d'hypoxie et d'anoxie dans le milieu.

(4) Le rôle de *R. cirrhosa* était primordial dans la diffusion des espèces épiphytes nuisibles, notamment *P. lima* et *T. nitzshioides*, et des diatomées dans la colonne d'eau de la lagune de Ghar El Melh. Les programmes de surveillance, surtout dans les lagunes et les eaux peu profondes, devraient inclure l'étude des microalgues épiphytes pour l'alerte précoce des algues nuisibles.

(5) Considérant la saisonnalité des différentes espèces phytoplanctoniques, il semble que les efflorescences peuvent se produire tout au long de l'année, et que les parties intérieures de la lagune semblent être les sites aux risques les plus élevés, lieux des événements phytoplanctoniques nuisibles. Pour les microalgues épiphytes, la présence des espèces nuisibles était clairement liée à la présence du *Ruppia* dans le site.

(6) En se basant sur les analyses statistiques, un scénario des événements néfastes dans la lagune de Ghar El peut être proposé (i) une prolifération hiver-printemps de la majorité des espèces phytoplanctoniques nuisibles. En effet, la diminution de la température de l'eau dans cette période de l'année (une moyenne de 15.54 ° C) semble favoriser la prolifération du taxon dominant *P. micans*. Les plus faibles concentrations d'azote total, avec une moyenne de 18.35 $\mu\text{mol l}^{-1}$, et d'orthophosphate, avec une moyenne de 0.27 $\mu\text{mol l}^{-1}$, dans ces saisons semblent favoriser le développement des deux dinoflagellés *S. trochoidea* et *A. sanguineum* et la cyanobactérie *Oscillatoria* sp. Cependant, les concentrations élevées d'ammonium avec une moyenne de 4.08 $\mu\text{mol l}^{-1}$ semblent favoriser la prolifération de *D. sacculus*. Il convient de noter que les concentrations maximales de *P. micans* et d'*A. sanguinium* ont été observées au printemps et que les proliférations les plus élevées de *D. sacculus* et *Oscillatoria* sp. ont été notées en hiver. L'apparition hiver-printemps des autres espèces nuisibles à savoir *P. triestinium*, *P. minimum* et *C. decepiens* ne semblait pas liée aux facteurs environnementaux suivis dans notre étude, (ii) une prolifération, en été, du dinoflagellé toxique *P. lima* et de la

diatomée nuisible *T. nitzshioides*. La prolifération de ces deux espèces dans les eaux de la lagune de Ghar El Melh a été corrélée significativement avec la température élevée enregistrée pendant la saison estivale. En plus de cette affinité apparente pour la température élevée, *R. cirrhosa* semble être un support important pour ces deux espèces et elle a, sans doute, un rôle dans leurs diffusion dans la colonne d'eau durant la période estivale. Cela a été confirmé par les corrélations hautement significatives entre l'abondance de ces deux espèces dans la colonne d'eau et sur les feuilles de *Ruppia*. La légère présence de *P. lima* et *T. nitzshioides* dans les autres saisons peut être attribuée à d'autres macrophytes. Les feuilles de *Ruppia* étaient l'habitat privilégié pour d'autres microalgues nocives telles que *P. concavum* et qui ont été détectées dans la colonne d'eau durant la même période, mais avec des corrélations non significatives et (iii) un développement, en automne, de la diatomée nuisible *C. closterium* liée à des températures élevées, enregistrées au cours de cette période (une moyenne de 21.01 °C).

L'étude des kystes de dinoflagellés nous a permis d'identifier :

(1) 14 morphotypes représentés surtout par les kystes de *Protoperidinium* spp. (48.82 %), *Scripsiella trochoidea* complex (9.56 %), RBC (round brown cysts) (7.22 %), *Lingulodinium machaerophorum* (5.36 %), *Alexandrium* spp. (5.35 %) et *Gymnodinium* spp. (4.03 %). Ce nombre est largement inférieur à celui des kystes retrouvés dans la lagune de Bizerte (42 morphotypes).

(2) La faible concentration des kystes de dinoflagellés enregistrée dans le sédiment de la lagune de Ghar El Melh (pas plus que 229 kystes par gramme de sédiment sec). Ceci reflète la composition du phytoplancton dans la colonne d'eau dominée par des espèces de dinoflagellés, ne produisant pas de kystes au cours de leur développement. A titre de comparaison, dans la lagune de Bizerte, les concentrations sont beaucoup plus importantes, atteignant plus de 20000 kystes par gramme de sédiment sec. D'autre part, nos résultats sont plus en concordance avec ceux enregistrés dans les zones tropicales.

(3) Une variation temporelle significative avec une abondance plus importante en automne. En comparaison avec la colonne d'eau, cette période de l'année est connue aussi pour les concentrations les moins importantes de microphytoplancton.

(4) La détection de quatre morphotypes qui sont susceptibles d'être nuisibles et/ou toxiques: *Alexandrium minutum*, *A. pacificum*, *A. pseudogonyaulax* et *Lingulodinium polyedrum*. Les formes végétatives de ces espèces, surtout celles d'*Alexandrium*, ont provoqué auparavant des

proliférations importantes dans la colonne d'eau de la lagune de Ghar El Melh, ce qui est peut être le résultat de la germination des kystes. *L. polyedrum* a été enregistrée dans les eaux de la lagune mais jamais avec des concentrations alarmantes.

L'étude d'ultraplancton (ultraphytoplancton + procaryotes hétérotrophes) nous a permis de mettre en évidence :

(1) L'importance du groupe ultraphytoplanctonique dans la lagune de Ghar El Melh avec des concentrations qui dépassent celles trouvées pour le microphytoplancton.

(2) La dominance de nanophytoplancton par rapport au picophytoplancton, ce qui est le cas pour la plupart des zones côtières et des lagunes. Le rapport nano/pico est largement supérieur à 1 reflétant l'état eutrophique de la lagune.

(2) Une diversité importante de l'ultraphytoplancton avec l'identification d'au moins 12 groupes. L'assemblage ultraphytoplanctonique dans un milieu lagunaire apparaît comme très diversifié par rapport au milieu pélagique.

(3) Des concentrations maximales des procaryotes hétérotrophes mais qui ne dépassent pas 9×10^6 cellules par cm^{-3} . Ces concentrations sont semblables à celles enregistrées dans d'autres baies et lagunes eutrophes, mais sont beaucoup plus élevées que celles rapportées pour les océans et les mers oligotrophes.

(4) Une présence remarquable de 5 groupes différents des procaryotes hétérotrophes (LNA1, LNA2, HNA1, HNA2 et HNA3).

(5) Considérant la saisonnalité des différents groupes ultraplanctoniques, il semble que les saisons été-hiver sont favorables pour les efflorescences ultraphytoplanctoniques alors que les saisons été-automne sont favorables pour les proliférations des procaryotes hétérotrophes. Il n'y a pas de variation spatiale significative pour les deux communautés.

(6) En se basant sur les analyses statistiques, la variation temporelle de l'ultraplancton peut être attribuée à (i) l'influence primordiale de la température de l'eau concernant les procaryotes hétérotrophes et l'effet combiné de la température de l'eau et de la salinité pour l'ultraphytoplancton et (ii) à l'effet probable de la prédation exercée par le microplancton sur l'ultraplancton. En effet, des corrélations significatives négatives ont été révélées entre l'ultraplancton et le microplancton notamment entre le microphytoplancton et les

picoeucaryotes et entre les ciliés avec les cryptophytes et HNA. Ceci reflète, remarquablement, la régression de l'ultraplancton en présence du microplancton et vice versa.

L'étude des populations des ciliés planctoniques et épiphytes nous a permis de connaître :

(1) L'importance du groupe des tintinnides dans la lagune de Ghar El Melh. Pour les ciliés planctoniques, ce groupe a été le plus important du point de vue diversité (15 taxa) et le deuxième groupe de point de vue abondance (27.55 %). Pour les ciliés épiphytes, le groupe des tintinnides a été le plus abondant (57.09 %), et représenté uniquement par l'espèce «*Tintinnopsis campanula*». L'importance de ce groupe est un signe d'eutrophisation pour plusieurs régions aquatiques du monde tenant compte de sa grande capacité d'adaptation dans ces milieux.

(2) La saison printanière comme la période favorable pour les proliférations maximales des ciliés planctoniques avec la présence de tous les groupes de différentes classes de taille et de différents modes de nutrition.

(3) *R. cirrhosa* comme support important pour les ciliés épiphytes atteignant des concentrations plus élevées que celles enregistrées dans la colonne d'eau.

(3) L'importance d'étudier les classes de taille de différentes communautés. Ceci nous a permis de collecter plus d'information sur la taille des microalgues adéquates comme nourriture pour les ciliés.

(4) Des corrélations hautement significatives entre les ciliés et les dinoflagellés nuisibles. Parmi les corrélations les plus importantes, on retrouve celle du cilié *S. conicum* et le dinoflagellé nuisible *P. micans* et celle du cilié *Aspidica* sp. et le dinoflagellé toxique *P. lima*.

(5) Que la lagune de Ghar El Melh est toujours sujette à des proliférations microalgales nuisibles que les ciliés sont incapables de contrôler par prédation. Cependant, les ciliés peuvent avoir une influence structurante sur les efflorescences ce qui a été observé dans plusieurs autres études.

En résumé, la lagune de Ghar El Melh, est un écosystème très perturbé souffrant d'une exacerbation de la salinité, la température, les nutriments et de l'invasion de *Ruppia* comme étant un bioindicateur de la détérioration de la qualité des eaux, qui combinés sont les principaux inducteurs des proliférations microalgales surtout les dinoflagellés nuisibles.

Malheureusement, cette situation peut facilement affaiblir l'intérêt économique de la lagune conduisant à l'abandon des projets d'aquaculture et ainsi, il devient absolument urgent de protéger ce site « Ramsar » de toutes sortes de pressions et d'adopter des mesures concrètes telles que :

- La mise en place d'une station d'épuration des eaux usées.
- Le dragage des surfaces proches des limites des berges notamment celles du Nord-Ouest...).

A l'issue de ce travail, plusieurs voies de recherche sont envisageables dans la lagune de Ghar El Melh :

- La recherche du profil toxique des espèces dominantes de la lagune (*P. lima* et *P. micans*) et de procéder à des tests écotoxicologiques qui peuvent donner une idée sur le rôle réel de ces espèces dans les événements néfastes et leur implication possible dans la mortalité des poissons toujours détectée dans la lagune de Ghar El Melh.
- Elaborer des essais de broutage, par exemple entre le cilié *S. conicum* et le dinoflagellé nuisible *P. micans* ou entre le cilié *Aspidica* sp. et le dinoflagellé toxique *P. lima*, ce qui pourrait améliorer notre compréhension des proliférations de dinoflagellés nuisibles dans la lagune.
- Conduire d'autres études sur les autres macrophytes de la lagune afin d'examiner plus de microalgues épiphytes potentiellement toxiques.
- Mener un suivi à haute résolution spatiale pour les kystes de dinoflagellés.

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ANNEXES

Annexe 1. Liste de toutes les espèces microphytoplanctoniques détectées dans la lagune Ghar El Melh (- absence; + 40-1000 cellules l⁻¹; *** > 1000 cellules l⁻¹).

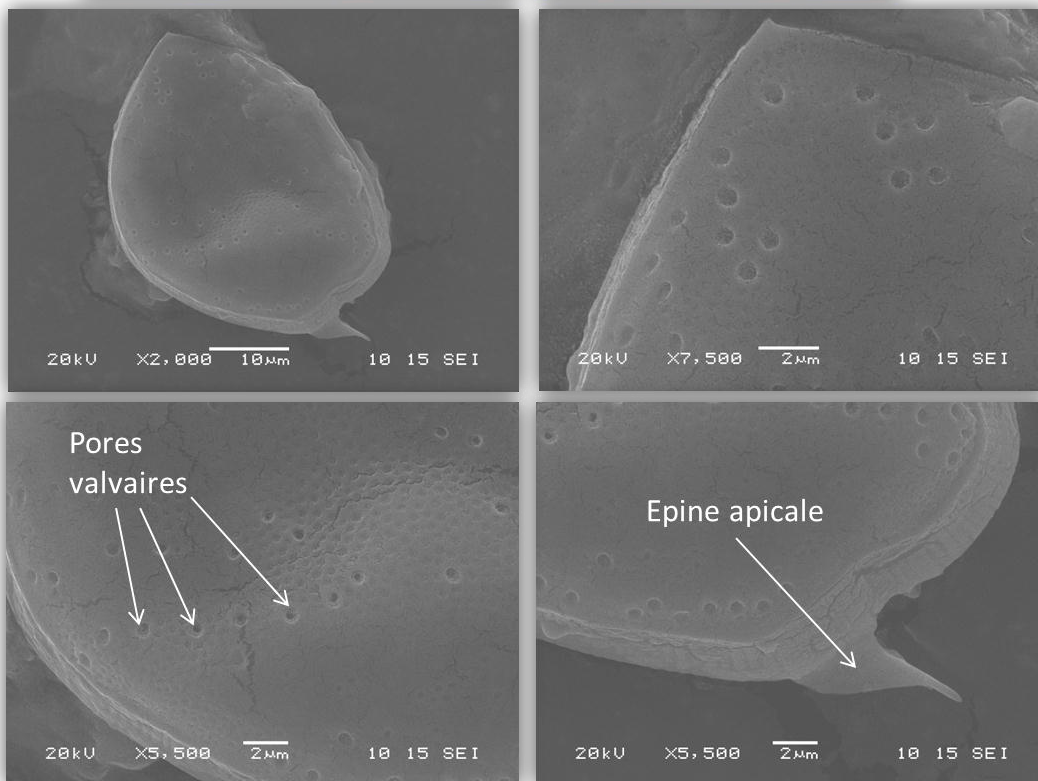
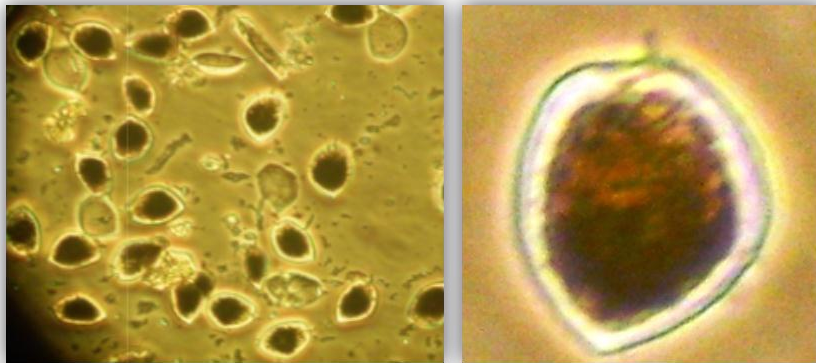
Phytoplancton	Longueur moyenne (µm)	Biovolume moyen (x10 ³ µm ³)	S1	S2	S3	S4	S5	Hiver	Printemps	Eté	Automne
Dinophyceae											
<i>Akashiwo sanguinium</i>	54	58.37	***	+	***	***	***	***	***	+	***
<i>Alexandrium pacificum</i>	29.75	16.44	-	+	-	+	-	-	+	+	-
<i>Tripos furca</i>	70	134.62	-	+	-	-	-	+	-	+	+
<i>Tripos fusus</i>	120	90.04	+	+	+	-	-	+	+	+	+
<i>Coolia monotis</i>	30	17.61	-	+	+	+	+	-	+	+	-
<i>Dinophysis sacculus</i>	37.9	5.84	***	+	***	***	***	***	+	+	+
<i>Dinophysis acuminata</i>	36	20.76	-	-	+	+	-	+	-	-	+
<i>Dinophysis rotundata</i>	25.5	9.19	+	-	-	-	+	+	-	-	-
<i>Diplopsalis lenticula</i>	30	14.13	+	-	+	+	+	-	+	-	+
<i>Gonyaulax polyedra</i>	45	28.84	-	-	+	+	-	-	-	+	+
<i>Gonyaulax spinifera</i>	45	28.84	+	-	-	-	+	-	-	+	-
<i>Gonyaulax</i> sp.	55	71.95	-	-	-	+	-	-	-	-	+
<i>Gymnodinium catenatum</i>	27.25	6.59	+	+	-	-	-	-	-	+	+
<i>Oxyphysis oxytoxoides</i>	35	16.48	+	+	+	+	+	+	+	+	-
<i>Prorocentrum concavum</i>	47.5	48.67	+	+	+	-	+	+	+	-	-
<i>Prorocentrum gracile</i>	55	18.35	+	+	+	+	-	+	+	+	+
<i>Prorocentrum lima</i>	39.81	19.013	+	+	+	***	***	+	***	***	+
<i>Prorocentrum rhathymum</i>	30	6.28	-	-	-	+	-	-	-	-	+
<i>Prorocentrum micans</i>	39.75	16.01	***	***	***	***	***	***	***	***	***
<i>Prorocentrum minimum</i>	16.65	1.16	+	-	+	***	+	+	-	-	***
<i>Prorocentrum triestinum</i>	25.29	1.98	***	+	+	-	+	***	+	+	-
<i>Protoperidinium conicum</i>	62	19.46	+	+	-	+	-	+	-	+	+
<i>Protoperidinium diabolium</i>	44.4	10.08	+	+	***	***	***	+	***	+	+

<i>Protoperidinium divergens</i>	45.5	16.11	-	+	+	-	-	+	-	+	+
<i>Protoperidinium minutum</i>	34.5	5.26	-	-	+	+	+	-	+	-	-
<i>Protoperidinium pentagonum</i>	70	28.02	-	-	+	+	+	-	+	-	-
<i>Protoperidinium pyriforme</i>	50	12.45	-	-	+	+	+	-	+	-	-
<i>Pyrocystis noctiluca</i>	65	143.72	+	-	+	+	+	+	+	+	-
<i>Pyrophacus horologium</i>	75	220.78	-	-	-	+	-	-	-	+	-
<i>Scrippsiella spinefera</i>	23.5	5.42	-	-	+	-	-	-	-	+	-
<i>Scrippsiella subsalsa</i>	35	11.44	-	-	+	-	-	-	-	+	-
<i>Scrippsiella trochoidea</i>	18.75	3.23	***	***	***	***	***	***	***	***	***
Bacillariophyceae											
<i>Bacillaria</i> sp.	83	17.22	+	-	-	-	-	-	+	+	-
<i>Chaetoceros decipiens</i>	70.2	27.96	***	+	+	+	+	+	***	***	+
<i>Chaetoceros socialis</i>	85	70.89	+	-	-	-	-	+	-	+	+
<i>Climacosphenia</i> sp.	208.5	14.71	+	+	***	***	***	***	+	***	***
<i>Cocconeis</i> sp.	21	4.84	+	-	+	+	+	+	-	+	-
<i>Cylindrotheca closterium</i>	286.5	4.17	+	+	***	***	***	***	***	***	***
<i>Denticulopsis</i> sp.	30	9	+	+	+	-	+	+	+	+	+
<i>Diploneis</i> sp.	35	6.18	+	-	-	+	+	+	+	-	-
<i>Guinardia</i> sp.	150	353.25	+	+	+	-	-	-	+	-	-
<i>Gyrosigma</i> sp.	188.4	39.58	***	+	+	***	***	+	+	***	***
<i>Leptocylindrus danicus</i>	250	735.93	***	+	-	-	***	-	***	-	-
<i>Licmophora gracilis</i>	47.85	1.19	+	+	+	***	***	***	***	+	***
<i>Navicula duerrenbergiana</i>	39	1.95	***	+	+	***	***	+	-	***	***
<i>Navicula phyllepta</i>	36.6	2.63	+	-	+	***	***	+	+	***	+
<i>Navicula</i> sp.	60	60.75	-	+	-	+	+	+	-	+	-
<i>Nitzschia dissipata</i>	48.9	0.88	***	+	+	+	***	+	***	***	-
<i>Nitzschia linearis</i>	121.2	71.95	***	+	***	***	***	+	***	***	***
<i>Plagiotropsis</i> sp.	55	9.71	-	-	-	***	+	***	-	-	+
<i>Pseudo-nitzschia</i> sp.	60	0.60	+	***	+	+	+	+	+	-	***
<i>Rhizosolenia</i> sp.	350	1923.25	+	***	+	+	+	***	***	-	-
<i>Striatella unipunctata</i>	85.05	253.75	+	+	***	***	***	+	+	***	+

<i>Thalassionema nitzschioides</i>	77.25	29.51	+	+	***	***	***	***	+	***	+
Cyanobacteriae											
<i>Anabaena</i> sp.	80	30.14	+	-	+	+	+	-	-	+	+
<i>Lyngbya</i> sp.	100	141.3	+	+	-	-	+	-	+	+	-
<i>Oscillatoria salina</i>	100	141.3	+	+	+	+	***	+	***	+	+
<i>Oscillatoria</i> sp.	100	32.49	+	+	***	+	***	***	+	***	+
<i>Spirulina</i> sp.	82	21.11	+	-	-	+	+	+	+	+	+
Chlorophyceae											
<i>Pediastrum simplex</i>	60	57.69	+	-	-	+	-	+	-	+	+
Euglenophyceae											
<i>Euglena</i> sp.	36	5.42	+	+	+	+	+	+	+	+	-

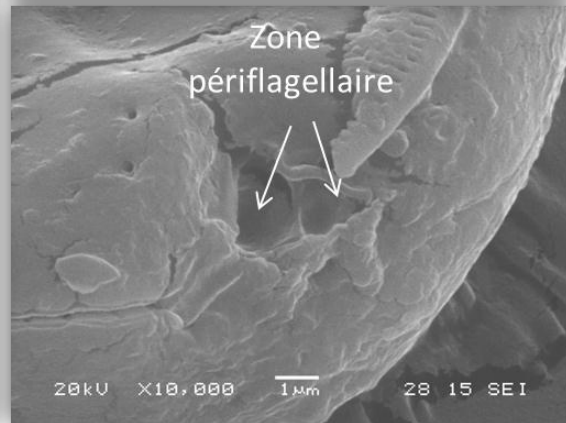
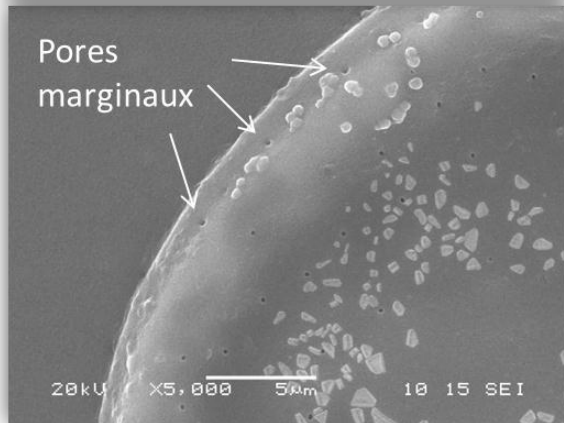
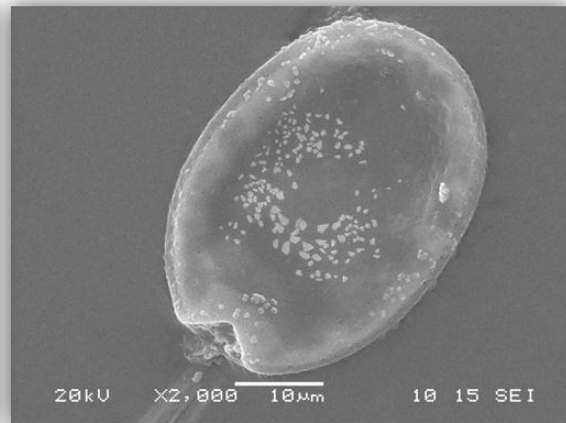
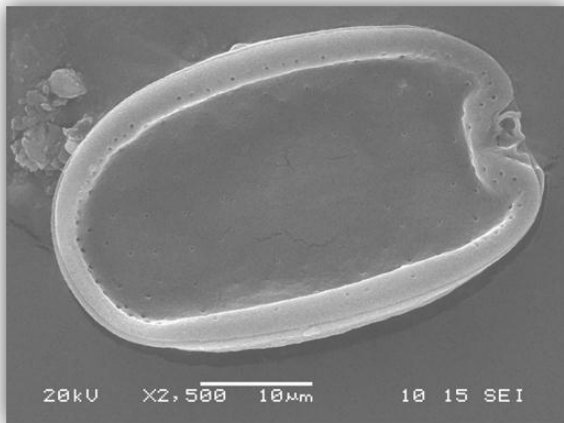
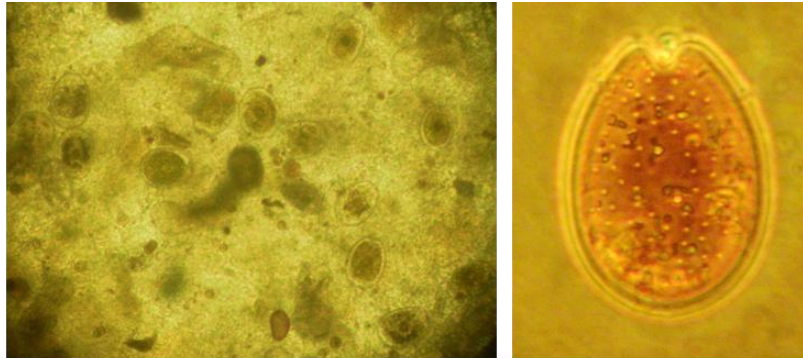
Annexe 2. Micrographies des principales espèces microphytoplanctoniques de la lagune de Ghar El Melh

Prorocentrum micans (dinoflagellés) :



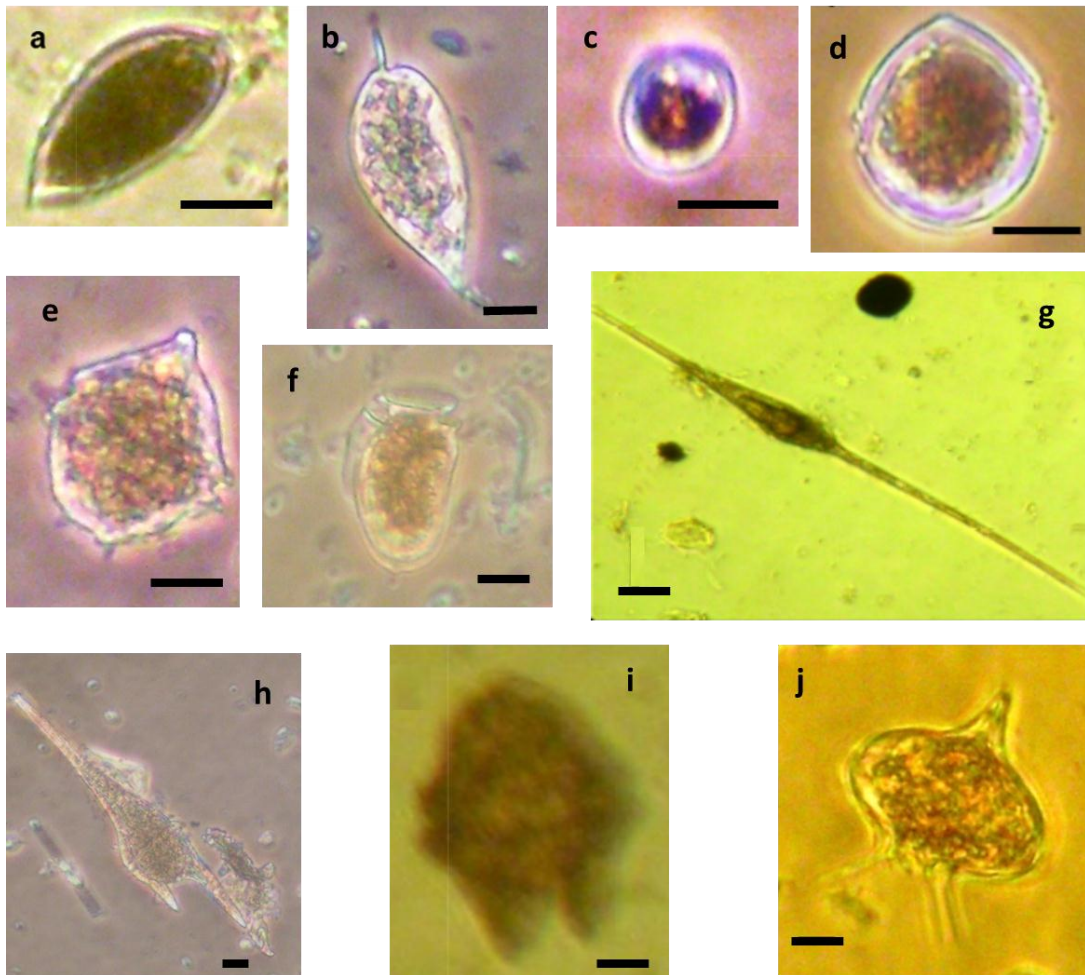
(Suite annexe 2)

Prorocentrum lima (dinoflagellés) :



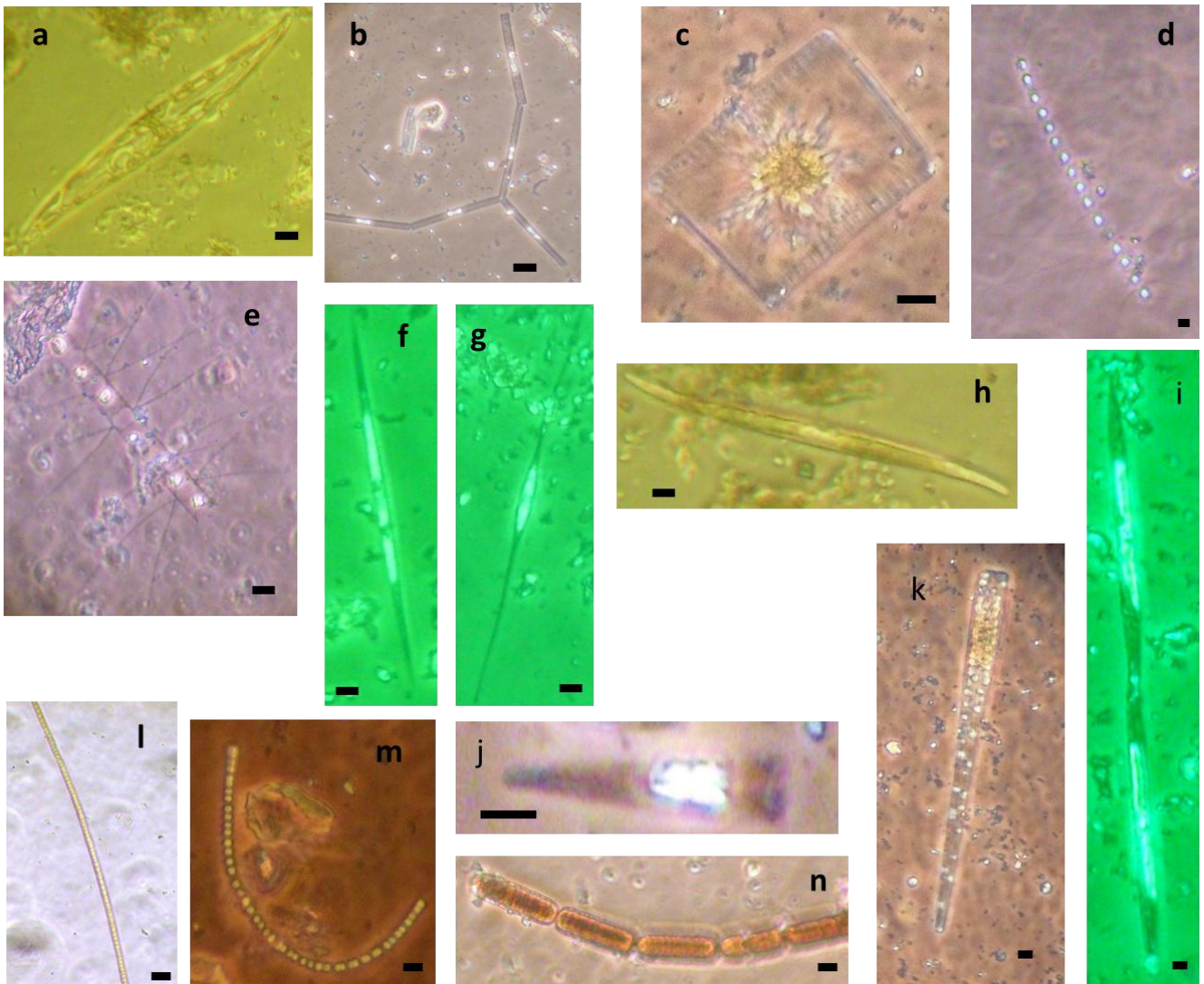
(Suite annexe 2)

Autres dinoflagellés : a. *Prorocentrum triestinum*, b. *P. gracile*, *P. minimum*, d. *Scrippsiella trochoidea*, e. *Gonyaulax spinifera*, f. *Dinophysis sacculus*, g. *Tripos fusus*, h. *Tripos furca*, i. *Akashiwo sanguinium*, j. *Protoperidinium diabolum*. Echelle = 10 µm.

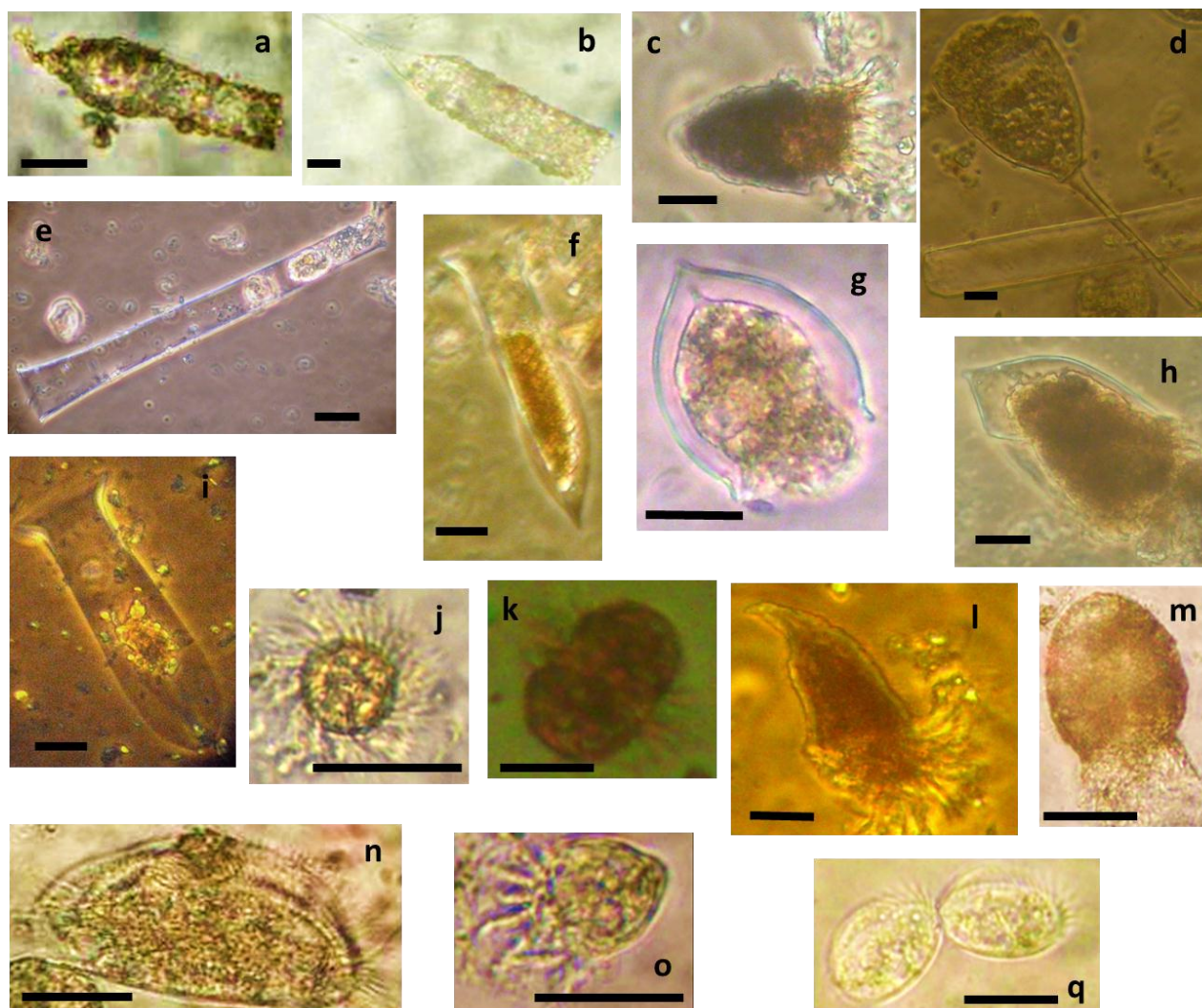


(Suite annexe 2)

Les principales diatomées et cyanobactéries de la lagune de Gha El Melh : **a.** *Gyrosigma* sp., **b.** *Thalassionema nitzschioides*, **c.** *Striatella unipunctata*, **d-e.** *Chaetoceros* spp., **f.** *Nitzschia dissipata*, **g.** *Cylindrotheca closterium*, **h.** *Nitzschia linearis*, **i.** *Pseudo-nitzschia* sp., **j.** *Licmophora gracilis* **k.** *Climacosphenia* sp., **l.** *Oscillatoria* sp., **m.** *Anabaena* sp., **n.** *Lyngbya* sp. Echelle = 10 µm.



Annexe 3. Les ciliés de la lagune de Ghar El Melh



a. *Tintinnopsis tocantinensis*, **b.** *Tintinnopsis radix*, **c.** *Tintinnopsis baltica*, **d.** *Tintinnopsis campanula*, **e.** *Eutintinnus macilentus*, **f.** *Amphorellopsis acuta*, **g.** *Metacylis mediterranea*, **h.** *Nitzschia linearis*, **i.** *Favella azorica*, **j.** *Leegaardiella sol*, **k.** *Mesodinium rubrum*, **l.** *Strombidium conicum*, **m.** *Codonella aspera*, **n.** *Aspidisca* sp., **o.** *Lohmanniella oviformis*, **q.** *Euplotes charon*. Echelle = 20 µm.

RESUME

Il s'agit de l'étude des successions écologiques du phytoplancton dans la lagune de Ghar El Melh au niveau de 5 stations pendant (i) un suivi bimensuel allant du janvier 2011 à janvier 2012 et (ii) un suivi saisonnier à partir du novembre 2012 à mars 2013. L'étude écologique a été consacrée au microphytoplancton en relation avec les facteurs abiotiques (température, salinité et nutriments) et biotiques (microalgues épiphytes, kystes des dinoflagellés, ultraplankton et ciliés). L'analyse hydrologique du milieu a montré une variation principalement saisonnière de la plupart des paramètres avec des valeurs élevées de la température et de la salinité en été et des concentrations exceptionnelles des nutriments en automne et en hiver. Le phytoplancton de la lagune de Ghar El Melh a été principalement dominé par les dinoflagellés et les diatomées avec une manifestation alarmante des espèces nuisibles atteignant plus que 70 % du peuplement. Ces proliférations ont été remarquées durant toute l'année avec une manifestation plus marquante dans les régions internes de la lagune ce qui est principalement tributaire à la température de l'eau, l'azote total, le nitrogène et l'orthophosphate. Une importante contribution des espèces épiphytes au phytoplancton a été signalée notamment celle du dinoflagellé toxique *Prorocentrum lima*. L'inventaire spécifique du phytoplancton dominé par l'espèce *Prorocentrum micans* (> 28 %) et d'autres espèces ne produisant pas de kyste a été en concordance avec les faibles valeurs enregistrées pour les kystes de dinoflagellés dans le sédiment où le maximum enregistré a été de l'ordre de 229 kystes g⁻¹. Une part importante de l'ultraplancton a été représentée par le nanophytoplancton et les hétérotrophes procaryotes, dépassant largement les concentrations microphytoplanctoniques, ce qui est un indice de l'eutrophisation du milieu. Des corrélations hautement significatives ont été trouvées entre le microphytoplancton et les organismes microzooplanctoniques évoquant un probable rôle de ces derniers dans le contrôle des proliférations microalgales nuisibles.

Mots clés : Lagune de Ghar El Melh, microphytoplancton, facteurs abiotiques, microalgues épiphytes, kystes de dinoflagellés, ultraplankton, ciliés.

ABSTRACT

We explored the distribution of the microphytoplankton community in relation to both abiotic (temperature, salinity and nutrients) and biotic factors (epiphytes microalgae, dinoflagellate cysts, ultraplankton and ciliates) in Ghar al Melh Lagoon at 5 stations during (i) a biweekly sampling from January 2011 to January 2012 and (ii) a seasonal sampling from november 2012 to march 2013. The results pointed out a seasonal variation of all parameters with high water temperature and salinity in summer and exceptional concentrations of nutrients in autumn and winter. Microphytoplankton was mainly dominated by dinoflagellates and diatoms with sequences of harmful species proliferation attaining more than 70 % of total phytoplankton. These sporadic proliferations occurred throughout the year with exacerbation in the inner parts of the lagoon mainly related to temperature and total nitrogen, nitrogen and orthophosphate. An important contribution of epiphytic species to phytoplankton abundance was recorded with especially the presence of the toxic dinoflagellate *Prorocentrum lima*. Microphytoplankton was dominated by *Prorocentrum micans* (> 28 %) and other non-cyst former species consistent with the low dinoflagellate cysts found at sediment surface (maximum = 229 cysts g⁻¹ DS). Urtraplankton was chiefly represented by nanophytoplankton and heterotrophic prokaryotes, with low microphytoplanktonic abundance. This indicates that the lagoon is undergoing eutrophication. Significant correlations were reported between phytoplankton and microzooplankton suggesting a potential role of the former in controlling harmful microalgae proliferations.

Key words: Ghar El Melh Lagoon, phytoplankton, abiotic factors, epiphyte microalgae, dinoflagellates cysts, ultraplankton, ciliates.