



Variability and plasticity of the nutrition of zooxanthellate jellyfishes : insights from experimental and field studies

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Par

Nicolas DJEGHRI

Variability and Plasticity of the Nutrition of Zooxanthellate Jellyfishes
Insights from experimental and field studies.

Variabilité et Plasticité de la Nutrition des Méduses à Zooxanthelles
Apports expérimentaux et de terrain.

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Note sur la Traduction Française

Ce manuscrit est écrit essentiellement en anglais. Toutefois, certaines sections sont traduites en français :

Les résumés des différents chapitres.

Les légendes des figures.

Les légendes des tableaux.

Les titres des sections principales.

Un résumé développé en français est également donné dans les pages suivantes, en avant-propos au corps du document de thèse

Note on the French Translation

This manuscript is written primarily in English. However, parts of it are translated in French:

The abstracts of the different chapters.

The figure captions.

The table captions.

The titles of the main sections.

A detailed abstract is also given in French in the following pages as a foreword to the manuscript

Résumé Développé

1. Introduction : Rôle des Méduses dans les Ecosystèmes Marins

Les méduses (ici employé au sens large de cnidaires pélagiques) ont historiquement été considérées comme n'ayant qu'un rôle négligeable dans les écosystèmes marins. Cette vision est à présent dépassée. La recherche des dernières dizaines d'années portant sur ces organismes a en effet démontré leur importance et leurs rôles dans les écosystèmes marins. Comme d'autres prédateurs pélagiques, les méduses sont à présent considérées comme des membres à part entière des réseaux trophiques, pouvant intervenir sur la dynamique des communautés, les cycles des nutriments ou l'export de matière organique vers l'océan profond. Toutefois, un certain nombre de caractéristiques distinguent les méduses des autres prédateurs pélagiques. L'une de ces caractéristiques notable est la tendance de certaines espèces de méduses à former des blooms. Un bloom de méduses est défini comme une augmentation importante de l'effectif de la population de ces organismes en lien avec leur phénologie. Deux caractéristiques fondamentales des méduses expliquent leur capacité à former ces blooms : (1) La formation d'un bloom nécessite un fort recrutement de jeunes méduses. Cela est assuré, chez la plupart des espèces, par la transition de la phase polype à la phase méduse (processus de strobilation chez les scyphozoaires). (2) Ces jeunes recrues doivent ensuite grandir rapidement pour que la population atteigne d'importantes biomasses. Chez les méduses, cette croissance rapide est permise par leur plan d'organisation simple et leur importante teneur en eau (> 95 %).

Tous ces aspects de l'écologie et de la dynamique des populations de méduses sont toutefois assujettis à leur nutrition ; i.e. à la capacité des individus à trouver dans leur environnement les ressources nécessaires à leur croissance et leur reproduction. La plupart des méduses sont strictement prédatrices, s'alimentant généralement sur du micro- ou du mésozooplancton. Ainsi, la formation d'un bloom de méduses est souvent corrélée à un pic dans la quantité de proies disponibles. Toutefois, de nombreuses espèces de méduses disposent d'une seconde source de nutrition *via* une photosymbiose avec des dinoflagellés autotrophes (zooxanthelles). Ce type de symbiose est généralement considéré comme mutualiste. Les méduses fournissent aux zooxanthelles un abri et un accès privilégié à leurs produits

d'excrétion. Les zooxanthelles fournissent aux méduses une part importante des produits de la photosynthèse. La combinaison de (1) la nutrition hétérotrophe des méduses (prédation de zooplancton), de (2) la nutrition autotrophe des zooxanthelles (photosynthèse), et (3) d'un échange de nutriments entre les deux partenaires, implique que ces méduses à zooxanthelles peuvent être considérées comme mixotrophes en tant qu'holobiontes (i.e. hôte et symbiontes considérés comme un seul organisme). L'étude de l'écologie des méduses à zooxanthelles et de leur nutrition mixotrophe constituent les thématiques principales de cette thèse organisée en quatre chapitres résumés ci-après.

2. Chapitre I : Revue de la Diversité, des Traits de Vie, et de l'Écologie des Méduses à Zooxanthelles

Le premier Chapitre de cette thèse présente une revue de la littérature concernant la diversité, les traits de vie, et l'écologie des méduses à zooxanthelles.

2.1. Diversité des Méduses à Zooxanthelles et de leurs Symbiontes

Au moins sept apparitions de la symbiose médusozoaire-zooxanthelles peuvent être identifiées au cours de l'histoire évolutive des médusozoaires. Deux concernent des groupes d'hydrozoaires ne présentant pas de phase méduse (taxons Filifera I & II, et Macrocolonia). Les cinq autres apparitions concernent des taxons contenant des méduses, et se répartissent dans tous les principaux groupes de médusozoaires à l'exception des staurozoaires : Une apparition est recensée chez les cubozoaires (taxon Carybdeida), deux apparitions chez les hydrozoaires (taxons Capitata et Laodiceida), et deux apparitions chez les scyphozoaires (taxons Coronatae et Kolpophorae). Dans la plupart des cas, les méduses à zooxanthelles sont apparemment peu diversifiées. Une exception importante toutefois est le taxon des Kolpophorae, qui est diversifié (ca. 40-50 espèces) et pourrait ne contenir que des espèces à zooxanthelles. Cela implique que 20 à 25 % des scyphozoaires sont des espèces à zooxanthelles (symbiontes facultatifs inclus). Chez les scyphozoaires, la nutrition mixotrophe n'est donc pas un caractère exceptionnel mais est, au contraire, plutôt commune.

Les zooxanthelles présentes dans les méduses à zooxanthelles sont généralement des Symbiodiniaceae (principalement les genres *Symbiodinium* et *Cladocopium*). Plus rarement, d'autres taxons peuvent être trouvés, notamment des zooxanthelles de la famille des Thoracosphaeraceae.

2.2. Traits de Vie Communs aux Méduses à Zooxanthelles

Les méduses à zooxanthelles, bien que diverses phylogénétiquement, semblent partager certaines caractéristiques communes. Trois traits de vie émergents sont ainsi discutés :

(1) Les méduses à zooxanthelles sont généralement mixotrophes durant leur phase méduse, tirant leur nutrition à la fois de la prédation et de la photosynthèse. Toutefois, une grande variabilité semble exister inter- et intra-spécifiquement autour de leur nutrition. Cette caractéristique permet aux méduses à zooxanthelles d'occuper une niche écologique unique de grands mixotrophes pélagiques.

(2) Les méduses à zooxanthelles, durant leur phase polype, sont peu dépendantes de leurs zooxanthelles. Les polypes sont donc essentiellement hétérotrophes. Cela implique que leurs populations pourraient être maintenues indépendamment de la disponibilité des ressources nécessaires à l'autotrophie (lumière, nutriments inorganiques).

(3) Les zooxanthelles sont en revanche importantes pour la strobilation (passage de la phase polype à la phase méduse). Dans la majorité des cas, elles semblent favoriser voire être nécessaires à la strobilation. Cela reste vrai y compris en l'absence de photosynthèse ce qui suggère que le rôle des zooxanthelles dans la strobilation n'est pas (ou pas uniquement) nutritif.

Une difficulté importante autour de ces traits de vie est de savoir à quel point ils sont généralisables aux méduses à zooxanthelles qui ont été moins étudiées (notamment les hydroméduses et cuboméduses à zooxanthelles).

2.3. Ecologie des Méduses à Zooxanthelles

Les traits de vie des méduses à zooxanthelles ont des implications pour leur écologie. Ces implications sont nombreuses et certaines sont discutées dans le Chapitre I. Notamment les méduses à zooxanthelles ont tendance à former moins de blooms que les méduses sans zooxanthelles. Ceci peut être expliqué par l'importance des zooxanthelles dans la strobilation, ou par la mixotrophie de la phase méduse. Les méduses à zooxanthelles présentent aussi des particularités dans leur réaction aux changements environnementaux. Par exemple, comme les coraux, elles peuvent blanchir (expulsion des zooxanthelles) en réponse à un stress thermique. Toutefois, l'impact final d'un blanchissement sur une population de méduses à zooxanthelles dépend des interactions complexes entre les zooxanthelles et le cycle de vie des méduses.

Les méduses à zooxanthelles peuvent aussi jouer des rôles particuliers dans les écosystèmes marins. Contrairement aux méduses sans zooxanthelles, elles participent à la production primaire d'un système. Elles pourraient également avoir des impacts particuliers sur les cycles des nutriments ou sur les communautés planctoniques via des effets « *top-down* » ou « *bottom-up* » directs ou indirects (e.g. cascades trophiques).

Ce chapitre fait l'objet d'un article publié dans la revue *Marine Biology*.

3. Chapitre II : Influence de la Disponibilité des Ressources sur des Polypes avec et sans Zooxanthelles : Réponses Similaires du Bourgeonnement et de la Survie de *Cassiopea* sp. et d'*Aurelia* sp.

L'un des traits de vie important des méduses à zooxanthelles mis en avant dans le Chapitre I est que leurs polypes ne sont que très peu dépendants de leurs zooxanthelles pour le maintien de leurs populations. Le corolaire de cette observation est que les polypes des méduses à zooxanthelles et les polypes des méduses sans zooxanthelles devraient présenter le même type de réponses vis-à-vis de variations de la disponibilité en ressources : Les deux devraient voir la croissance de leur population favorisée par une augmentation de la disponibilité en

proies (ressource liée à l'hétérotrophie) et ne devraient pas être affectés par la disponibilité des ressources liées à l'autotrophie (lumière, nutriments inorganiques dissous).

Cette hypothèse a été testée expérimentalement dans le Chapitre II. Les polypes de deux espèces de scyphozoaires, avec et sans zooxanthelles (respectivement *Cassiopea* sp. et *Aurelia* sp.), ont été incubés en présence ou absence de proies, lumière et nutriments inorganiques dissous supplémentaires. La reproduction asexuée des polypes, et la croissance de la population qui en a découlée ont été suivis pendant 55 jours. Chez *Cassiopea* sp., comme chez *Aurelia* sp., la reproduction asexuée, et donc la croissance de la population, n'a été impactée positivement que par la présence de proies. Aucun effet de la présence de lumière ou de nutriments inorganiques dissous n'a été observé.

L'expérience a été poursuivie au-delà des 55 jours pour les polypes des traitements sans proies afin d'observer l'effet de la lumière et des nutriments inorganiques dissous sur leur temps de survie. Chez *Cassiopea* sp., comme chez *Aurelia* sp., la lumière a diminué le temps de survie, probablement indirectement, via la compétition avec des algues.

Cette expérience corrobore l'hypothèse de départ et suggère que les dynamiques de population des polypes de méduses avec ou sans zooxanthelles ne devraient pas différer beaucoup dans leurs réactions aux ressources disponibles.

4. Chapitre III : $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, et Ratios C:N en tant qu'Indicateurs de la Nutrition des Méduses à Zooxanthelles : Apports d'une Approche Expérimentale

Un autre trait de vie important des méduses à zooxanthelles identifié dans le Chapitre I est la mixotrophie de la phase méduse, qui tire sa nutrition à la fois de la prédation et de la photosynthèse. La part relative de l'autotrophie et de l'hétérotrophie dans la nutrition des méduses à zooxanthelles semble pouvoir varier beaucoup selon l'espèce, ou même selon la population de méduses considérée. Cela suggère que les méduses à zooxanthelles pourraient avoir une importante plasticité dans leur nutrition. Toutefois, cette plasticité reste mal caractérisée à ce jour.

Pour caractériser cette plasticité, des outils sont nécessaires. Les isotopes stables sont d'excellents candidats. L'effet de variations de la part relative de l'autotrophie et de

l'hétérotrophie sur les isotopes stables sont notamment connus chez les coraux, mais n'ont pas été étudiés expérimentalement dans le cas des méduses à zooxanthelles.

Le Chapitre III présente donc une expérience visant à caractériser l'effet de la variation de la part relative de l'autotrophie et de l'hétérotrophie sur les signature en isotopes stables ($\delta^{13}\text{C}$ et $\delta^{15}\text{N}$) et la composition élémentaire (rapports C:N) des méduses à zooxanthelles. De jeunes méduses du genre *Cassiopea* ont donc été incubées en présence ou absence de lumière et de proies pendant 24 jours. Tous les quatre jours, trois méduses de chaque traitement ont été échantillonnées au hasard et ont été analysées en spectrométrie de masse afin de suivre leurs signatures isotopiques et élémentaires. L'analyse des résultats a montré qu'en présence de lumière seule, les méduses atteignaient des $\delta^{13}\text{C}$ et des rapports C:N élevés et des $\delta^{15}\text{N}$ faibles. En présence de proies seules, l'inverse était observé avec des $\delta^{13}\text{C}$ et des rapports C:N faibles et des $\delta^{15}\text{N}$ élevés. Les méduses incubées en présence de lumière et de proies avaient des $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ et rapports C:N intermédiaires. Cela suggère qu'une nutrition dominée par l'autotrophie serait caractérisée par des $\delta^{13}\text{C}$ et des rapports C:N élevés et des $\delta^{15}\text{N}$ faibles. En revanche, une nutrition dominée par l'hétérotrophie serait caractérisée par $\delta^{13}\text{C}$ et des rapports C:N faibles et des $\delta^{15}\text{N}$ élevés. Il est à noter que des résultats similaires ont été obtenus chez des coraux photosymbiotiques, et l'étude menée ici confirme qu'ils peuvent être étendus aux méduses à zooxanthelles. Les résultats expérimentaux décrits ici permettent par ailleurs de faciliter l'interprétation des signatures isotopiques et des rapports C:N des méduses à zooxanthelles sur le terrain.

Ce chapitre fait l'objet d'un article publié dans la revue *Journal of Experimental Marine Biology and Ecology*.

5. Chapitre IV : Photosynthèse ou Prédation ? Plasticité Nutritionnelle de *Mastigias papua* (Scyphozoa : Rhizostomeae) de Palaos à l'aide d'Isotopes Stables et d'Acides Gras

Le Chapitre I a permis de montrer que les méduses à zooxanthelles avaient apparemment une grande plasticité dans leur nutrition mais que cette plasticité était peu caractérisée dans la littérature scientifique. Plus précisément, elle est souvent caractérisée par des cas extrêmes documentant l'absence ou la présence des zooxanthelles. Le Chapitre IV a pour objectif de

caractériser plus finement cette plasticité de la nutrition en étudiant la nutrition de différentes populations de méduses à zooxanthelles dans leur environnement naturel.

5.1. Site d'Etude, Echantillonnage et Méthodes

Les méduses à zooxanthelles de l'espèce *Mastigias papua* ont été échantillonnées en 2018 dans quatre lacs marins (Clear Lake, Goby Lake, Ongeim'l Tketau, Uet era Ngermeuangel) et dans le lagon de l'archipel de Palaos dans l'océan Pacifique ouest. Ces cinq sites sont isolés les uns des autres et présentent des différences écologiques (e.g. différentes quantités et qualités de lumière et proies disponibles). De plus, leur isolement implique que les populations de méduses qu'ils abritent ont leur propre histoire éco-évolutive. Ces caractéristiques variables suggèrent que la nutrition des méduses à zooxanthelles est susceptible de présenter des différences marquées d'un site à l'autre. En plus des différents sites, des méduses de toutes tailles ont été échantillonnées afin de mesurer de potentiels effets de la taille individuelle sur la nutrition

Pour caractériser la nutrition des méduses de ces différents sites, deux types d'indicateurs ont été utilisés. Les premiers sont les compositions isotopiques et élémentaires. Les résultats et enseignements issus du Chapitre III ont donc été réutilisés ici lors de l'interprétation des données issues du milieu naturel. En supplément des compositions isotopiques et élémentaires, la composition en acides gras des méduses a été utilisée. Certains acides gras sont en effet connus pour être marqueurs des zooxanthelles tandis que d'autres sont marqueurs de leurs hôtes ; faisant d'eux de potentiels indicateurs de la nutrition. Un total de quatorze indicateurs a été utilisé, ce qui a permis de caractériser la nutrition mais aussi le stress des différentes populations et classes de taille de *Mastigias papua* à Palaos. Les différents indicateurs de la nutrition ont donné des résultats concordants (généralement, coefficient de corrélation de Pearson $> 0,5$ en valeur absolue), ce qui renforce la confiance dans les conclusions obtenues. De plus, l'interprétation des résultats a été facilitée par la présence d'une population de méduses entièrement aposymbiotique (dénuée de zooxanthelles) dans Clear Lake. Cette population a pu servir de ligne de base représentant des méduses purement hétérotrophes.

5.2. Résultats et Implications pour l'Écologie des Méduses à Zooxanthelles

Les méduses des différents sites échantillonnés ne présentaient pas toujours les mêmes tendances liées à leur taille : par exemple, les méduses de Uet era Ngermeuangel devenaient plus hétérotrophes en grandissant tandis que l'inverse était observé chez les méduses de Goby Lake ou de Ongeim'l Tketau. La comparaison des indicateurs de la nutrition des méduses des différents sites a révélé que les populations des différents lacs étaient ordonnées, des plus autotrophes aux plus hétérotrophes, de la façon suivante : Uet era Ngermeuangel et Goby Lake, puis Ongeim'l Tketau et enfin Clear Lake. Cette étude démontre que *Mastigias papua* peut présenter un large spectre de nutrition ; de 100 % hétérotrophe (méduses de Clear Lake), à dominé par l'autotrophie (méduses de Uet era Ngermeuangel et Goby Lake).

L'ordre présenté ci-dessus n'est toutefois pas figé dans le temps. Des observations datant d'années précédentes (par exemple l'alternance de présence et absence de zooxanthelles dans les méduses de Clear Lake) montrent que la nutrition des populations de *Mastigias papua* au sein d'un même site peut varier. Cela suggère que les variations de nutrition caractérisées ici ne sont pas strictement liées au génotype des méduses mais sont aussi liées à la variabilité de leur environnement. Le spectre de nutrition caractérisée plus haut reflète donc une plasticité dans la nutrition de ces méduses.

Si l'environnement détermine une grande part de la nutrition des méduses à zooxanthelles, quels peuvent-être les mécanismes sous-jacents ? Un premier mécanisme est le blanchissement lié à un stress thermique. En effet, Clear Lake était le plus chaud de nos sites d'échantillonnage et abritait une population de méduses blanchies. D'autres mécanismes existent : Une analyses des données isotopiques des méduses de Ongeim'l Tketau des années 2010 à 2018 révèle que, plus la densité de la population de méduses est importante, plus les individus sont autotrophes (et vice-versa). Cela suggère que les méduses peuvent épuiser le stock de proies zooplanctoniques dans les lacs marins de Palaos quand elles sont abondantes via des mécanismes « *top-down* ». En retour, la raréfaction des proies peut diminuer la part de l'hétérotrophie dans la nutrition mixotrophe des méduses.

Pour conclure, les résultats du Chapitre IV caractérisent pour la première fois la grande plasticité de la nutrition de *Mastigias papua*. Ils donnent aussi des pistes de recherche pour les mécanismes environnementaux qui déterminent la nutrition de ces méduses.

Ce chapitre fait l'objet de deux articles en cours de préparation.

6. Discussion Générale

L'objectif de cette thèse était de contribuer à l'étude de l'écologie, et plus particulièrement, de la nutrition des méduses à zooxanthelles. Plusieurs importantes contributions peuvent être retenues :

Le Chapitre I donne un état de l'art des connaissances sur la diversité et l'écologie des méduses à zooxanthelles. Un tel état de l'art n'était pas disponible précédemment dans la littérature scientifique. Les résultats du Chapitre II tendent à confirmer la faible importance des zooxanthelles pour les polypes de méduses à zooxanthelles. Les résultats du Chapitre III permettent d'aider à l'interprétation des composition isotopiques et élémentaires des méduses à zooxanthelles sur le terrain. Enfin, les résultats du Chapitre IV caractérisent la plasticité de la nutrition de *Mastigias papua*, qui peut aller de la pure hétérotrophie à une nutrition dominée par l'autotrophie. Les résultats du Chapitre IV indiquent également l'existence de processus écologiques liés à la densité de méduses à zooxanthelles dans un écosystème donné.

Ces nouvelles informations ont des implications pour l'écologie des méduses à zooxanthelles. Par exemple, la plasticité de la nutrition des méduses à zooxanthelles caractérisée au Chapitre IV apporte un nouvel éclairage sur la tendance des méduses à zooxanthelles à ne pas former de blooms. Plusieurs hypothèses pour expliquer cette tendance sont avancées dans la littérature scientifique et au Chapitre I. L'une d'elles suggère que les méduses à zooxanthelles peuvent changer de source de nutrition principale si l'une (e.g. abondance en proies ou en nutriments, luminosité) devient rare. Cela permettrait de rendre l'apport énergétique des méduses à zooxanthelles plus régulier. Il en résulterait des dynamiques de populations plus stables et donc, une tendance à bloomer réduite. Cette hypothèse est appuyée par les résultats du Chapitre IV.

En revanche, de nombreux aspects de l'écologie et de la diversité des méduses à zooxanthelles restent toujours peu connus. Notamment, la plupart des études, y compris celles conduites pendant cette thèse, sont focalisées sur quelques espèces de grands scyphozoaires à zooxanthelles (e.g. *Cassiopea*, *Mastigias*). Par opposition, les cuboméduses ou hydroméduses

à zooxanthelles sont peu étudiées. Cela pose la question de la généralisation des résultats présentés ici. Un résultat semble cependant certain : les méduses à zooxanthelles présentent une très grande diversité, tant en termes phylogénétiques qu'en termes d'écologie. Cela implique que la compréhension de leurs rôles dans les écosystèmes ne sera pas atteinte par la seule étude de quelques espèces modèles. L'étude d'espèces plus rares, ou moins emblématiques, est également d'une importance centrale pour mieux comprendre la biologie et l'écologie de ce groupe d'organismes.

Publication of the Results of the Thesis

Publication des Résultats de la Thèse

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Djaghri N, Pondaven P, Stibor H, Dawson MN (2019) Review of the diversity, traits, and ecology of zooxanthellate jellyfishes. [Marine Biology](#)

Djaghri N, Stibor H, Lebeau O, Pondaven P (2020) $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratios as nutrition indicators of zooxanthellate jellyfishes: insights from an experimental approach. [Journal of Experimental Marine Biology and Ecology](#)

Articles in preparation / Articles en préparation :

Djaghri N, Pondaven P, Stockenreiter M, Behl S, Huang JYT, Hansen T, Patris S, Ucharm G, Stibor H (in preparation) Density-dependent nutrition in a mixotrophic jellyfish holobiont

Djaghri N, Pondaven P, Le Grand F, Bideau A, Duquesne N, Stockenreiter M, Behl S, Huang Y-T, Hansen T, Patris S, Ucharm G, Stibor H (in preparation) Photosynthesis or predation? Nutritional plasticity of Palau's *Mastigias papua* (Scyphozoa: Rhizostomeae) using stable isotopes and fatty acids

Conference presentations / Présentations en conférences :

Djaghri N, Pondaven P, Stibor H, Dawson MN (2019) Sunbathing jellies: What do we know of zooxanthellate jellyfishes? [Talk at the 6th International Jellyfish Bloom Symposium, Cape Town, South Africa](#)

Djaghri N, Pondaven P, Le Grand F, Bideau A, Duquesne N, Stockenreiter M, Behl S, Huang Y-T, Hansen T, Patris S, Ucharm G, Stibor H (2019) Photosynthesis or predation? Nutritional plasticity of Palau's *Mastigias papua* (Scyphozoa: Rhizostomeae) using stable isotopes and fatty acids. [Poster presentation at the 6th International Jellyfish Bloom Symposium, Cape Town, South Africa \(Awarded 2nd prize for a poster presentation\)](#)

Djaghri N, Pondaven P, Stibor H, Stockenreiter M, Behl S, Huang Y-T (2019) Can $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratios be used as indicators of nutrition in zooxanthellate jellyfishes? [Talk at the ASLO 2019 Aquatic Science Meeting, San Juan, Puerto Rico](#)

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General introduction

Introduction générale



1. Jellyfishes in Marine Ecosystems

Jellyfishes—here, used in the sense of pelagic cnidarians (Lucas and Dawson 2014)—have had a long history of being considered as having little role in marine environments (Haddock 2004). Nowadays however, jellyfishes are increasingly recognized as important components of pelagic ecosystems. This increasing scientific interest has been partly motivated by new available tools (from the early blue-water diving, Hamner et al. 1975, to latest molecular tools, McInnes et al. 2016) but also, by the possibility of a human-induced global increase of jellyfish blooms (Mills 2001). In spite of many claims, often unsubstantiated, that jellyfish blooms globally increase (see Sanz-Martin et al. 2016, Pitt et al. 2018), the question remains unresolved, and a definitive answer is presently difficult to give due to a lack of historic data (see however Brotz et al. 2012, Purcell 2012, Condon et al. 2013). Independently of the global trends, the research on jellyfish biology and ecology of the last decades has allowed to analyse the roles of jellyfishes in marine ecosystems.

For instance, jellyfishes have long been considered as trophic “dead-ends” being only preyed upon by specialists (e.g. Verity and Smetacek 1996). However old and more recent evidences indicate that this is not the case (Arai 2005, Hays et al. 2018). This, allied to the fact that jellyfishes are important pelagic predators (Purcell 1997, Purcell and Arai 2001, Choy et al. 2017) demonstrates that jellyfishes have an integrated role in pelagic food webs (Fig. 1). Beyond food webs, jellyfishes can impact other aspects of pelagic ecosystems’ functioning such as nutrient cycling (Pitt et al. 2009) or carbon export (Lebrato et al. 2012). In that sense, jellyfishes have ecological roles similar to other pelagic consumers (Fig. 1).

However, in many aspects, jellyfishes—and more generally, gelatinous zooplankton—differ from other non-gelatinous zooplankton or from nekton. A major difference is the ability of many jellyfish species to bloom (Boero et al. 2008, Dawson and Hamner 2009, Lucas and Dawson 2014). A true bloom is defined as a sudden increase in biomass associated with the phenology of the species (to be distinguished from an apparent bloom where the biomass can have been e.g. accumulated through physical processes, see Lucas and Dawson 2014). During a bloom, jellyfish biomass and abundances can be huge (up to tens of individuals per m³, see e.g. Olesen et al. 1994, Kawahara et al. 2006, Churnside et al. 2016) potentially perturbing human activities (Purcell et al. 2007, Fig. 1). Jellyfish blooms are often followed by sudden

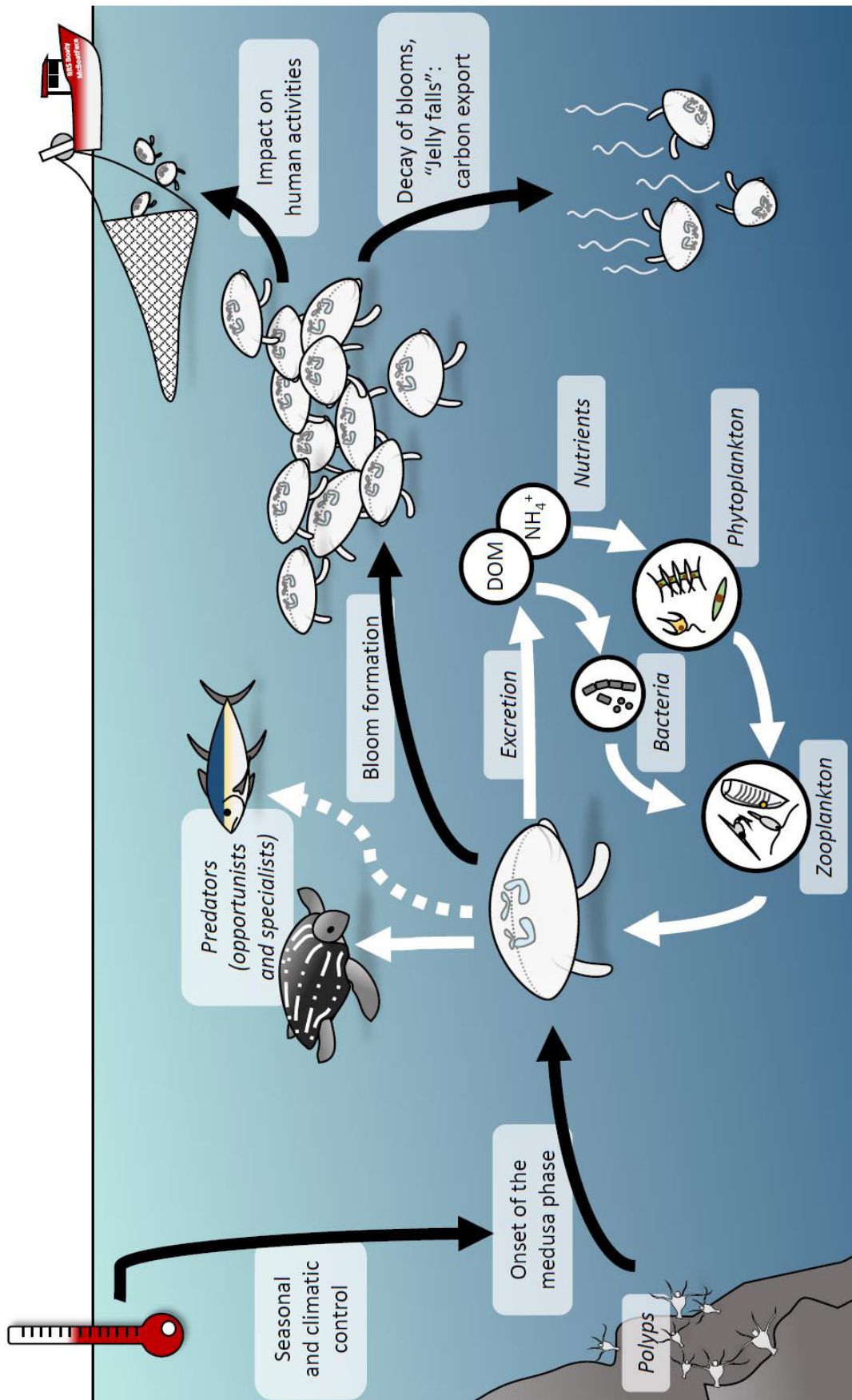


Fig. 1 Some of the roles of jellyfishes in marine ecosystems' functioning. White arrows = trophic links, black arrows = non-trophic links

Fig. 1 Quelques rôles des méduses dans les écosystèmes marins. Flèches blanches = liens trophiques, flèches noires = liens non trophiques

biomass collapses (Pitt et al. 2014). These sudden increases and collapses of the jellyfish populations results, in most cases, in very irregular and hard to predict dynamics (Boero et al. 2008). These dynamics are an important and normal aspect of jellyfishes' ecology and biology (Boero et al. 2008, Lucas and Dawson 2014) which apparently originated very early in their evolution (fossil traces of mass jellyfish strandings are found in Cambrian sediments, Sappenfield et al. 2016). This propensity of jellyfishes to bloom is linked to two important traits related to their life-cycle and body-plan.

2. Role of Jellyfish Specific Life-Cycles and Body-Plans for their Population Dynamics

Being able to achieve high reproduction output is important in order to bloom (Lucas and Dawson 2014). In most jellyfishes, high reproduction outputs are attained when the pelagic medusae phase is formed from the benthic polyp phase. Indeed, the classic life-cycle of jellyfishes is metagenetic, involving the alternation of a benthic, asexually reproducing polyp phase and a pelagic, sexually reproducing phase (Fig. 2). As a function of the peculiar taxa considered (e.g. Cubozoa, Hydrozoa or Scyphozoa) the detail of the life-cycle can change. Most notably, the transition from the polyp phase to the medusa phase is done through metamorphosis in Cubozoa, budding in Hydrozoa and strobilation in Scyphozoa (e.g. Boero et al. 2016). Moreover, many deviations from this classic life-cycle exist (mainly in Hydrozoa, Bouillon et al. 2006; but also in Scyphozoa, Rottini Sandrini and Avian 1983). In scyphozoans, which comprise most of the bloom-forming jellyfishes (Dawson and Hamner 2009) the role of polyps in maintaining the medusa population has been reviewed by Lucas et al. (2012). Scyphozoan polyp populations are often perennial, not experiencing as much fluctuations as the more conspicuous medusa phase, and are able to reproduce independently of the medusae through a variety of asexual reproduction processes (Lucas et al. 2012). Polyp populations can reach high densities (up to tens of polyps per cm⁻², although quantitative field estimates are rare, Lucas et al. 2012). They thus constitute a population reserve from which medusae populations can arise (Boero et al. 2008, Lucas et al. 2012). The onset of strobilation, and thus the formation of the medusae, is under the control of environmental factors (e.g. temperature, light, amount of prey, Lucas et al. 2012, Fig. 1). In many cases, this allows for few seasonal synchronized strobilation event (although many deviations exist, with more extended strobilation periods also common, Lucas et al. 2012). Given that, in most species,

one polyp can give several (up to 30, in some semeostomes) ephyrae when strobilating ([Lucas et al. 2012](#)), it is understandable, that a synchronous strobilation event of a whole polyp population could result in an important ephyrae release.

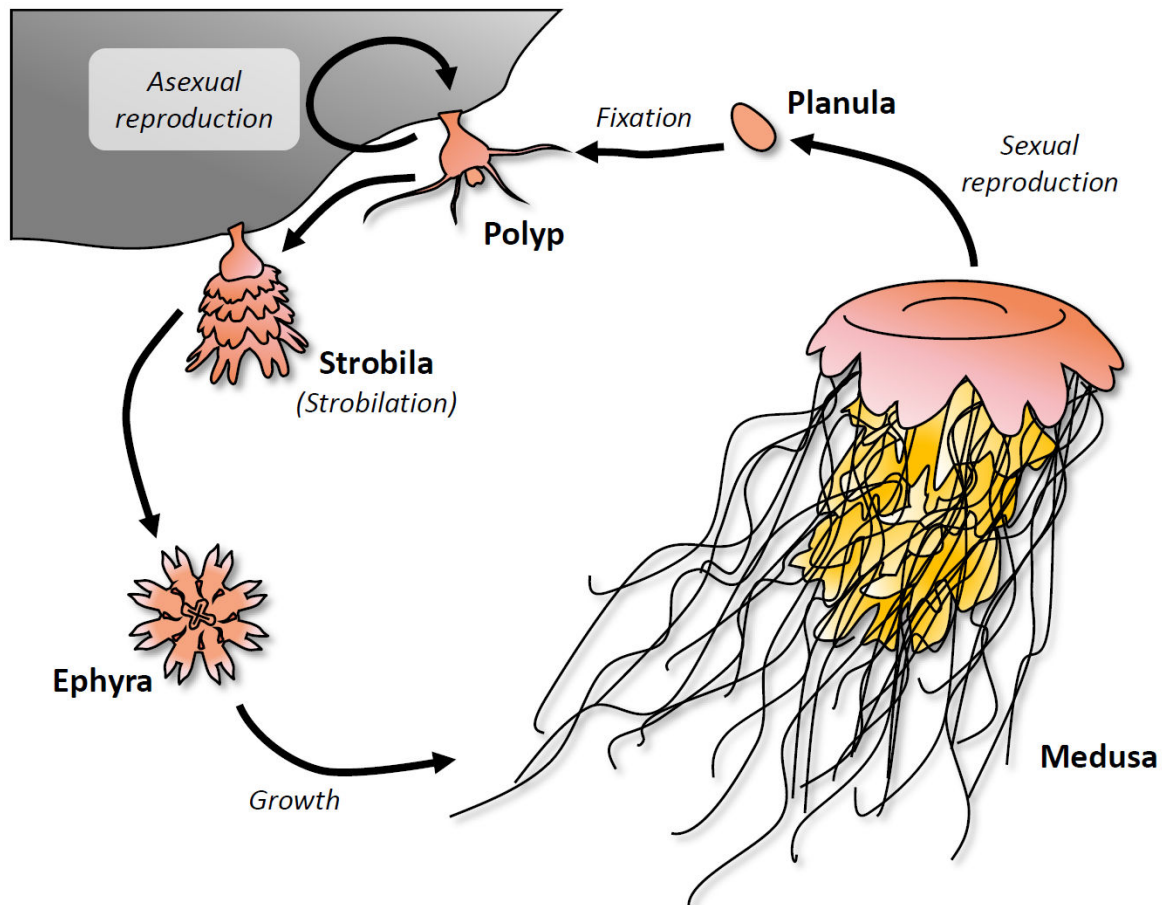


Fig. 2 Typical life-cycle in Scyphozoa (example of *Cyanea* sp., see e.g. [Gröndahl and Hernroth 1987](#)). The different phases are indicated in bold. Growth and reproduction are indicated in italic

Fig. 2 Cycle de vie typique des scyphozoaires (exemple de *Cyanea* sp., voir e.g. [Gröndahl et Hernroth 1987](#)). Les différentes phases sont indiquées en gras. La croissance (*growth*) et la reproduction sont indiquées en italique

Once the ephyrae are released, they still need to grow to achieve high biomass. This is where the body-plan of jellyfishes comes into play. Jellyfishes have notoriously high water content (generally > 95 % of the wet mass, only overcome by ctenophores, [McConville et al. 2017](#)). Although high water content is not the sole explanation, this water-inflated body allows jellyfishes to be larger than other zooplankters of similar carbon content ([Pitt et al. 2013](#)). This allows jellyfishes to operate at higher Reynolds number relative to other zooplankters making

for energy-efficient movement and increased contact rates with potential prey (Costello et al. 2008, Acuña et al. 2011, Pitt et al. 2013). Largely inflated body also allows jellyfishes to filter important water volumes for prey (Acuña et al. 2011, Pitt et al. 2013). Importantly for bloom formation, the combination of low wet mass-specific nutrition requirements, and high predation efficiency results in very high growth rates (Pitt et al. 2013).

Therefore, the blooming ability of many jellyfishes is linked to two key characteristics: their high reproductive output (achieved through strobilation in most scyphozoans) and their body-plan which allows high growth rates. However, all this under the control of other factors. One of the most important, and of particular relevance to the present thesis, is the nutrition of jellyfishes.

3. Heterotrophic Nutrition of Jellyfishes

Jellyfishes, as any other living organism, need to acquire energy. Jellyfishes are recognized as heterotrophic consumers. They acquire their energy through predation, mainly on other zooplankters (reviewed in Purcell 1997). Jellyfish's predation involves direct contact with its prey, capture, digestion and assimilation (Arai 1997, see also Kiørboe 2011). The contact with prey is generally performed by specialized organs characterized by high surface area, which essentially act like nets. In jellyfishes, these organs are tentacles and/or oral arms (Arai 1997). The way jellyfishes behave to favor the encounter of prey with their capture surface lead to the recognition of two predation modes (Costello et al. 2008, Kiørboe 2011), strongly influenced by the jellyfish morphology and behavior (Costello et al. 2008): (1) passive ambush feeding; where the jellyfish stays motionless waiting for a motile prey to fall in its tentacles (e.g. genera *Agalma*, *Forskalia*, *Liriope*, *Solmaris* or *Stomotoca* see Madin 1988) or (2) current feeding; where the jellyfish, through the contraction of its umbrella, forces water, and prey through its filtering apparatus (tentacles and/or oral arms, e.g. genera *Aequora* or *Pelagia*, Madin 1988). The latter is the most common in the large scyphozoan jellyfishes on which this thesis will be mostly focused (see e.g. Costello and Colin 1995). The capture of the prey is then assured in two ways (Arai 1997): (1) smallest preys (e.g. microplankton) are simply attached to the jellyfish mucus; (2) larger preys (e.g. mesozoplankton mostly but also nekton) are captured through the action of nematocytes, a cell type unique to cnidarians. Nematocytes

act like small harpoons charged with venom that fire upon contact with prey (Arai 1997). Captured prey is then brought to the mouth through ciliary motion and, for larger prey, movement of the oral arms (Arai 1997). Digestion in jellyfish can begin on oral arms, before ingestion, but is mostly done in the jellyfish's stomach (Arai 1997). Jellyfishes have no circulatory system; the repartition of the digestion products in the jellyfish body is instead assured by a set of radial and circular canals originating from the stomach (Arai 1997). These canals tend to get more numerous and complex in larger jellyfish species (Dawson and Hamner 2009).

All aspects of jellyfish ecology are impacted by their heterotrophic nutrition from asexual reproduction in polyps (Lucas et al. 2012, Schiariti et al. 2014), to the success of the medusa phase and the duration of blooms (Pitt et al. 2014). However, whereas heterotrophic predation is the sole mode of nutrition for most jellyfishes, for many others, heterotrophy is complemented through autotrophy. Many jellyfish species are indeed involved in a symbiotic relationship with zooxanthellae (dinoflagellates, mostly Symbiodiniaceae e.g. LaJeunesse et al. 2001). These zooxanthellate jellyfishes therefore obtain a part of their nutrition through their zooxanthellae's photosynthesis (e.g. Kremer et al. 1990, Kikinger 1992, Verde and McCloskey 1998), making them mixotrophs as holobionts (host + symbionts considered together, Fig. 3).

4. Nutrition of Zooxanthellate Jellyfishes, and the Structure and Objectives of this Thesis

The nutrition of zooxanthellate jellyfishes is the focus of this thesis. Zooxanthellate jellyfishes are known to be mixotrophs as holobionts, deriving their nutrition from both photosynthesis and predation (e.g. Verde and McCloskey 1998, Kremer 2005). However, this general picture appears to hide many variations. Indeed, the relative importance of autotrophy and heterotrophy appear to vary as a function of ontogeny (e.g. polyp phase versus medusa phase or in-between different medusae size-classes, Sugiura 1969, Hofmann and Kremer 1981, McCloskey et al. 1994) but also, as a function of the environment, or of the populations (Bolton and Graham 2004). The extent to which zooxanthellate jellyfishes can cope with such variations of their nutrition can be expected to have a strong influence on their ecological success and potential impacts on ecosystems. However, these variations are little

documented and are mostly characterized by extreme cases (e.g. presence or absence of zooxanthellae, [Bolton and Graham 2004](#)). To characterize the full extent of this variability, it is key to resolve more finely the question of the nutrition of zooxanthellate jellyfishes.

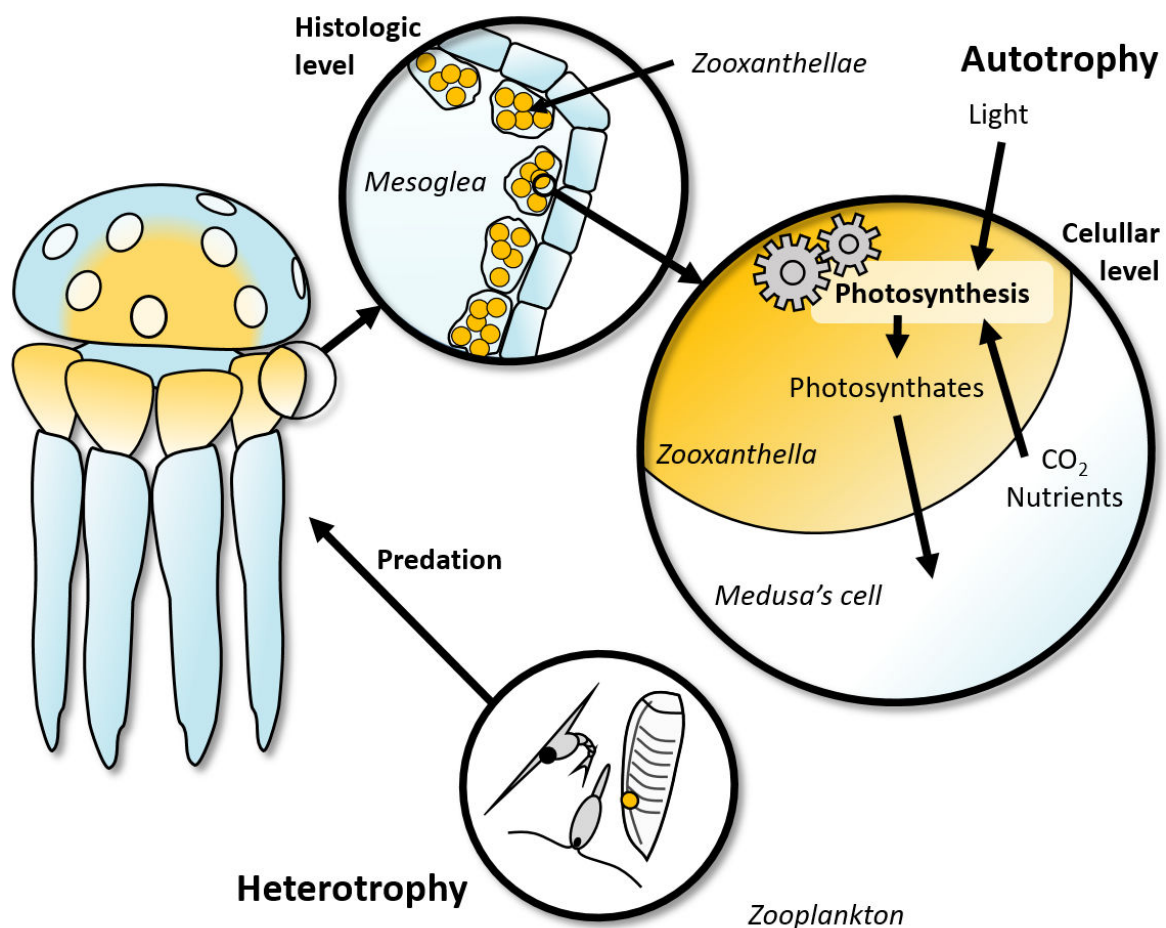


Fig. 3 Mixotrophic nutrition of zooxanthellate jellyfishes. Two modes of nutrition exist in zooxanthellate jellyfishes: heterotrophy (through predation) and autotrophy (through the photosynthesis of their zooxanthellae). A central question of this thesis is to know how these two sources of nutrition vary with the jellyfish's ontogeny or environment. Example of *Mastigias papua* (see [Muscatine et al. 1986](#), [McCloskey et al. 1994](#))

Fig. 3 Nutrition mixotrophe des méduses à zooxanthelles. Deux modes de nutrition coexistent chez les méduses à zooxanthelles : l'hétérotrophie (via la prédation) et l'autotrophie (via la photosynthèse de leur zooxanthelles). Une question centrale de cette thèse est de savoir comment ces deux sources de nutrition varient avec le développement ou l'environnement de la méduse. Exemple de *Mastigias papua* (voir [Muscatine et al. 1986](#), [McCloskey et al. 1994](#))

In the course of this work, it became quickly apparent that the available information on zooxanthellate jellyfishes is very scattered. Often, the literature is split between the scientific community focusing on corals and the scientific community focusing on jellyfishes. With the exception of the section “symbiosis” of Mary N Arai’s “A Functional Biology of Scyphozoa” (Arai 1997), no integrative review of biology and ecology of zooxanthellate jellyfishes was found in the scientific literature. The first step in this thesis was therefore to synthesize the scientific literature on zooxanthellate jellyfishes’ diversity, traits, and ecology. This synthesis constitutes the Chapter I of this thesis. The direction and objectives of the next chapters are then derived from the insights gained in Chapter I, with three main questions:

- How does the relative significance of heterotrophic and autotrophic nutrition of zooxanthellate jellyfishes vary with their ontogeny (e.g. polyp versus medusae, size gradients)? What are the ecological consequences of these variations?
- Environment is an important determinant of the nutrition sources available to zooxanthellate jellyfishes. How can zooxanthellate jellyfishes cope with environmental variations affecting their nutrition?
- Finally, how does the variability and plasticity of the nutrition of zooxanthellate jellyfishes help explain their ecologies?

The first approach, experimental and focused on the polyp stage, is presented in Chapter II. The hypothesis that, at the polyp stage, zooxanthellate jellyfishes rely little on their zooxanthellae—and therefore should not present many ecological differences as compared to non-zooxanthellate jellyfishes—is tested. The second approach is focused on fieldwork and on the medusa stage. Chapter III describes a controlled experiment assessing how isotopic and elemental composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N ratios) can be used to track the variations of zooxanthellate jellyfishes nutrition in the field. The findings of the Chapter III are then reinvested in Chapter IV. There, the nutrition of zooxanthellate jellyfishes is assessed—using isotopic and elemental, but also fatty acid indicators—in a field study conducted on *Mastigias papua* medusae from Palau (Micronesia). The sampling of all medusae size classes, and of different environments (four marine lakes plus the lagoon) allows for the description of the plasticity of the nutrition of *Mastigias papua* according to their size and environmental gradients. Finally, the major findings of this thesis are synthesized in a general discussion on the nutrition of zooxanthellate jellyfishes and its ecological implications.

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Chapter I: Review of the diversity, traits, and ecology of zooxanthellate jellyfishes

Chapitre I : Revue de la diversité, des traits de vie, et de l'écologie des méduses à zooxanthelles

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Abstract

Many marine organisms form photosymbioses with zooxanthellae, but some, such as the medusozoans, are less well known. Here, we summarize the current knowledge on the diversity of zooxanthellate jellyfishes, to identify key traits of the holobionts, and to examine the impact of these traits on their ecology. Photosymbiosis with zooxanthellae originated at least seven times independently in Medusozoa; of these, five involve taxa with medusae. While most zooxanthellate jellyfishes are found in clades containing mainly non-zooxanthellate members, the sub-order Kolpophorae (Scyphozoa: Rhizostomeae) is comprised—bar a few intriguing exceptions—of only zooxanthellate jellyfishes. We estimate that 20–25 % of Scyphozoa species are zooxanthellate (facultative symbiotic species included). Zooxanthellae play a key role in scyphozoan life-cycle and nutrition although substantial variation is observed during ontogeny, or at the intra and inter-specific levels. Nonetheless, three key traits of zooxanthellate jellyfishes can be identified: (1) zooxanthellate medusae, as holobionts, are generally mixotrophic, deriving their nutrition both from predation and photosynthesis; (2) zooxanthellate polyps, although capable of hosting zooxanthellae, rarely depend on them; (3) zooxanthellae play a key role in the life-cycle of the jellyfish by allowing or facilitating strobilation. We discuss how these traits might help explain some aspects of the ecology of zooxanthellate jellyfishes—notably their generally low ability to bloom, and their reactions to temperature stress or to eutrophication—and how they could in turn impact marine ecosystem functioning.

Résumé

De nombreux organismes marins forment des symbioses avec des zooxanthelles, mais certains, comme les médusozoaires, sont moins connus. Ici, nous faisons l'état de l'art concernant la diversité des méduses à zooxanthelles dans le but d'identifier leurs traits de vie centraux et d'examiner l'impact de ces traits sur leur écologie. La photosymbiose avec des zooxanthelles est apparue au moins sept fois au cours de l'évolution des médusozoaires dont cinq fois dans des taxons incluant des méduses. Tandis que dans la plupart des cas, les méduses à zooxanthelles sont isolées au sein de taxons principalement sans zooxanthelles, le sous-ordre des Kolpophorae (Scyphozoa : Rhizostomeae) est compris—à l'exception de quelques cas particuliers—uniquement de méduses à zooxanthelles. Nous estimons que 20 à 25 % des espèces de scyphozoaires ont des zooxanthelles (symbiontes facultatifs inclus). Les zooxanthelles jouent un rôle important dans la nutrition et le cycle de vie des scyphozoaires bien qu'une importante variabilité soit observée durant l'ontogénie ou à l'échelle intra- et interspécifique. Néanmoins, trois traits de vie importants des méduses à zooxanthelles peuvent être identifiés : (1) les méduses à zooxanthelles, durant leur phase méduse et en tant que holobiontes, sont généralement mixotrophes, dérivant leur nutrition à la fois de la prédation et de la photosynthèse ; (2) les polypes des méduses à zooxanthelles, bien que capables d'héberger des zooxanthelles, n'en sont que peu dépendants ; (3) les zooxanthelles jouent un rôle central dans le cycle de vie des méduses en permettant, ou facilitant, la strobilation. Nous étudions comment ces traits de vie des méduses à zooxanthelles pourraient aider à expliquer certains aspects de leur écologie—notamment leur faible tendance à former des blooms, et leur réactions aux stress thermiques, ou à l'eutrophisation—et comment les méduses à zooxanthelles pourraient, à leur tour, impacter le fonctionnement des écosystèmes marins.

1. Introduction

Many organisms, from corals and giant clams to protists, form photosymbioses with endosymbiotic, autotrophic dinoflagellates or “zooxanthellae” (Venn et al. 2008, Stoecker et al. 2009). Most of our knowledge on this kind of relationships comes from the study of photosymbiotic scleractinian corals, but other cnidarians also form photosymbioses including jellyfishes (e.g. Arai 1997). Zooxanthellate jellyfishes are historically linked to the study of cnidarian-zooxanthellae symbiosis as the zooxanthellae first described as *Symbiodinium* was obtained from the upside-down jellyfish *Cassiopea* (Freudenthal 1962). This jellyfish genus *Cassiopea* is still relevant today as a model organism (Ohdera et al. 2018) while other symbiotic scyphozoan species have potential to illuminate pelagic symbioses (e.g. Muscatine et al. 1986). Moreover, their relevance is increasing as zooxanthellate jellyfishes’ populations have also increased in some environments (Arai 2001, Pérez-Ruzafa et al. 2002) raising the question of their roles in ecosystem functioning (see e.g. Pitt et al. 2009).

However, despite increasing interest, zooxanthellate jellyfish remain little studied relative to other, non-zooxanthellate, jellyfishes or to scleractinian corals. Zooxanthellate jellyfishes are of particular interest for two, often distinct, communities of scientists: the first, focused on jellyfishes, the second focused on photosymbiosis. This has led to a somewhat scattered literature with few works bridging the gap. Our goal with this review is to establish a more integrative, synthetic, foundation for future studies focusing on the diversity and on the ecology of zooxanthellate jellyfishes.

All zooxanthellate jellyfishes share two key characteristics. First a metagenetic life-cycle, as many other, non-zooxanthellate, jellyfishes, with two distinct body-plans and ecologies: the polyp and the medusa (Box 1). Second a photosymbiotic relationship with zooxanthellae, as in scleractinian corals (Box 2). The combination of these two characteristics and their interplay is likely to give zooxanthellate jellyfishes a unique set of ecological traits. Indeed, zooxanthellate jellyfishes have a variety of unique morphologies and behaviors (Arai 1997), a narrower latitudinal distribution (e.g. Holland et al. 2004, Swift et al. 2016), a propensity to not bloom (Dawson and Hamner 2009), and a different nutrition (e.g. Verde and McCloskey 1998, Kremer 2005) from their non-zooxanthellate counterparts. However, these jellyfishes also occupy multiple branches of the cnidarian tree of life. This raises the question of whether

these characteristics are attributable to the symbiosis itself or to other lineage-specific evolutionary history.

To better understand these characteristics of zooxanthellate jellyfishes, we divide our review into three parts. First, we review the phylogenetic distribution of zooxanthellate jellyfishes. Second, we review the roles of zooxanthellae in zooxanthellate jellyfishes, primarily with respect to their complex life-cycle and in their nutrition. Finally, once the key traits have been characterized, we review how these traits could impact the ecology of zooxanthellate jellyfishes and their influence on marine communities and ecosystem functions.

Box 1: Jellyfishes and their Metagenetic Life-Cycle

Jellyfish may be seen as an ambiguous word. Here, we refer to jellyfish as pelagic cnidarians ([Lucas and Dawson 2014](#)). Many of these organisms display a complex life-cycle with an alternation of a benthic, asexually reproducing, polyp phase and a pelagic, sexually reproducing, medusa phase. Differences in the details of the life-cycle exist between groups. For instance, the transition between the polyp and the medusa phase is generally done by lateral budding in Hydrozoa, by metamorphosis in Cubozoa, and by strobilation in Scyphozoa (see e.g. [Boero et al. 2016](#)). Many variations from this general depiction of life-cycles exist; for instance, reduction or loss of one of the phases (very common in Hydrozoa, [Bouillon et al. 2006](#)).

The alternation of polyp and medusae phases has important repercussions for the ecology of these organisms. Generally, populations of asexually reproducing polyps are perennial, and represent the source of the pelagic medusae populations. Then, pelagic medusae, may bloom, aggregate, or swarm (see e.g. [Lucas and Dawson 2014](#)), with potential for ecological and human consequences.

Box 2: Cnidarian-Zooxanthellae Photosymbiosis

Photosymbiosis can be defined predominantly as a mutualistic nutritional association. In the case of the cnidarian-zooxanthellae photosymbiosis the symbionts are found within host cells (endosymbionts). This involves complex processes of symbiont recognition, acquisition and regulation (see [Davy et al. 2012](#)). The main advantage of this kind of symbiosis is nutritional. Zooxanthellae photosynthesize and share some of the photosynthates with their host. These photosynthates include diverse molecules such as carbohydrates, lipids and amino-acids that can be metabolized by the host ([Davy et al. 2012](#)). The symbionts can then use the host excretion and respiration products (CO₂, dissolved inorganic nitrogen) for their autotrophic metabolism ([Yellowlees et al. 2008](#), [Davy et al. 2012](#)). Consequently, these associations allow a recycling of nutrients within the holobiont (symbionts + host). They are thus considered particularly advantageous in nutrient poor environments ([Yellowlees et al. 2008](#)).

2. Diversity of Zooxanthellate Jellyfishes**2.1. Diversity of Hosts**

Jellyfish (i.e. pelagic cnidarians, [Lucas and Dawson 2014](#)) belong to the taxon Medusozoa. Albeit the aim of this review is to focus on zooxanthellate jellyfishes, their evolutionary history cannot be separated from either: (1) other medusozoans that lack zooxanthellae and, (2) other medusozoans that lack a pelagic phase. Thus, all groups of medusozoans are taken into account, at this first stage, to discuss phylogenetic patterns of zooxanthellate medusozoans (Table 1, Fig. 1).

Considering only confirmed reports of zooxanthellate presence (Table 1), we estimate that symbiosis between medusozoans and zooxanthellae originated independently at least seven times (Fig. 1). Of these seven originations, five involved groups containing pelagic taxa (i.e. jellyfishes) (Fig. 1): one in Cubozoa (two species of Carybdeid jellyfish), two in Hydrozoa (in Capitata with the family Porpitidae and in Loadiceida with the genus *Wuvula*) and two in Scyphozoa (in Coronatae with the Linuchidae plus some *Nausithoe* and in Kolpophorae). The two other originations are from hydrozoan groups lacking a medusae phase (see [Bouillon et](#)

al. 2006): in Filifera I (family Eudendriidae) and in Macrocolonia (e.g. *Aglaophenia*, *Halecium*, *Pseudoplumaria*). No zooxanthellate staurozoans have been found.

It is important to emphasize that seven originations of symbiosis with zooxanthellae in Medusozoa is a minimum. Firstly, symbioses between zooxanthellae and medusozoans are often little documented in the literature. It is thus likely that we have underestimated the number of zooxanthellate species. Secondly, the phylogeny of hydrozoans in particular is more complex than presented here. Thus, some zooxanthellate groups may be subdivided more finely phylogenetically as more data are gathered. For instance, Macrocolonia is a very diversified group (Maronna et al. 2016) and it is likely that the macrocolonian genera reported to be zooxanthellate are not closely related (see Maronna et al. 2016, Moura et al. 2018). Similarly, the Porpitidae family and the genus *Millepora* (fire corals) both belong to Capitata and host zooxanthellae but might not be closely related (Nawrocki et al. 2010). This remark is also valid for zooxanthellate Coronatae until phylogenetic relationships are better resolved (Fig. 1). Finally, *Dichotomia cannoides* (Hydrozoa, Leptothecata, Dipleurosomatidae) may represent another origination of the medusozoan-zooxanthellae symbiosis. However, until its phylogeny is better resolved (Maronna et al. 2016), it cannot be confirmed.

Despite multiple originations, only a minority of medusozoans are found in symbiosis with zooxanthellae. Often, photosymbiotic species are clustered in mostly non-zooxanthellate clades. Even in close relatives (within a family or a genus) it is common that some species are zooxanthellate while most others are not (see e.g. *Aglaophenia* - Svoboda and Cornelius 1991, *Alatina* - Carrette et al. 2014, *Eudendrium* - Marques et al. 2000, Laodiceidae - Bouillon et al. 2006, *Nausithoe* - Silveira and Morandini 1997). In these groups, symbiosis with zooxanthellae does not appear to have favored adaptive radiation. In which context, Kolpophorae appears as an exception: it is a diversified group with ca. 40–50 species in five families (Daly et al. 2007) and likely many more yet to be described (Gómez Daglio and Dawson 2017), that is predominantly, and possibly only, constituted of zooxanthellate species. Six of the nine genera of Kolpophorae are reported to be zooxanthellate, photographic evidence suggest that two other genera might have zooxanthellae (Appendix). We hence infer parsimoniously that all Kolpophorae might be zooxanthellate (facultative included; Table 1).

Considering all Kolpophorae plus the zooxanthellate Coronatae we estimate that between 20 and 25% of scyphozoan species are zooxanthellate (including facultative symbionts, species richness obtained from [Daly et al. 2007](#)). Such an estimate is hard to give at present for hydrozoans and cubozoans as their zooxanthellate representatives are less documented.

Table 1 → List of zooxanthellate medusozoan genera found in the literature and inferred in this study (including facultative symbionts). The inference of the presence of zooxanthellae was made for the members of the sub-order Kolpophorae where all the genera on which relevant information is available are zooxanthellate. Unless stated otherwise, it is assumed that all species in each genus may have zooxanthellae. This list is likely far from complete as many groups, particularly in Hydrozoa remain little known. Reports of zooxanthellae presence are classified as follows: “Confirmed”: when zooxanthellae have been directly observed and/or studied (e.g. microscopic imagery, zooxanthellae presence used as a taxonomic criterion, measures of photosynthesis available). “Potentially”: when authors were unsure that the observed structure were zooxanthellae. “Indirect report”: report of the presence of zooxanthellae without direct observation. “Doubtful”: indirect reports with contradicting direct observations. Additional indirect evidence (photographs) for the presence of zooxanthellae in some little studied Kolpophorae is discussed in Appendix

Table 1 → Liste des genres de médusozoaires à zooxanthelles trouvés dans la littérature et inférés dans cette étude (symbiontes facultatifs inclus). L’inférence de la présence de zooxanthelles a été faite pour les membres du sous-ordre des Kolpophorae, ou tous les genres sur lesquels une information adéquate était disponible ont des zooxanthelles. Sauf si indiqué autrement, il est considéré que toutes les espèces de chaque genre pourraient avoir des zooxanthelles. Cette liste est probablement loin d’être complète comme beaucoup de groupes, en particuliers chez les hydrozoaires, restent peu connus. Les signalements de la présence des zooxanthelles sont classés comme suit : « Confirmed » : quand les zooxanthelles ont été directement observées et/ou étudiées (e.g. microscopie, présence de zooxanthelles en tant que critère taxonomique, mesures de photosynthèse disponibles). « Potentially » : quand les auteurs étaient incertains si la structure observée était une zooxanthelle. « Indirect report » : signalement de la présence de zooxanthelles sans observation directe. « Doubtful » : « Indirect reports » avec des observations directes contradictoires. Des indications supplémentaires (photographies) sur la présence de zooxanthelles dans quelques Kolpophorae peu étudiés sont présentés dans l’Appendix

| CLASS, Order, Family | Genus | Presence of zooxanthellae? (Reference(s)) |
|-------------------------|------------------------|---|
| CUBOZOA | | |
| Carybdeida | | |
| Alatinidae | <i>Alatina</i> | Confirmed in <i>Alatina morandinii</i> (Straehler-Pohl and Jarms 2011, Straehler-Pohl and Toshino 2015) |
| Carukiidae | <i>Malo</i> | Confirmed in <i>Malo maxima</i> (only in a minority of polyps, Underwood et al. 2018) |
| HYDROZOA | | |
| Anthoathecata | | |
| Capitata incertae sedis | <i>Paulinum</i> | Potentially (see Kramp 1961 (as <i>Dicodonium</i>), Brinckmann-Voss and Arai 1998) |
| Eudendriidae | <i>Eudendrium</i> * | Confirmed in some species (Marques et al. 2000) |
| | <i>Myrionema</i> * | Confirmed (Fitt and Cook 2001) |
| Milleporidae | <i>Millepora</i> ** | Confirmed (Lewis 2006) |
| Porpitidae | <i>Porpita</i> | Confirmed (Bouillon et al. 2006) |
| | <i>Velella</i> | Confirmed (Larson 1980, Bouillon et al. 2006, Lopes et al. 2016) |
| Ptilocodiidae | <i>Hydrichthella</i> * | Indirect report (Muscatine 1974) |
| Stylasteridae | <i>Sporadopora</i> * | Indirect report (Muscatine 1974) |

Table 1 Continued / Suite

| CLASS, Order, Family | Genus | Presence of zooxanthellae? (Reference(s)) |
|----------------------|--------------------------|---|
| Leptothecata | | |
| Agloapheniidae | <i>Aglaophenia</i> * | Confirmed in some species (Svoboda and Cornelius 1991) |
| Dipleurosomatidae | <i>Dichotomia</i> | Confirmed (Brooks 1903 , Bouillon 1984) |
| Haleciidae | <i>Halecium</i> * | Confirmed in some species (Pagliara et al. 2000) |
| Laodiceidae | <i>Wuvula</i> | Confirmed (Bouillon et al. 1988) |
| Plumulariidae | <i>Pseudoplumaria</i> * | Confirmed in <i>Pseudoplumaria marocana</i> (Medel and Vervoort 1995) |
| Sertularellidae | <i>Sertularella</i> * | Indirect report (Muscantine 1974 , see also Calder 1990) |
| Sertulariidae | <i>Dynamena</i> * | Potentially (Galea and Ferry 2015) |
| Thyroscyphidae | <i>Symmetroscyphus</i> * | Confirmed (Calder 1991) |
| Siphonophorae | | |
| Physaliidae | <i>Physalia</i> | Doubtful (Wittenberg 1960 but see Lopes et al. 2016) |
| SCYPHOZOA | | |
| Coronatae | | |
| Linuchidae | <i>Linuche</i> | Confirmed (Ortiz-Corp's et al. 1987 , Kremer et al. 1990) |
| Nausithoidae | <i>Nausithoe</i> | Confirmed in some species (Werner 1973 , Silveira and Morandini 1997) |

Table 1 Continued and end / Suite et fin

| CLASS, Order, Family | Genus | Presence of zooxanthellae? (Reference(s)) |
|------------------------------------|---------------------|---|
| Rhizostomeae | | |
| Catostylidae | <i>Catostylus</i> | Doubtful (Muscatine 1974 but see Pitt et al. 2005) |
| Rhizostomatidae | <i>Rhizostoma</i> | Doubtful (Trench 1971 but see Fuentes et al. 2011) |
| Cassiopeidae (Kolpophorae) | <i>Cassiopea</i> | Confirmed (Freudenthal 1962 , Mergner and Svoboda 1977 , Verde and McCloskey 1998) |
| Cepheidae (Kolpophorae) | <i>Cephea</i> | Confirmed (Sugiura 1969) see also Appendix |
| | <i>Cotylorhiza</i> | Confirmed (Kikinger 1992) see also Appendix |
| | <i>Marivagia</i> | No photographic evidence of the presence of zooxanthellae in the medusa (Appendix) – inference: zooxanthellae likely present in the polyp as in close relatives |
| | <i>Netrostoma</i> | Confirmed (Straehler-Pohl and Jarms 2010) see also Appendix |
| Mastigiidae (Kolpophorae) | <i>Mastigias</i> | Confirmed (Sugiura 1964 , McCloskey et al. 1994) |
| | <i>Phyllorhiza</i> | Confirmed (Pitt et al. 2005) |
| Thysanostomatidae (Kolpophorae) | <i>Thysanostoma</i> | Photographic evidence for the presence of zooxanthellae available (Appendix) |
| Versurigidae (Kolpophorae) | <i>Versuriga</i> | Photographic evidence for the presence of zooxanthellae available (Appendix) |
| ? | <i>Bazinga</i> | Confirmed (Gershwin and Davie 2013) |

* = groups with no medusae phase (see [Bouillon et al. 2006](#)). ** = group with short lived medusae phase (see [Soong and Cho 1998](#))

* = groupes sans phase méduse (voir [Bouillon et al. 2006](#)). ** = groupe avec une phase méduse à vie courte (voir [Soong and Cho 1998](#))

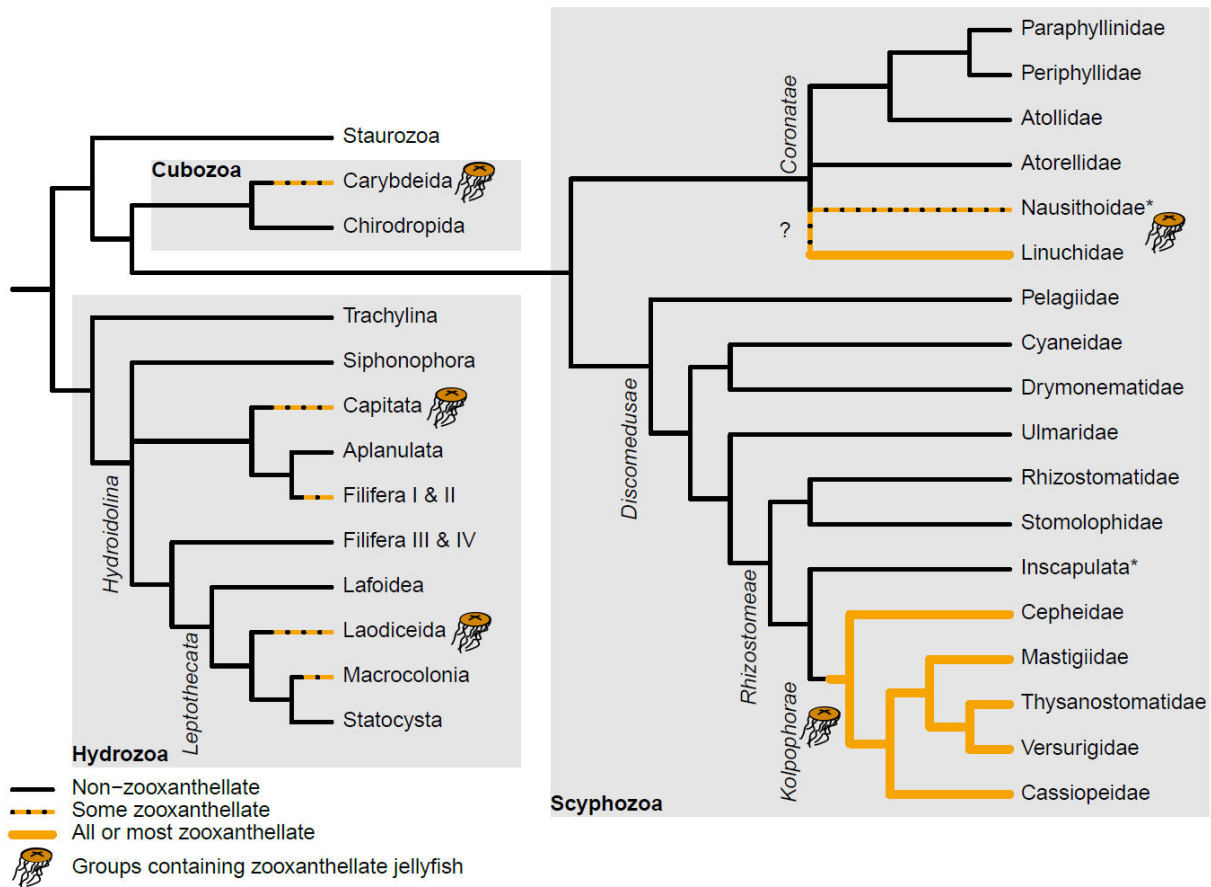


Fig. 1 Phylogenetic position of zooxanthellate medusozoans with emphasis on Scyphozoa. The topology used here is based on [Kayal et al. \(2018\)](#) for the relationships among major groups, [Kayal et al. \(2015\)](#) for Hydroidolina, [Maronna et al. \(2016\)](#) for Leptothecata, [Bayha et al. \(2010\)](#) for Coronatae and [Gómez Daglio and Dawson \(2017\)](#) for Discomedusae. '*' = non-monophyletic group. '?' = unclear origin of symbiosis with zooxanthellae in Coronatae. See Table 1 for the literature identifying zooxanthellae presence. Only confirmed reports are taken into account in this figure with the exception of Kolpophorae where we parsimoniously infer the presence of zooxanthellae in all or most species (possibly facultative, see Appendix)

Fig. 1 Position phylogénétique des médusozoaires à zooxanthelles avec l'accent sur les scyphozoaires. La topologie utilisée ici est basée sur [Kayal et al. \(2018\)](#) pour les relations entre grands groupes, [Kayal et al. \(2015\)](#) pour Hydroidolina, [Maronna et al. \(2016\)](#) pour Leptothecata, [Bayha et al. \(2010\)](#) pour Coronatae et [Gómez Daglio and Dawson \(2017\)](#) pour Discomedusae. « * » = groupe non monophylétique. « ? » = origine incertaine de la symbiose avec des zooxanthelles chez les coronates. Voir Table 1 pour les références sur la présence des zooxanthelles dans les différents groupes. Seuls les symbioses confirmées sont prises en compte dans cette figure à l'exception des Kolpophorae ou nous inférons parcimonieusement la présence de zooxanthelles chez toutes, ou la plupart des espèces (potentiellement facultative, voir Appendix)

Box 3: Glossary

We give here some of the technical terms used in this review. For more information on jellyfish anatomy, development and taxonomy, see [Arai 1997](#) and [Bouillon et al. 2006](#).

Ecology:

Bloom: A true, natural, jellyfish bloom is defined as an increase in jellyfish biomass linked to phenology (see [Lucas and Dawson 2014](#)).

Development:

Planula: The larval stage of cnidarians. Generally pyriform, ciliated, and motile.

Planuloid budding: A mode of asexual reproduction in scyphozoan polyps. A planuloid bud is formed by a polyp, detaches and swims to finally reattach to the substrate and form a new polyp. This is the dominant mode of polyp formation in Kolpophorae, a group of monodisk strobilating, often photosymbiotic, jellyfishes.

Ephyra: Young medusa-like stage in Scyphozoa.

Strobilation: A mode of asexual reproduction whereby a scyphozoan polyp forms ephyrae through transverse fission (see [Helm 2018](#)). Strobilation can be monodisc, when one ephyra is formed at a time or polydisc, when multiple ephyrae are formed at the same time.

Anatomy:

Mesoglea: An extracellular matrix, primarily composed of collagen, located between the endoderm and ectoderm in cnidarian. In medusae, the mesoglea can be thick and generally provides most of the volume of the animal.

Oral arms: In medusae, expansions of the mouth involved in prey capture and, at times, digestion. Not to be confounded with tentacles.

Exumbrella: In medusae, the superior (aboral) part of the umbrella.

Subumbrella: In medusae, the inferior (oral) part of the umbrella.

Coronal muscle: A circular muscle that ensures umbrella contractions.

2.2. Diversity of Symbionts

Zooxanthellae associated with jellyfish species belong mostly to the family Symbiodiniaceae (see the recent revision of the family by [LaJeunesse et al. \(2018\)](#)). The most common symbionts found in zooxanthellate scyphozoan jellyfishes in the field appear to belong to the genera *Symbiodinium* (previously *Symbiodinium* clade A) and *Cladocopium* (previously *Symbiodinium* clade C) although other Symbiodiniaceae can be found ([LaJeunesse et al. 2001](#), [Santos et al. 2003](#), [Thornhill et al. 2006](#), [Mellas et al. 2014](#)). Furthermore, laboratory experiments have demonstrated that the associations between the jellyfish *Cassiopea* spp. and Symbiodiniaceae genera are not specific. Indeed, *Cassiopea* spp. polyps have been successfully infected with a variety of isolated and mixed Symbiodiniaceae genera including *Symbiodinium*, *Cladocopium*, *Breviolum* (previously *Symbiodinium* clade B) and *Durusdinium* (previously *Symbiodinium* clade D) ([Thornhill et al. 2006](#), [Mellas et al. 2014](#), [Lampert 2016](#)). However, adult medusae tend to harbour only one phylotype of symbiont suggesting that a mechanism such as competitive exclusion occurs within the host ([Thornhill et al. 2006](#)). Thus some flexibility appears to exist in the zooxanthellae-jellyfish association. This is further illustrated by the symbionts found in the hydrozoan *Velella velella*. Zooxanthellae from *Velella velella* can indeed belong to Symbiodiniaceae ([LaJeunesse et al. 2001](#)) but they can also belong to the genera *Brandtodinium* and *Scrippsiella* (or *Ensiculifera*) from the family Thoracosphaeraceae ([Banaszak et al. 1993](#), [Probert et al. 2014](#)).

2.3. Biogeography and Habitat

Generally, zooxanthellate jellyfishes are found in tropical and sub-tropical waters between 40° N and 40° S (see e.g. [Bieri 1977](#), [Bouillon et al. 1988](#), [Bolton and Graham 2004](#), [Holland et al. 2004](#), [Bayha and Graham 2014](#), [Heins et al. 2015](#), [Straehler-Pohl and Toshino 2015](#), [Boero et al. 2016](#), [Swift et al. 2016](#)). The zooxanthellate coronates and kolpophoran rhizostomes in particular are tropical clades ([Dawson and Hamner 2009](#)). However, exceptions can exist as some zooxanthellate jellyfishes may be found in temperate waters either occasionally (e.g. [Purcell et al. 2012a](#)) or possibly as resident species (see [Brinckmann-Voss and Arai 1998](#)).

At finer geographic scales, zooxanthellate jellyfishes are typically shallow-water species ([Dawson and Hamner 2009](#)). They have been reported in a number of coastal habitats

including lagoons, estuaries, coral reefs, mangroves or marine lakes (see e.g. [García 1990](#), [Kikinger 1992](#), [Fleck and Fitt 1999](#), [Pitt et al. 2004](#), [Swift et al. 2016](#), [Morandini et al. 2017](#)). Such coastal habitats are most likely linked to the fact that most zooxanthellate jellyfishes have a benthic polyp phase, which limits their extension toward the open ocean. However, the medusa phase in some instances has been reported in the open sea (e.g. in Cepheidae – [Tokio et al. 1964](#), [Boero et al. 2016](#), in *Linuche* – [Larson 1992](#)). Furthermore, hydrozoans of the family Porpitidae realize their whole life-cycle in the open ocean ([Bieri 1977](#)), exemplifying that the presence of benthic polyps in the life-cycle, rather than symbiosis with zooxanthellae, more likely restricts jellyfishes to coastal waters.

3. Roles of the Zooxanthellae in Jellyfish Symbioses

3.1. Acquisition, Location, Transmission and Abundance of Zooxanthellae along the Jellyfish Life-Cycle

3.1.1. Acquisition of the zooxanthellae

The acquisition of zooxanthellae is the first step of the symbiosis. A host may acquire zooxanthellae by two means: (1) vertical transmission, where the symbiont is directly transferred from the parents to the offspring (usually from the mother to the egg), or (2) horizontal transmission, where the symbiont is taken from the environment. While vertical transmission may occur in zooxanthellate hydrozoans (see e.g. [Mangan 1909](#), [Bouillon 1984](#), [Lewis 1991](#)), it is likely that most other zooxanthellate jellyfishes acquire their symbionts via horizontal transmission. In Kolpophorae, the symbiont is not provided by parents but acquired from the environment at the polyp stage (e.g. [Sugiura 1963](#), [Ludwig 1969](#), [Sugiura 1969](#), [Fitt 1984](#), [Colley and Trench 1985](#), [Kikinger 1992](#), [Astorga et al. 2012](#), [Newkirk et al. 2018](#)). The coronate *Linuche unguiculata* presents a somewhat intermediate mode of acquisition of the symbiont since fertilized eggs are released in mucus strand replete with maternal zooxanthellae that contaminate the larvae very early in development, generally before the 128 cells stage ([Montgomery and Kremer 1995](#)).

3.1.2. Location of zooxanthellae in jellyfishes

In hydromedusae, zooxanthellae are found in endodermal cells (Bouillon et al. 1988, Banaszak et al. 1993, see also Brooks 1903, Brinckmann-Voss and Arai 1998, Fig. 2a). In scyphozoans, zooxanthellae first enter polyps' endodermal cells, which then migrate and become mesogleal amaeobocytes (Colley and Trench 1985, Fig. 2a and b). In ephyrae, these amaeobocytes filled with zooxanthellae stay mostly closely associated with the endoderm (see Kikinger 1992, Silveira and Morandini 1998, Straehler-Pohl and Jarms 2010). This remains the case for later stage medusae in the Coronatae (*Linuche unguiculata*; Costello and Kremer 1989), and in the Cepheidae (*Cotylorhiza tuberculata*; Kikinger 1992). In other, non-cepheid, Kolpophorae, the zooxanthellae end up closely associated with the ectoderm (e.g. coronal muscle, subumbrella, exumbrella, oral arms; Blanquet and Riordan 1981, Muscatine et al. 1986, Blanquet and Phelan 1987, Estes et al. 2003, Souza et al. 2007, Fig. 2c). This suggests that the close association of zooxanthellae with the ectoderm could be a synapomorphy of the clade of non-cepheid Kolpophorae. The reason for this evolution is unclear, but perhaps could have adaptive value in allowing better exposure of zooxanthellae to light or nutrients, or providing energy more directly to the host tissues that require it the most.

3.1.3. Abundance and transmission of the zooxanthellae during the jellyfish life-cycle

Zooxanthellae abundance in their hosts is affected by the complex life-cycles of jellyfishes. In the best studied zooxanthellate jellyfishes, the Kolpophorae, the symbionts are taken up at the polyp stage. At this stage, the abundance of zooxanthellae can range from zero (aposymbiotic polyp) to tens of thousands of zooxanthellae per polyp (Newkirk et al. 2018). Polyps form other polyps asexually through a variety of processes (e.g. Schiariti et al. 2014) and in Kolpophorae, the dominant process is by far the production of planuloid buds (Schiariti et al. 2014, Heins et al. 2015). During this process, zooxanthellae are transferred from the parent polyp to the forming bud. Thus polyps formed asexually by zooxanthellate polyps are also zooxanthellate (e.g. Sugiura 1964, Ludwig 1969, Silveira and Morandini 1998, Heins et al. 2015). Then, during strobilation, zooxanthellae multiply in the oral region of the polyp where the ephyra is formed (Ludwig 1969). The ephyrae formed are thus also zooxanthellate (e.g. Sugiura 1964, Ludwig 1969, Sugiura 1969, Kikinger 1992, Silveira and Morandini 1997, 1998,

Straehler-Pohl and Jarms 2010). Finally, during the growth of medusae, zooxanthellae densities tend to stay constant or to decrease slightly in most species (densities in the order of 10^7 cells.g⁻¹ wet mass, see Muscatine et al. 1986, Kremer et al. 1990, Verde and McCloskey 1998). However, some species such as *Cephea cephea* may lose their zooxanthellae at the medusae stage (Sugiura 1969); this is likely the case of many other Cepheidae too (see Appendix). The ontogenic loss of zooxanthellae suggests that the symbiosis might present trade-offs and might not always be advantageous (see e.g. Lesser et al. 2013). The presence or absence of zooxanthellae during the life-cycle of some zooxanthellate jellyfish have been compiled in Table 2.

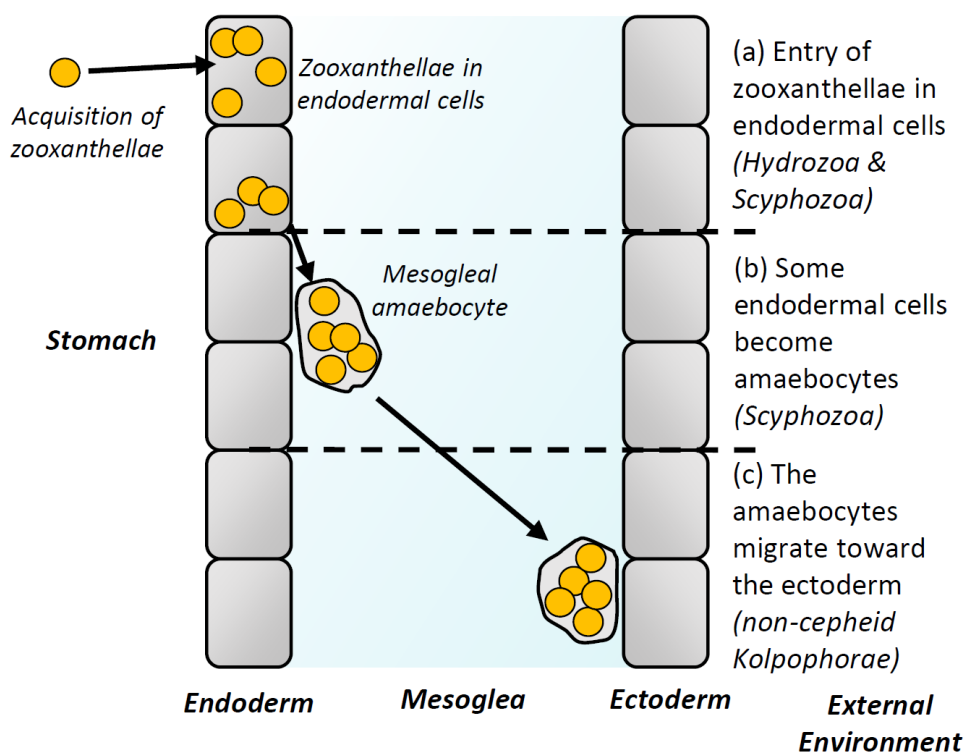


Fig. 2 Positions of zooxanthellae in jellyfishes' tissues as a function of taxa and ontogeny. (a) Zooxanthellae enter in endodermal cells. (b) Some endodermal cells filled with zooxanthellae become mesogleal amaebocytes. (c) The mesogleal amaebocytes migrate toward the ectoderm at the medusae stage in non-cepheid Kolpophorae. In brackets are the taxa that perform a given step

Fig. 2 Position des zooxanthelles dans les tissus des méduses en fonction du taxon et de l'ontogénie. (a) Les zooxanthelles entrent dans les cellules endodermes. (b) Certaines cellules endodermes pleines de zooxanthelles deviennent des amaebocytes mésogléaux. (c) Les amaebocytes mésogléaux migrent vers l'ectoderme au stade méduse chez les Kolpophorae non-céphéides. Entre parenthèse sont les taxons concernés par une étape donnée

Table 2 Presence of zooxanthellae along the life-cycle in some species of zooxanthellate jellyfish. Brackets indicates a state inferred from closely related species but without direct confirmation found in the literature

Table 2 Présence des zooxanthelles au cours du cycle de vie de quelques espèces de méduses à zooxanthelles. Les parenthèses indiquent un état inféré des espèces proches mais sans confirmation directe dans la littérature

| Species | Planula | Polyp | Ephyra | Medusa | Reference(s) |
|---------------------------|---------|---------|--------|---------|---|
| CUBOZOA | | | | | |
| Carybdeida | | | | | |
| <i>Alatina morandinii</i> | ? | present | N/A | present | Straehler-Pohl and Jarms 2011 , Straehler-Pohl and Toshino 2015 |
| <i>Malo maxima</i> * | ? | present | N/A | ? | Underwood et al. 2018 |
| HYDROZOA | | | | | |
| Anthoathecata | | | | | |
| <i>Porpita porpita</i> | ? | present | N/A | present | Bouillon et al. 2006 |
| <i>Veella veella</i> | ? | present | N/A | present | Larson 1980 , Bouillon et al. 2006 |
| Leptothecata | | | | | |
| <i>Wuvula fabietii</i> | ? | ? | N/A | present | Bouillon et al. 1988 |

Table 2 Continued and end / Suite et fin

| Species | Planula | Polyp | Ephyra | Medusa | Reference(s) |
|--------------------------------|----------|---------|---------|---------|--|
| SCYPHOZOA | | | | | |
| Coronatae | | | | | |
| <i>Linuche unguiculata</i> | present | present | present | present | Ortiz-Corp's et al. 1987 , Montgomery and Kremer 1995 , Silveira and Morandini 1998 |
| <i>Nausithoe aurea</i> | ? | present | present | present | Silveira and Morandini 1997 |
| Rhizostomeae | | | | | |
| <i>Cassiopea</i> spp. | absent | present | present | present | Ludwig 1969 , Hofmann et al. 1996 , Verde and McCloskey 1998 |
| <i>Cephea cephea</i> * | absent | present | present | absent | Sugiura 1969 |
| <i>Cotylorhiza tuberculata</i> | absent | present | present | present | Kikinger 1992 |
| <i>Mastigias papua</i> ** | absent | present | present | present | Sugiura 1963 , Sugiura 1964 , Dawson et al. 2001 |
| <i>Phyllorhiza punctata</i> ** | (absent) | present | present | present | Bolton and Graham 2004 , Schiariti et al. 2014 |

? = unknown. * = species where presence of the symbiont is facultative. ** = species with some populations without zooxanthellae. N/A = not applicable (cubozoans and hydrozoans do not have ephyra)

? = inconnu. * = espèces où la présence du symbionte est facultative. ** = espèces avec certaines populations sans zooxanthelles. N/A = non-applicable (les cubozoaires et les hydrozoaires n'ont pas d'éphyres)

3.2. Nutrition of Zooxanthellate Jellyfishes

3.2.1. Photosynthesis

Zooxanthellate jellyfishes differ from non-zooxanthellate jellyfishes by the additional energy source they can access through the photosynthesis of their zooxanthellae (either through exchange of metabolites but also through digestion of zooxanthellae, see [Davy et al. 2012](#)). In polyps however, only a small part of photosynthates is directed to the host ([Hofmann and Kremer 1981](#)). At the medusae stage, by contrast, photosynthesis can constitute an important, if not the major part, of the nutrition of zooxanthellate medusae. Photosynthetic rates are often equal or superior to respiration rates ([Drew 1972](#), [Cates 1975](#), [Mergner and Svoboda 1977](#), [Kremer et al. 1990](#), [Kikinger 1992](#), [McCloskey et al. 1994](#), [Verde and McCloskey 1998](#), [Welsh et al. 2009](#), [Jantzen et al. 2010](#)). This indicates that in most cases, respiration requirements in carbon may be fulfilled, and even exceeded, by the photosynthetic activity.

When the holobiont's photosynthesis rates exceed respiration rates, the host's metabolites cannot fulfill the photosynthetic demand of zooxanthellae. Thus zooxanthellate jellyfishes must take additional inorganic nutrients (inorganic carbon, nitrogen or phosphorus) from the surrounding water (reviewed in [Pitt et al. 2009](#), see [Hofmann and Kremer 1981](#), [Muscatine and Marian 1982](#), [Wilkerson and Kremer 1992](#), [Pitt et al. 2005](#), [Todd et al. 2006](#), [Welsh et al. 2009](#), [Jantzen et al. 2010](#), [Freeman et al. 2016](#)). Uptake rates of various nutrients can be influenced by some environmental factors. For instance, darkness can induce net nitrogen excretion ([Cates and McLaughlin 1976](#), [Pitt et al. 2005](#), [Welsh et al. 2009](#) but see [Muscatine and Marian 1982](#), [Wilkerson and Kremer 1992](#)), while light has been found to increase ammonium and inorganic carbon uptake ([Jantzen et al. 2010](#), [Freeman et al. 2016](#)). All this indicates that photosynthetically active zooxanthellae play an important role in inorganic nutrient uptake.

Given the nutritional importance of the symbionts, it is not surprising that their hosts present some behavioral and morphological characteristics that help their zooxanthellae (see e.g. [Furla et al. 2011](#) for scleractinian corals). Zooxanthellate jellyfishes, for instance, tend to maximize their light exposure by swimming near the surface (e.g. [Hamner et al. 1982](#), [Larson 1992](#), [Haddad and Nogueira Júnior 2006](#) but see [Bieri 1977](#)), but also by performing more complex horizontal and vertical daily migrations ([Hamner and Hauri 1981](#), [Hamner et al. 1982](#),

Dawson and Hamner 2003). Similarly, zooxanthellae patches found in *Linuche unguiculata* tissue contract with a daily rhythm (Costello and Kremer 1989). One consequence of these behaviors is high exposure to potentially damaging UV radiation. It has thus been hypothesized that some zooxanthellate jellyfishes' pigments might have a photoprotective role (Blanquet and Phelan 1987, Dawson 2005 but see Lampert et al. 2012) as might small behavioral adjustments of depth (Dawson and Hamner 2003). Other behavioral and morphological characteristics of zooxanthellate medusae have been suggested to help their zooxanthellae to access inorganic nutrients. For instance, zooxanthellae within their hosts are found in high concentration near the coronal muscle, which is an important source of excretion products (Blanquet and Riordan 1981, Muscatine et al. 1986, Blanquet and Phelan 1987). The zooxanthellate jellyfish *Mastigias papua* performs reverse diel vertical migrations (Hamner et al. 1982, Tomascik and Mah 1994, Dawson and Hamner 2003) which help it to access deep nutrients at night in stratified environments (Hamner et al. 1982, Muscatine and Marian 1982), possibly imprinting a daily rhythm in the cell division of its symbionts (Wilkerson et al. 1983). And finally, the pumping action of *Cassiopea* facilitates its access to nutrient-rich pore water (Jantzen et al. 2010). Additional access to nitrogen might also be provided by symbiotic nitrogen fixing bacteria (Freeman et al. 2017).

3.2.2. Predation

Zooxanthellate jellyfishes are predators that can eat a variety of prey. Both the polyp and the medusae stages do feed on zooplankton. Information on the polyp diet in the field is scarce, but they are routinely fed zooplankton such as *Artemia* sp. nauplii in the laboratory (e.g. Schiariti et al. 2014). In zooxanthellate medusae, it has been suggested that some of the characteristics that favor their autotrophy might be detrimental to their heterotrophy (Arai 1997). An extreme case might be the modified body shape of the benthic *Cassiopea* sp., which might reduce its contact efficiency with prey as compared to other rhizostomes (see Bezio et al. 2018). For planktonic zooxanthellate jellyfishes, behaviors such as a reversed diel vertical migration might induce spatial mismatch with zooplanktonic prey during the day (Hamner et al. 1982). Zooxanthellate medusae might also have less stinging ability than non-zooxanthellate medusae from other species (Peach and Pitt 2005), or even non-zooxanthellate conspecifics (Bolton and Graham 2004), possibly affecting the amount of captured prey.

However, zooxanthellate medusae have been found to feed on a large variety of prey from large diatoms to fish larvae (see e.g. [Kikinger 1992](#), [Larson 1997](#), [Pérez-Ruzafa et al. 2002](#), [Graham et al. 2003](#), [Kremer 2005](#), [Peach and Pitt 2005](#), [Purcell et al. 2012a, 2015](#), [Zeman et al. 2018](#)). Moreover, the clearance rates of zooxanthellate jellyfishes are often comparable to those of similarly sized non-zooxanthellate jellyfishes ([García and Durbin 1993](#), [Santhanakrishnan et al. 2012](#), [Bezio et al. 2018](#)). Finally, while several studies indicate that photosynthesis can provide most if not all the carbon requirement, predation seems necessary to meet nitrogen and phosphorus requirements ([Kremer 2005](#), [Welsh et al. 2009](#)).

3.3. Variability in the Roles of Zooxanthellae in Jellyfish Symbioses

In previous sections we noted that the abundance and position of zooxanthellae can vary during the jellyfish life-cycle or as a function of the jellyfish species (Table 2). In terms of nutrition, zooxanthellate jellyfishes appear to be generally mixotrophic deriving their nutrition from both photosynthesis and predation. However, variations of the relative importance of autotrophy versus heterotrophy can be identified. This variability can be detected along the ontogeny of jellyfishes but also at the intra- and inter-specific level, particularly at the medusae stage.

3.3.1. Reduced role of zooxanthellae at the polyp stage

At the polyp stage, the zooxanthellae transmit only a small fraction of the photosynthates to the host ([Hofmann and Kremer 1981](#)). Most studies also show that zooxanthellae presence or activity have little impact on polyp's budding or survival ([Sugiura 1963](#), [Hofmann et al. 1978](#), [Rahat and Adar 1980](#), [Prieto et al. 2010](#), but see [Mellas et al. 2014](#)). Moreover, the zooxanthellae *Durusdinium* (previously *Symbiodinium* clade D) can increase the mortality of *Cassiopea* sp. polyps ([Lampert 2016](#)). All this suggests that, at the polyp stage, symbionts and autotrophy are of little direct importance for most zooxanthellate jellyfishes. However, zooxanthellae in polyps can still provide some benefits for the host such as resistance to hypoxia or acidification ([Klein et al. 2017](#)). Such reduced importance of zooxanthellae has also been reported for coral larvae, indicating that importance of the symbiosis can vary through host life-cycle ([Hartmann et al. 2019](#)).

This limited importance of zooxanthellae for polyps is further confirmed by the ecology of zooxanthellate jellyfish polyps in the field. Zooxanthellate jellyfish polyps tend to be found more often on the undersides of surfaces (Kikinger 1992, Fleck and Fitt 1999, see also Astorga et al. 2012) and the settlement of planulae appears favored in the dark (Duarte et al. 2012). These observations indicate that the polyps are generally poorly exposed to sunlight in the field.

3.3.2. *Important, but still unclear, role of zooxanthellae during strobilation*

Several environmental cues control the strobilation in scyphozoans but one of the most important is temperature (Lucas et al. 2012). This is also true of zooxanthellate scyphozoans (Sugiura 1965, Sugiura 1969, Rahat and Adar 1980, Rippingale and Kelly 1995, Prieto et al. 2010, Purcell et al. 2012b). However, zooxanthellae appear also involved in the strobilation process. Indeed, zooxanthellae tend to multiply and accumulate into the forming ephyra during strobilation (Ludwig 1969). Several laboratory studies show that the presence of zooxanthellae helps, and is even often indispensable, for the onset of strobilation (Sugiura 1964, Ludwig 1969, Sugiura 1969, Hofmann et al. 1978, Rahat and Adar 1980, Hofmann and Kremer 1981, Fitt 1984, Colley and Trench 1985, Kikinger 1992; see also Hofmann et al. 1996 and Astorga et al. 2012) though exceptions apparently exist (e.g. Dawson et al. 2001). Interestingly, zooxanthellae still favor strobilation when they are unable to photosynthesize (Sugiura 1969, Hofmann and Kremer 1981, Hofmann et al. 1996) and time to strobilation is independent of zooxanthellae density in *Cassiopea xamachana* (Newkirk et al. 2018). This suggests that the role of zooxanthellae during strobilation is not only to provide photosynthates (Hofmann et al. 1996). However, their exact role is still unclear and warrants further research.

3.3.3. *Variable role of zooxanthellae during the medusa stage*

At the medusa stage there can be important variation in the relative importance of autotrophy and heterotrophy among populations or individuals of the same species. The most extreme case may be *Phyllorhiza punctata* where some populations have zooxanthellae while others do not (Bolton and Graham 2004). Similarly, individuals or populations of *Mastigias papua* can

occasionally be non-zooxanthellate (Dawson et al. 2001). Beyond these extreme cases, it is expected that variations in the autotrophy/heterotrophy balance can vary depending on physiologic (e.g. abundance of zooxanthellae) or environmental (e.g. light exposure, availability of prey) conditions. For instance, populations of *Mastigias papua* appear to differ in the proportion of their energetic demand that can be met by their zooxanthellae (McCloskey et al. 1994). However, few comparative data exist to confirm this point. Individual size can also impact the relative importance of heterotrophy and autotrophy. In some species, the photosynthesis can increase faster than the respiration with medusae size suggesting that larger individuals are more autotrophic than small ones (McCloskey et al. 1994). On the other hand, some other species such as *Cephea cephea* can lose their symbionts at some point in their development (Sugiura 1969) suggesting that smaller individuals rely more on their zooxanthellae than large ones.

Variation in reliance on zooxanthellae can also be seen inter-specifically. Some species appear to be heavily dependent on their zooxanthellae such as *Cassiopea* spp. (e.g. low level of light can induce shrinking; Mortillaro et al. 2009). At the other extreme, *Cephea cephea* (and probably many Cepheidae, Appendix) may lose its symbionts at the medusae stage (Sugiura 1969) and becomes then strictly heterotrophic. The fact that both zooxanthellate and non-zooxanthellate individuals and populations can be found in *Mastigias papua* and *Phyllorhiza punctata* (Dawson et al. 2001, Bolton and Graham 2004) leads us to hypothesize that Kolpophorae are generally ordered Cassiopeidae > Mastigiidae > Cepheidae from the most to the least dependent on zooxanthellae. However, this hypothesized order stays speculative as relevant information exists for only a limited number of species.

From the information compiled here, it is possible to summarize the life-cycle of Kolpophorae, indicating the transmission, and variation of the role of zooxanthellae (Fig. 3). Due to the little information available it is however impossible to propose such a synthetic view for zooxanthellate cubozoans or hydrozoans.

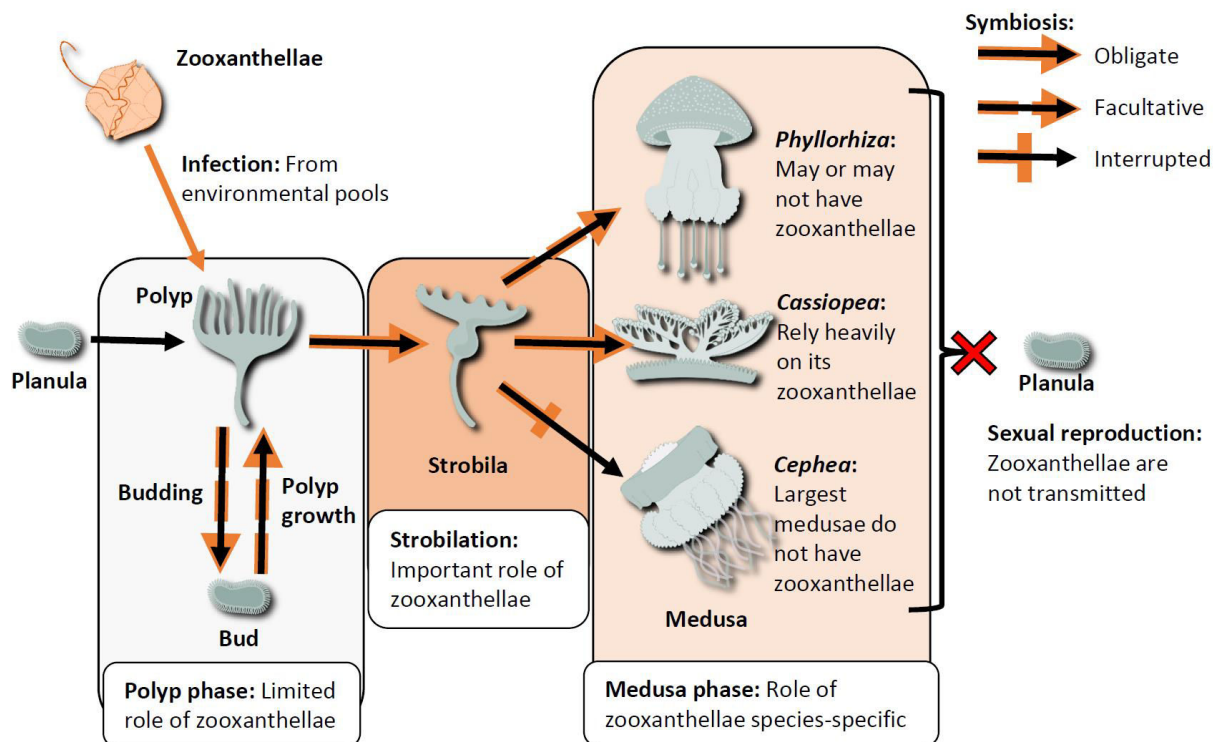


Fig. 3 Synthetic representation of zooxanthellate Kolpophorae (Scyphozoa: Rhizostomeae) life-cycle with presence and importance of zooxanthellae. Black arrows indicate jellyfish life-cycle while orange arrows indicate presence of the symbiont. The infection occurs at the polyp stage. The symbiont is transmitted during asexual reproduction (budding and strobilation) but not during sexual reproduction. Symbionts are generally important for strobilation but not for budding. During the medusae phase, the nutritional importance of the symbiont is species specific (three examples are given)

Fig. 3 Représentation synthétique du cycle de vie des Kolpophorae (Scyphozoa : Rhizostomeae) à zooxanthelles indiquant la présence et l'importance des zooxanthelles. Les flèches noires indiquent le cycle de vie de la méduse tandis que les flèches orange indiquent la présence des symbiontes. L'infection se produit durant la phase polype. Le symbionte est transmis durant la reproduction asexuée (bourgeonnement et strobilation) mais pas durant la reproduction sexuée. Les symbiontes sont généralement importants pour la strobilation mais pas pour le bourgeonnement. Durant la phase méduse, l'importance nutritionnelle des symbiontes est espèce-spécifique (trois exemples sont donnés)

3.4. Zooxanthellate Jellyfishes as a Unique Combination of Traits

Although evolution is typically considered to proceed through the gradual acquisition of small modifications, the evolution of photosymbioses in the jellyfishes combined traits that previously were present only in very distinct lineages. The novel zooxanthellate jellyfishes thus can be compared and contrasted with other groups of cnidarians—non-zooxanthellate jellyfishes, and photosymbiotic scleractinian corals—with which they share newly assorted characteristics. The novel combination brought together (1) the complex life-cycle, the body-plan, and the mobility of the former with (2) the often oligotrophic, shallow-water, photosymbiosis of the latter. We believe that the integration, in zooxanthellate jellyfishes, of these two characteristics became more than the sum of the parts: this unique combination of complex traits originated three major novelties in the ecologies of zooxanthellate jellyfishes.

Large pelagic mixotrophs: The combination of the medusa and photosymbiosis not only conjoined a pelagic life-style with mixotrophy, but additionally did so in relatively large and fast growing, annual, organisms. Other pelagic photosymbioses are found in protists ([Stoecker et al. 2009](#)), but zooxanthellate jellyfishes are orders of magnitudes larger; corals in contrast, are benthic, perennial and slow growing. In this regard, zooxanthellate jellyfishes occupy a unique ecological niche, which, for some (especially Kolpophorae), may have represented a new adaptive zone (sensu [Simpson 1953](#)).

Heterotrophic benthic photosymbioses: In spite of their ability to host zooxanthellae, zooxanthellate jellyfish polyps generally do not rely much on them. They can thus be seen as (mostly) heterotrophic benthic photosymbioses, in stark contrast to the mixotrophic benthic corals. One can speculate on why it is the case. Scyphozoan polyps are generally found on undersides of surfaces (e.g. [Arai 1997](#)) where they are not exposed to light and zooxanthellate jellyfish polyps may simply have retained this trait from their non-zooxanthellate ancestors. Irrespective of the reason, this implies that the polyp would most likely behave more as a non-zooxanthellate jellyfish polyp than as a small scleractinian coral.

A symbiont-dependent strobilation: The strobilation process in zooxanthellate jellyfishes is facilitated or permitted by the symbionts. The underpinning process is still unknown but it is not only due to energetic requirements. This may be due to the fact that the medusae needs the symbionts more than the polyp but cannot acquire them; that the polyp

is not competent to strobilate without zooxanthellae assures that the medusae would have the symbionts. Irrespective of the mechanism, this implies that the transition from the polyp to the medusa is under the control of a supplementary factor as compared with non-zooxanthellate jellyfishes.

These three novel complex traits are likely shared to differing degrees by different zooxanthellate jellyfishes. They are most characteristics of the large zooxanthellate scyphozoans (e.g. *Cassiopea*, *Mastigias*) from which most of the data were gleaned. Exploring the extent to which these conclusions can be applied to other, less studied, zooxanthellate hydrozoan and cubozoan jellyfishes is a key research agenda.

4. Ecology of Zooxanthellate Jellyfishes

The unique combination of traits found in zooxanthellate jellyfishes is expected to shape various aspects of their ecology. Notably, differences in strobilation, nutrition and dependencies on environmental conditions might impact their population dynamics as compared to non-zooxanthellate jellyfishes. Likewise, differences in life-cycle or nutrition might impact the way zooxanthellate jellyfishes react to environmental perturbations as compared with non-zooxanthellate jellyfishes or corals. Finally, their unique combination of traits could lead to unique impacts on marine ecosystems and communities.

4.1. Population Dynamics

4.1.1. Phenology

Across their range of habitats, zooxanthellate jellyfishes can exhibit varied phenology. In some cases, medusae are present year-round ([Hamner and Hauri 1981](#), [Hamner et al. 1982](#), [Fitt and Costley 1998](#)) sometimes with mixed size-classes, suggesting long or continuous recruitment periods. Contrastingly, in other environments, the medusae are present seasonally ([García 1990](#), [Pérez-Ruzafa et al. 2002](#), [Pitt et al. 2004](#), [Purcell et al. 2012b](#)) with generally one cohort identifiable each year (e.g. [Ruiz et al. 2012](#)). On top of these intra-annual population fluctuations, recruitment differences ([Ruiz et al. 2012](#)) or long term environmental variations such as El Niño Southern Oscillation ([Dawson et al. 2001](#), [Martin et al. 2006](#)) can shape

populations sizes over years or decades. Such phenology and population fluctuations are also observed in non-zooxanthellate jellyfishes (e.g. [Dawson and Martin 2001](#), [Purcell et al. 2012b](#), [Condon et al. 2013](#)).

4.1.2. *Blooming ability*

An important aspect of many jellyfish species population dynamics is their tendency to bloom ([Dawson and Hamner 2009](#), [Lucas and Dawson 2014](#)). Blooms are defined as sudden increases of medusa biomass linked with seasonal life-cycle ([Lucas and Dawson 2014](#)). These “true blooms” are a consequence of the coincidence of favorable environmental conditions (e.g. a pulse in prey abundance) with a jellyfish species whose traits enable it to take advantage of the conditions ([Dawson and Hamner 2009](#)). Zooxanthellate jellyfishes, however, are generally unlikely to bloom to the same degree as non-zooxanthellate jellyfishes, suggesting that the photosymbiosis limits their ability to respond to, or benefit from, pulses of prey ([Dawson and Hamner 2009](#)). Due to the correlated nature of character complexes—e.g. zooxanthellate scyphozoans are also predominantly monodisc strobilators whereas bloom forming, non-zooxanthellate scyphozoans are predominantly polydisc strobilators—it is challenging to identify the cause of this low blooming ability. Nonetheless, we believe that it would be valuable to formulate testable hypotheses to give a foundation for future research. [Dawson and Hamner \(2009\)](#) hypothesized several possible causes for the low blooming ability of zooxanthellate jellyfishes, as follows.

One hypothesis is that the mixotrophy of zooxanthellate jellyfishes implies trade-offs that physiologically limit their ability to take advantage of pulses of prey. For instance, several behavioral characteristics of zooxanthellate jellyfishes may limit their predation efficiency (see section 3.2.2.). The strobilation of many zooxanthellate jellyfishes is regulated by zooxanthellae (see section 3.3.2.) potentially decorrelating strobilation period from pulse of prey. Moreover, evidence from zooxanthellae-coral symbioses suggests that zooxanthellae can at times have negative effects on their hosts ([Lesser et al. 2013](#), [Hartmann et al. 2019](#)). The existence of such trade-offs might also explain why many Cepheidae apparently tend to lose their symbionts at the medusa stage ([Sugiura 1969](#), Appendix). These trade-offs are,

however, little known in zooxanthellate jellyfishes and more studies are needed to characterize them.

The other hypotheses relate to the ecological availability of resources and how zooxanthellate jellyfishes can use them. [Dawson and Hamner \(2009\)](#) proposed two mechanisms: (1) zooxanthellae may access a more stable resource stream as compared to prey abundances (Fig. 4a); (2) zooxanthellate jellyfishes may be able to switch from one resource to another (see section 3.3.3., Fig. 4b). In both cases, the generalism of zooxanthellate jellyfishes allow them to achieve a temporally smoother energetic income (as opposed to non-zooxanthellate jellyfishes which, in this context, may be seen as specialists). This would in turn result in smoother population dynamics and thus less abrupt population increase, i.e. no unusual blooms. Along with these two hypotheses from [Dawson and Hamner \(2009\)](#) we propose a third one: that some zooxanthellate jellyfishes might need both autotrophy and heterotrophy (e.g. [Kremer et al. 2005](#), [Welsh et al. 2009](#)). Thus to bloom, zooxanthellate jellyfishes would need both resources concomitantly in abundance. In the context of the match-mismatch hypothesis developed for fisheries (see e.g. [Cushing 1990](#)) the success of a given cohort is function of the timing between the spawning and a peak in available prey. This can be transposed directly to non-zooxanthellate jellyfishes ([Dawson and Hamner 2009](#)) where the strobilation corresponds to the spawning event. Zooxanthellate jellyfishes, however, under the hypothesis that they need both autotrophic and heterotrophic resources to bloom, would be dependent on appropriate timing of not one, but two matching peaks of resources. Thus, because they rely on more diverse resources, zooxanthellate jellyfishes would be more likely to encounter a mismatch than non-zooxanthellate jellyfishes (Fig. 4c). Elevated possibility of mismatch for zooxanthellate jellyfishes might also arise as a consequence of the additional control of zooxanthellae on strobilation as compared to non-zooxanthellate jellyfishes (e.g. temperature cue for strobilation and zooxanthellae presence might not always match, see section 3.3.2.) or changing seasonality and phenology in the oceans (e.g. [Mackas et al. 2012](#)). This higher chance of getting a mismatch would reduce zooxanthellate jellyfishes' likeliness to bloom.

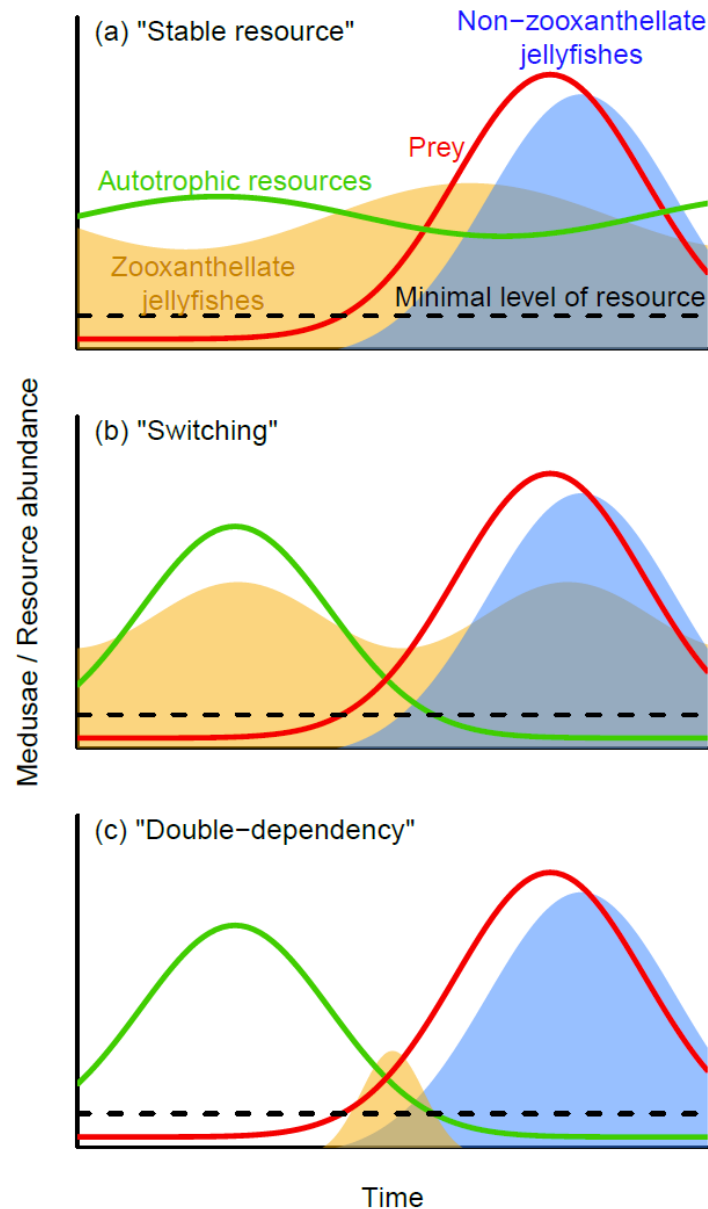


Fig. 4 Three hypotheses on zooxanthellate jellyfishes' reduced blooming ability as compared to non-zooxanthellate jellyfishes. Zooxanthellate jellyfishes (orange) are able to exploit both autotrophic resources (light, dissolved inorganic nutrients; green) and prey (red) whereas non-zooxanthellate jellyfishes (blue) can only exploit prey. Black dashed line indicates the minimum level of any resource to support a medusae population. (a) Hypothesis 1: Zooxanthellae provide access to a more stable resource (the autotrophic resources), smoothing population dynamics. (b) Hypothesis 2: Zooxanthellae allow a diversification of resources, when one is insufficient, the other might compensate; again smoothing population dynamics. (c) Hypothesis 3: Zooxanthellate jellyfishes need both resource streams which exposes them to higher chances of mismatches reducing their likeliness to bloom. Schematics are illustrative only, and provided in simplified form to emphasize key circumstances that may shape jellyfish population responses

Fig. 4 ← Trois hypothèses sur la tendance à bloomer réduite chez les méduses à zooxanthelles compare aux méduses sans zooxanthelles. Les méduses à zooxanthelles (orange) sont capables d'exploiter à la fois des ressources pour l'hétérotrophie (lumière, nutriments inorganiques dissous ; vert) et les proies (rouge) tandis que les méduses sans zooxanthelles (bleu) ne peuvent exploiter que les proies. La ligne pointillée noire indique le niveau minimum de ressources pour supporter une population de méduse. (a) Hypothèse 1 : Les zooxanthelles permettent l'accès à une ressource plus stable (les ressources de l'hétérotrophie), stabilisant ainsi les dynamiques de population. (b) Hypothèse 2 : Les zooxanthelles permettent une diversification des ressources, quand l'une est insuffisante, l'autre peut compenser ; à nouveau cela stabilise les dynamiques de population. (c) Hypothèse 3 : Les méduses à zooxanthelles ont besoin des deux ressources en même temps ce qui les expose à de plus grandes chances de « mismatch » et réduit leur susceptibilité à bloomer. Ces schémas simples ne sont donnés que dans un but illustratif pour illustrer des circonstances contrastées qui pourraient influencer les réponses des populations de méduses

These different hypotheses might not be mutually exclusive. For instance, resource availability might vary across ecosystems or from year to year. The hypothesis in which zooxanthellate jellyfishes are able to switch resources (Fig. 4b) might represent a species that can tolerate a lot of variation in its nutrition (e.g. *Phyllorhiza punctata* - Bolton and Graham 2004). By opposition, the hypothesis in which zooxanthellate jellyfishes need both resources (Fig. 4c) would represent a species that cannot tolerate much variation in its nutrition. Thus, rather than being opposed these hypotheses might represent extremes of a continuum. It is also important to note that some species might not be within the scope of these hypotheses. For instance, *Cephea* (and possibly other Cepheidae; see Appendix) loses its zooxanthellae at the medusa stage (Table 2), and might thus bloom (e.g. Cruz-Rivera and El-Regal 2015) as many other, non-zooxanthellate, jellyfishes. Similarly, *Phyllorhiza punctata* has invasive, non-zooxanthellate, populations that have been reported to bloom (Graham et al. 2003, Verity et al. 2011). Disentangling the conditions, species and environments in which a given hypothesis might best describe population dynamics and blooming ability is an important direction for future research on zooxanthellate jellyfishes.

4.2. Reaction to Environmental Perturbations

4.2.1. Temperature driven bleaching

As scleractinian corals, zooxanthellate jellyfish also can bleach (expel zooxanthellae) in response to a heat stress (Dawson et al. 2001, McGill and Pomoroy 2008, Newkirk et al. 2018, Klein et al. 2019). Few jellyfish bleaching event have been documented in the field; it is unclear whether such events are rarer, or simply less reported than in corals, but this would be an important question to resolve. The first report was of *Mastigias papua* from Clear Lake, a marine lake in Palau (Dawson et al. 2001). Subsequent experimental manipulations also elicited bleaching in conspecific polyps from nearby lakes at temperatures higher than 31.5 °C, providing a possible explanation for the subsequent *Mastigias papua* population collapse in the adjacent Ongeim'l Tketau marine lake during the extreme El Niño-La Niña oscillation of 1997-1999. Interestingly, the co-occurring non-zooxanthellate *Aurelia* sp. population did not experience such a collapse (Dawson et al. 2001). Another jellyfish bleaching event—of *Cassiopea* sp. medusae in the Red Sea, Saudi Arabia—occurred during a heat wave, after three consecutive days of atmospheric temperatures > 44 °C. Mass mortality of the medusae occurred five to seven days after the bleaching event (Klein et al. 2019).

Recovery of a medusa population following a bleaching-induced collapse depends on replenishment from polyps and raises an intriguing scenario. As polyps do not rely on zooxanthellae to sustain their populations (section 3.3.1.) it can be expected that the requisite polyp populations will not have been depleted even if they bleached. However, because strobilation usually depends on zooxanthellae (section 3.3.2.), even a large population of bleached polyps may not be able to replenish the medusae population. In such cases, the recovery of the medusa population would depend on the reacquisition of zooxanthellae by the polyps (excepting the strobilation of aposymbiotic medusae, see Dawson et al. 2001).

However, as some zooxanthellate jellyfish species can sometimes realize their life-cycle without zooxanthellae (Table 2), bleaching may not always result in a population collapse. This is confirmed by observation of populations of apparently bleached, heterotrophic, mixed size-classes (from ca. 1 to 20 cm bell diameter) of *Mastigias papua* in Clear Lake, in Palau (Djeghri, Dawson, unpublished data). Moreover, remaining zooxanthellae in artificially bleached *Cassiopea* medusae are able to recolonize their host (Estes et al. 2003). Thus zooxanthellate

jellyfishes might be able to survive a bleaching event either by the recovery of their zooxanthellae or by switching their nutrition towards predation.

4.2.2. Eutrophication

Some zooxanthellate jellyfish populations have been reported to increase after eutrophication events (García 1990, Arai 2001, Pérez-Ruzafa et al. 2002) or to have higher abundances in human-impacted sites, possibly due to higher nutrient concentrations (Stoner et al. 2011). This contrasts with what is seen in other photosymbiotic animals such as scleractinian corals (Fabricius et al. 2005, Lapointe et al. 2019) but is consistent with the tendency of some jellyfish species to be favored by eutrophication (Arai 2001, Purcell 2012). This apparent contradiction can be resolved if we consider that, generally speaking, zooxanthellate jellyfishes are less likely than benthic photosymbiotic organisms (such as corals) to suffer from the usual negative effects of eutrophication. For example, zooxanthellate medusae are not subject to competition with macroalgae. They can also compensate for turbidity by adjusting their depth either by swimming in planktonic species (Dawson and Hamner 2003), or by settling in shallower waters in the case of the benthic *Cassiopea*. Water turbidity could affect benthic polyps but, as discussed in previous sections, they do not rely much on their zooxanthellae and therefore, do not need much light. Moreover, scyphozoan polyps tend to be resistant to the conditions associated with eutrophication (Purcell 2012, see also Klein et al. 2017). Eutrophic ecosystems could even present advantages for zooxanthellate jellyfishes as they are characterized by a greater availability of nutrients, which can promote the growth of zooxanthellae in zooxanthellate jellyfishes (see e.g. Freeman et al. 2017) and may, in normal circumstances, be limiting (see section 3.2.).

4.3. Impacts on Ecosystems

In some ecosystems, zooxanthellate jellyfish populations reach densities up to tens or hundreds of medusae per 1000 m⁻³ (e.g. Hamner et al. 1982, García 1990, Pérez-Ruzafa et al. 2002, Gueroun et al. 2014, Cimino et al. 2018). Such densities likely strongly impact these ecosystems in various ways. Some of these impacts can be very similar to those induced by non-zooxanthellate jellyfishes such as high predation pressure on zooplankton (e.g. García and

Durbin 1993). However, due to their particular traits, zooxanthellate jellyfishes could impact ecosystems in ways that differ from what is known from their non-zooxanthellate counterparts.

4.3.1. Primary productivity

Individual zooxanthellate jellyfishes can reach primary productivity levels comparable to those of scleractinian corals (Kremer et al. 1990). Therefore, when abundant, zooxanthellate jellyfishes can represent an important fraction of the primary productivity of the ecosystem they inhabit. For instance, *Mastigias papua* medusae can contribute 16 % of the primary productivity in the marine lake Ongeim l'Tketau in Palau (McCloskey et al. 1994). However, this high contribution may approach the upper bound of primary production contributions by zooxanthellate jellyfishes: the population density of *Mastigias papua* medusae in Ongeim l'Tketau can be very high (on average ca. 1000 medusae per 1000 m⁻³, Hamner et al. 1982, Cimino et al. 2018). In other ecosystems, even when zooxanthellate jellyfishes are numerous they rarely attain densities as high (maximum densities of ca. 100 medusae per 1000 m⁻³ e.g. García 1990, Pérez-Ruzafa et al. 2002). Moreover, whereas jellyfish productivity can be directed towards higher trophic levels (Hays et al. 2018), when their populations attain high densities, most of their production may be instead directed towards microbial respiration (Condon et al. 2011) or exported (e.g. Billett et al. 2006). Hence, it is unlikely that zooxanthellate jellyfishes would contribute as much as scleractinian corals to productivity in their ecosystems.

4.3.2. Nutrient cycling

Zooxanthellate jellyfishes can affect nutrient cycling in the ecosystems they inhabit. One example already mentioned is the pumping action of the benthic medusae *Cassiopea* spp. which releases nutrient-rich pore water for its zooxanthellae but also for the pelagic community (Jantzen et al. 2010). However, this positive impact of zooxanthellate jellyfishes on pelagic nutrient fluxes should probably be considered as an exception. Pitt et al. (2009) suggested that the recycling and uptake of nutrients by zooxanthellate jellyfishes implies that

they act more as sinks for nutrients than as sources (unlike non-zooxanthellate jellyfishes). This, in turn, can impact planktonic communities.

4.3.3. Planktonic communities

Pitt et al. (2009) suggested that if zooxanthellate jellyfishes act as sinks for dissolved inorganic nutrients, then this implies fewer nutrients are available for phytoplankton growth. Moreover, some characteristics of zooxanthellate jellyfishes might reduce their predation efficiency (Dawson and Hamner 2009, see section 3.2.2.). Thus Pitt et al. (2009) concluded that the presence of zooxanthellate jellyfish would reduce phytoplankton population densities by two means. First, a bottom-up effect where the zooxanthellate jellyfishes make nutrients unavailable to phytoplankton. Second, a top-down effect where predation on zooplankton by zooxanthellate jellyfishes is relatively low (as compared with non-zooxanthellate jellyfishes); zooplankton populations would thus be larger and predation pressure on phytoplankton would increase. This hypothesis has been successfully tested in mesocosm experiments (West et al. 2009).

It is however important to stress that the impacts discussed here concern mainly quite specific ecosystems (i.e. lagoons) that are characterized by important densities of jellyfishes and that are more or less enclosed. In more open coastal areas, or in the open ocean, only little is known of zooxanthellate jellyfishes' population fluctuations and potential impacts on community dynamics and ecosystem functioning.

5. Summary and Knowledge Gaps

Zooxanthellate jellyfishes can be found in lineages across the medusozoan phylogeny (Fig. 1). Most of the zooxanthellate jellyfish species are isolated in mostly non-zooxanthellate clades with the notable exception of Kolpophorae (Rhizostomeae: Scyphozoa). Zooxanthellate jellyfishes' reliance on their symbionts can vary across species, populations and ontogeny (Fig. 3, Table 2). Three key traits can be identified: (1) As holobionts, the medusae are generally mixotrophic, although many variations can be observed; (2) in contrast, the polyps, although

being able to host zooxanthellae, do not rely much on zooxanthellae for survival, growth and budding; and (3) zooxanthellae play a key role during strobilation. Due to these traits, zooxanthellate jellyfishes may have different ecologies when compared to non-zooxanthellate jellyfishes (e.g. different blooming ability, Fig. 4) or to other photosymbiotic cnidarians such as scleractinian corals (e.g. reaction to eutrophication).

However, there are still substantial gaps in our current understanding of zooxanthellate jellyfish ecology. Hydrozoan and cubozoan zooxanthellate jellyfishes are, in most cases, only described to occur, with no in-depth study of their ecology. Basic information on the life-cycle and nutrition is still lacking for most species. Most of our knowledge comes from large, coastal, scyphozoan species which may not be representative of other groups or of other environments such as the open ocean. The best studied genus is *Cassiopea* since it has been used as a model organism for the study of cnidarian-zooxanthellae symbiosis (see recent review; [Ohdera et al. 2018](#)). However, due to the benthic life-style of its medusae, this genus may not be the best model for understanding pelagic zooxanthellate jellyfish ecology. Our understanding of zooxanthellate jellyfish diversity and ecology would benefit from more systematic assessment—e.g. using microscope study or molecular methods—of the presence of zooxanthellae in a wide range of medusozoans. Albeit generally mixotrophic, zooxanthellate medusae display a great variability in their nutrition, which also needs to be characterized better given its implications for the ecologies of these species. Traditional measures of predation, photosynthesis, respiration and excretion (e.g. [Kremer 2005](#)) would be beneficial though time consuming. More recent methods, involving trophic markers such as stable isotopes or fatty acids have been applied successfully to other photosymbiotic animals such as corals (see e.g. [Ferrier-Pagès and Leal 2018](#), [Mies et al. 2018](#)) and would usefully be applied to zooxanthellate jellyfishes (e.g. [Mortillaro et al. 2009](#), [Freeman et al. 2017](#), [Zeman et al. 2018](#)). The last, but possibly major caveat with the study of zooxanthellate jellyfishes may be due to the way we divide our scientific fields. Zooxanthellate jellyfishes are somewhat caught between jellyfish focused researchers and coral focused researchers. To gain insight of zooxanthellate jellyfishes' biology and ecology, we need to bring together ideas from these two communities.

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Appendix: *Photographic evidence of zooxanthellae presence or absence in less studied Kolpophorae medusae*

Method

In the absence of samples that could be tested using a suite of modern techniques (see main text) possible presence of zooxanthellae was assessed using photographs of the less studied genera (or species) of Kolpophorae. Photographs were searched for in scientific publications (as priority) and online. These genera and species belong to the families Cepheidae, Thysanostomatidae and Versurigidae. Brown coloration was considered as potential evidence for the presence of zooxanthellae. Absence of brown coloration was considered as evidence for few or no zooxanthellae.

The genera and species investigated for photographs were the following:

Cepheidae: *Cephea* spp., *Cotylorhiza erythraea*, *Marivagia stellata*, *Netrostoma* spp.

Thysanostomatidae: *Thysanostoma* spp.

Versurigidae: *Versuriga* spp.

Importantly, this method does not allow us to conclude that a species is or is not zooxanthellate as photos generally are of insufficient resolution to see zooxanthellate and, also, zooxanthellae may still be present in the polyp.

Results

Cepheidae:

Cephea spp.: The photographs show generally very clear medusae, with possibly a few zooxanthellae in the tip of oral arms (Cruz-Riveira and El-Regal 2015, Gul et al. 2015a, 2015b). Zooxanthellae are however present in the polyp and ephyra (Sugiura 1969). This supports the conclusion of Sugiura (1969) stating that *Cephea cephea* lose most of its symbionts during the medusa phase.

See also:

http://thescyphozoan.ucmerced.edu/Syst/Rhi/C_cephea_i.html

Cotylorhiza eythraea: Photographs of both clear and brownish individuals found (Galil et al. 2016) suggesting that this species is a facultative symbiont at the medusa stage. Zooxanthellae apparently present in oral arms.

Marivagia stellata: The photographs found show only very clear individuals (Galil et al. 2010) suggesting that this species is not zooxanthellate at the medusa stage (zooxanthellae may still be present in polyps and ephyra, see *Cephea* spp. and *Netrostoma* spp.).

Netrostoma spp.: The photographs found show very clear individuals, with possibly a few zooxanthellae in the tip of oral arms (Gul et al. 2015a). Zooxanthellae present in polyp and ephyra (Straehler-Pohl and Jarms 2010). This suggests that this genus loses most of its symbionts at the medusae stage, as does its close relative *Cephea* spp.

Thysanostomatidae:

Thysanostoma spp.: No color photograph found in scientific papers. Online photographs suggest the presence of zooxanthellae in oral arms and coronal muscle:

https://fr.wikipedia.org/wiki/Thysanostoma#/media/File:Thysanostoma_loriferum_Maldives.JPG

[http://doris.ffesmm.fr/Especies/Thysanostoma-cf.-loriferum-Thysanostome-bleue-4883/\(rOffset\)/0](http://doris.ffesmm.fr/Especies/Thysanostoma-cf.-loriferum-Thysanostome-bleue-4883/(rOffset)/0)

[http://doris.ffesmm.fr/Especies/Thysanostoma-thysanura-Thysanostome-rouge-3539/\(rOffset\)/1](http://doris.ffesmm.fr/Especies/Thysanostoma-thysanura-Thysanostome-rouge-3539/(rOffset)/1)

http://thescyphozoan.ucmerced.edu/Syst/Rhi/T_Thysanura_i.html

Versurigidae:

Versuriga spp.: Photographs of brown individuals (Sun et al. 2018) suggest this genus is zooxanthellate. Zooxanthellae apparently present in oral arms and along the coronal

muscle. Another photograph shows a pale individual, suggesting that the genus may be a facultative symbiont:

http://thescyphozoan.ucmerced.edu/Syst/Rhi/V_anadyomene_i.html

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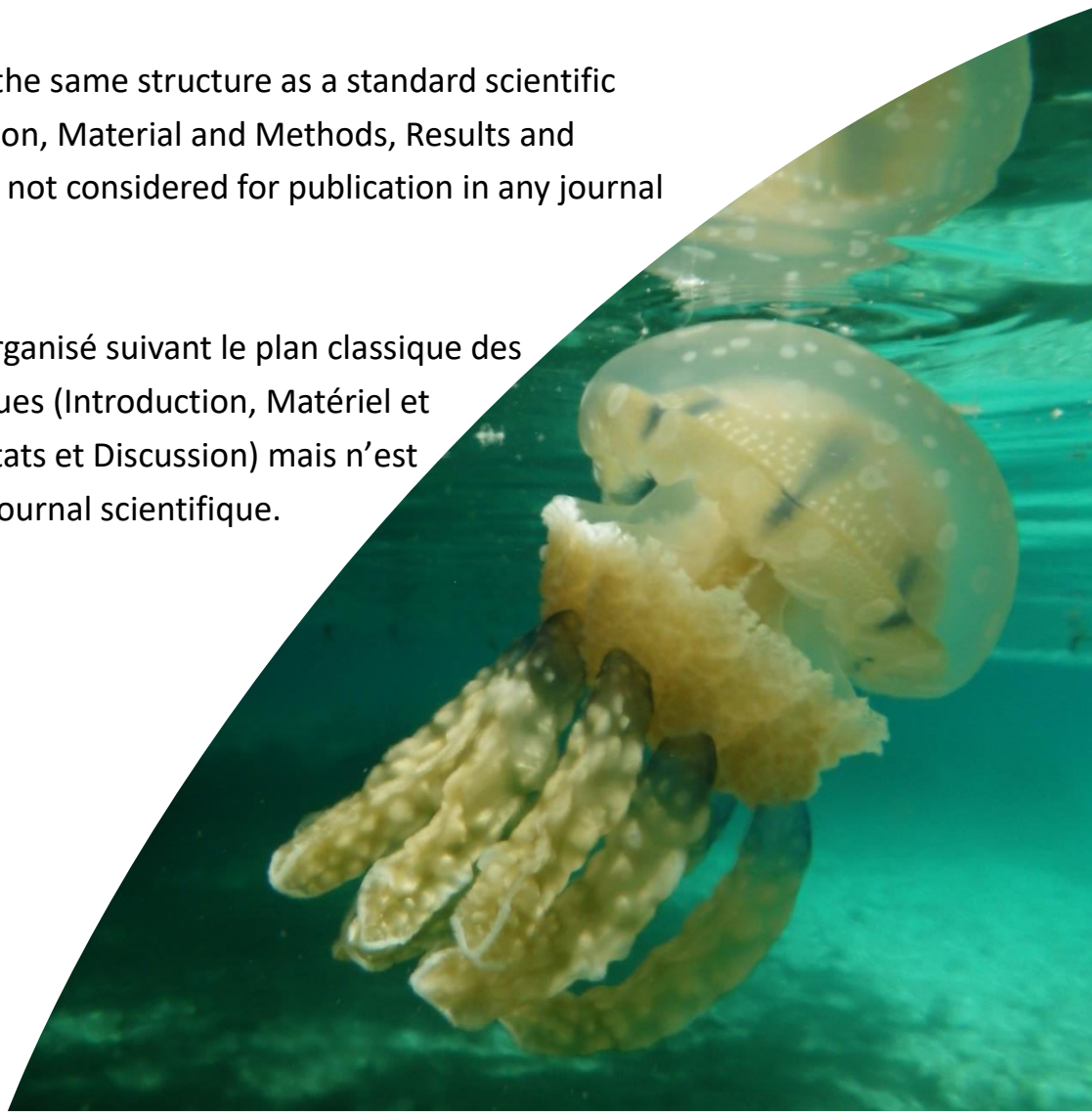
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Chapter II: Influence of resource availability on zooxanthellate and non-zooxanthellate scyphozoan polyps: Similar budding and survival responses of *Cassiopea* sp. and *Aurelia* sp.

Chapitre II : Influence de la disponibilité des ressources sur des polypes avec et sans zooxanthelles : Réponses similaires du bourgeonnement et de la survie de *Cassiopea* sp. et d'*Aurelia* sp.

This chapter has the same structure as a standard scientific article (Introduction, Material and Methods, Results and Discussion) but is not considered for publication in any journal at present.

Ce chapitre est organisé suivant le plan classique des articles scientifiques (Introduction, Matériel et Méthodes, Résultats et Discussion) mais n'est pas soumis à un journal scientifique.



Abstract

Most scyphozoan polyps are strict heterotrophs, whereas some others, hosting zooxanthellae in their tissues, are both autotroph and heterotroph. Zooxanthellate scyphozoan polyps can thus exploit light or dissolved inorganic nutrients which are unavailable to non-zooxanthellate scyphozoan polyps. However, evidence from previous studies suggest that autotrophy contributes little to zooxanthellate scyphozoan polyps' nutrition. I therefore hypothesized that zooxanthellate and non-zooxanthellate scyphozoan polyps' responses to resource availability might be very similar. To test this hypothesis, I compared the budding responses of the zooxanthellate *Cassiopea* sp. and the non-zooxanthellate *Aurelia* sp. in the presence or absence of added nutrients, light and prey. The presence of prey increased the budding whereas light and added nutrients had no significant effect in both species. The survival time of starved polyps was decreased in the treatments with light in both species. Furthermore, zooxanthellae growth was observed in a strobilating polyp incubated in the dark suggesting that heterotrophy of zooxanthellae occurs during zooxanthellate scyphozoans strobilation. The similarity noted between *Cassiopea* sp. and *Aurelia* sp. in term of budding and survival is strong evidence for their equivalent responses to resources availability. I thus suggest that the state zooxanthellate or non-zooxanthellate might have little impact on scyphozoan polyp population dynamics.

Résumé

La plupart des polypes des scyphozoaires sont des hétérotrophes stricts tandis que d'autres abritent des zooxanthelles et sont donc à la fois autotrophes et hétérotrophes. Les polypes des scyphozoaires à zooxanthelles peuvent donc exploiter la lumière ou les nutriments inorganiques dissous qui sont inaccessibles aux polypes de scyphozoaires sans zooxanthelles. Toutefois, des études précédentes suggèrent que l'autotrophie contribue peu à la nutrition des polypes de scyphozoaires à zooxanthelles. Je fais donc l'hypothèse que les polypes de scyphozoaires avec et sans zooxanthelles devraient répondre de façon similaire à la disponibilité des ressources. Pour tester cette hypothèse, j'ai comparé la reproduction asexuée de polypes de *Cassiopea* sp. (avec zooxanthelles) et de *Aurelia* sp. (sans zooxanthelles) en présence ou absence de nutriments supplémentaires, lumière et proies. La présence des proies a augmenté la reproduction asexuée des deux espèces tandis que la lumière et les nutriments supplémentaires n'ont pas eu d'effet. Le temps de survie des polypes non-nourris a été diminué, chez les deux espèces, par la présence de lumière. De plus, la croissance des zooxanthelles dans une strobila au noir suggère que les zooxanthelles peuvent se développer de façon hétérotrophe dans leurs hôtes. Les similitudes notées entre *Cassiopea* sp. et *Aurelia* sp. en termes de reproduction asexuée et de survie est une forte indication de l'équivalence de leurs réponse à la disponibilité des ressources. Je suggère donc que l'état avec, ou sans, zooxanthelles pourrait n'avoir que peu d'impact sur les dynamiques de population des polypes de scyphozoaires.

1. Introduction

Population fluctuations and blooms of jellyfishes have recently gained increasing interest. Jellyfish population dynamics have a complex determinism resulting from the interaction of the species' biology with multiple, local to global, environmental factors ([Purcell 2012](#), [Lucas and Dawson 2014](#)). The metagenetic life cycle is a central trait of many jellyfishes, particularly scyphozoans. It is characterized by an alternation of a benthic, asexually reproducing, polyp stage and a pelagic, sexually reproducing, medusae stage ([Arai 1997](#)). The polyp stage populations play an important role in sustaining the pelagic medusae populations ([Lucas et al. 2012](#)). Therefore, understanding the factors affecting the polyp populations is key for a mechanistic understanding of jellyfishes' population dynamics.

Most scyphozoans polyps are strict heterotrophs, which make their growth, asexual reproduction, survival and subsequent population dynamics dependent on the availability of suitable prey ([Lucas et al. 2012](#), [Schiariti et al. 2014](#)). Some scyphozoans undergo a mutualistic symbiosis with photosymbiotic dinoflagellates from the family Symbiodiniaceae (called zooxanthellae) hosted in their tissues (e.g. [Lajeunesse 2001](#), see also the recent review of the family: [Lajeunesse et al. 2018](#)). The process is similar to that known in corals; zooxanthellae recycle their hosts' excretion products while providing them with photosynthates ([Davy et al. 2012](#)). Via zooxanthellae's photosynthesis, zooxanthellate polyps are able to exploit light and inorganic dissolved nutrients as an additional nutrition resource ([Hofmann and Kremer 1981](#)). Based on their ability to exploit different resources one might hypothesize that zooxanthellate and non-zooxanthellate polyps respond differently to resource availability inducing different population dynamics and ecological impacts.

At the medusae stage, many zooxanthellate scyphozoans are highly dependent on their zooxanthellae for nutrition and energy budgets ([Kremer et al. 1990](#), [Verde and McCloskey 1998](#), [Mortillaro et al. 2009](#)). This can lead to differences between zooxanthellate and non-zooxanthellate medusae in terms of their impact on planktonic communities and nutrient cycling ([Pitt et al. 2009](#), [West et al. 2009](#)).

At the polyp stage however, previous studies suggest that the zooxanthellae's role in nutrition minor. Indeed, the acquisition of symbionts in most zooxanthellate scyphozoan occurs during the polyp stage (e.g. [Sugiura 1963](#), [Kikinger 1992](#)); thus the metamorphosis from planula to

polyp occurs without the help of zooxanthellae. Moreover, previous studies have shown that presence or activity of zooxanthellae have little or no effect on asexual polyp production (hereafter referred to as budding) (Hofmann et al. 1978, Rahat and Adar 1980, Prieto et al. 2010). Using Carbon-14 labeling, Hofmann and Kremer (1981) have estimated that 5 to 10 % of net photosynthates are translocated to the host in *Cassiopea andromeda* polyps. Among zooxanthellate cnidarians, this is at the low end of translocation rates obtained by this method (Davy et al. 2012). Finally, survival time of the zooxanthellate *Cotylorhiza tuberculata* polyps was not affected by light or nutrients (Prieto et al. 2010). All this suggest that during the polyp stage of scyphozoans, zooxanthellae are of little importance for nutrition.

I therefore tested experimentally whether, zooxanthellate and non-zooxanthellate scyphozoan polyps differ in their response to available resources. To date, no such comparison of zooxanthellate and non-zooxanthellate scyphozoan polyps' responses to resources availability have been performed. I used the zooxanthellate *Cassiopea* sp. and the non-zooxanthellate *Aurelia* sp. in a full-factorial experiment assessing the effect of presence or absence of prey, light or added nutrients on the budding. I also assessed the survival time of starved polyps. I acknowledge that *Cassiopea* sp. and *Aurelia* sp. differ in many regards. Importantly, they have different asexual reproduction modes as *Cassiopea* sp. rely almost only on planuloid buds (Schiariti et al. 2014, Heins et al. 2015) while *Aurelia* sp. displays a variety of asexual reproduction modes such stolons, lateral budding and podocysts (Schiariti et al. 2014). They also differ in morphology and physiological traits such as thermic optimums. Taking into account these differences, my goal is not to perform quantitative comparisons but rather to compare the qualitative budding response of the two species. The rationale here is that if autotrophy is important to *Cassiopea* sp. polyps, then light and dissolved organic nutrients could favor its budding or survival while having no effects on the strict heterotroph *Aurelia* sp.

2. Materials and Methods

2.1. Experimental Setup

Polyps of the zooxanthellate *Cassiopea* sp. and the non-zooxanthellate *Aurelia* sp. were provided by Océanopolis (Brest Aquarium, France). *Cassiopea* sp. in Océanopolis were found

in the wall of a tropical aquarium not directly exposed to light and at a temperature of 25 °C. *Aurelia* sp. polyps come from Océanopolis' culture where they were maintained at 23-24 °C in the dark and fed daily with newly hatched *Artemia* sp. nauplii. The experimental polyps were gently scraped from their support using a razor blade. The polyps were inserted individually in separate culture wells filled with 10 mL of filtered (1 µm mesh size) sea water at a temperature of 23-24 °C. A total of 96 wells were prepared (48 with *Cassiopea* sp. and 48 with *Aurelia* sp.). The wells were covered to avoid evaporation and stored in two incubators (®Lucky Reptile Herp Nursery II) at 23-24 °C in the dark. The polyps were then allowed to acclimate and reattach for one week.

After one week, the polyps were subjected to different conditions that combined presence or absence of light, prey or added dissolved inorganic nutrients (Table 1). For each treatment, six replicates were realized. Here it should be noted that some polyps of *Aurelia* sp. died during or soon after (< one week) the acclimatization week. The early deaths were considered to be a result difficulties encountered during the acclimatization process and were thus excluded from the final results. The early polyp mortality reduced the number of replicates to five or four in some treatments (Table 1).

Table 1 Experimental setup. The incubators were regulated at a temperature of 23-24 °C. + and - indicate respectively presence and absence of an experimental factor

Table 1 Plan expérimental. Les incubateurs étaient régulés à 23-24 °C. + et - indiquent respectivement la présence et l'absence d'un facteur expérimental

| | | Incubator 1 | | | | Incubator 2 | | | |
|----------------------|----------------------|-------------|---|---|---|-------------|---|---|---|
| Experimental factors | Light | + | | | | - | | | |
| | Prey | + | | - | | + | | - | |
| | Nutrients | + | - | + | - | + | - | + | - |
| Number of replicates | <i>Cassiopea</i> sp. | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | <i>Aurelia</i> sp. | 4 | 4 | 5 | 6 | 4 | 4 | 4 | 5 |

In the first incubator, the light was provided by a blue-enriched fluorescent lamp (*ca.* 60 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ of PAR measured with a hyperspectral radiometer; RAMSES SAM ACC VIS, ®TriOS, Rastede, Germany) on a 12:12 h light:dark cycle while the second was covered with aluminum foil to provide full darkness. This level of light, although low, is a good representation of field conditions encountered by scyphozoan polyps (see [Fleck and Fitt 1999](#), [Purcell 2007](#)) and is sufficient for zooxanthellae growth ([Iglesias-Prieto and Trench 1994](#)). The used prey consisted of young (< 48 h after hatching) *Artemia* sp. nauplii fed *ad libitum* for one hour three times a week. The nutrients were added by spiking natural filtered sea water (1 μm mesh size) with 10 $\mu\text{mol.L}^{-1}$ of phosphate (NaH_2PO_4) and 100 $\mu\text{mol.L}^{-1}$ of nitrate (NaNO_3) in final concentration. Water was changed three times a week, after each feeding, by pipetting out used sea-water and pipetting in 10 mL of new filtered sea-water at the correct temperature. This protocol discarded uneaten prey and unattached planuloid buds.

Preceding each feeding and water change, polyps, released planuloid buds, and ephyrae were counted under a dissecting microscope. The temperature in the incubators was monitored at each water change and varied little during the experiment (23.7 ± 0.6 °C in the lit incubator and 23.6 ± 0.6 °C in the darkened incubator; mean \pm s.d.).

All treatments were monitored as described and kept for 55 days. After this period, starved treatments were kept and monitored using the same protocol until the death of all polyps to assess their survival to starvation time.

Photosynthetic activity of the zooxanthellae in five of the released *Cassiopea* sp. ephyrae was assessed by measuring relative Electron Transport Rates (rETR) of the entire ephyra exposed to a gradient of light (*i.e.* Rapid Light-response Curves, *RLC*). They were measured using the “LC3” protocol of an AquaPen-C AP-C100 (®Photon Systems Instruments, PSI, Brno, Czech Republic) with a 450 nm excitation wavelength. This protocol estimates the quantum yield (a proxy for the photochemical yield of photosystem II, Fv'/Fm') at different light intensities ($I = 10, 20, 50, 100, 300, 500$ and $1000 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$). $\text{Fv}'/\text{Fm}' < 0.1$ (usually recorded for $I = 1000 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$) were considered unreliable and were excluded from the analysis. Then, rETR is calculated with $\text{rETR} = (\text{Fv}'/\text{Fm}') \times I$. The ephyrae were kept at least 20 minutes in the dark before the measurement.

2.2. Statistical Analysis

All the statistical analyses were performed in R (R Core Team 2017). The effect of the presence or absence of light, prey or added nutrients and their interactions on polyp number per well and summed produced planuloid buds were investigated for each species using generalized linear models (GLM) on the abundances at day 55. A Poisson error structure was used to take in account the specificities of count data (e.g. non-normal error).

The effects of light and added nutrients on survival time of the starved polyps was investigated for each species using a two-way ANOVA. Normality of residuals and homoscedasticity were tested using respectively Shapiro's and Bartlett's tests.

3. Results

3.1. Budding

The polyps were produced asexually in both species. *Cassiopea* sp. reproduced via planuloid buds whereas *Aurelia* sp. reproduced mainly by stolons and lateral budding. Thus no planuloid bud production data are available for *Aurelia* sp.

Within the 55 experimental days, the number of polyps in experimental wells reached a mean value comprised between 7 and 20 for *Cassiopea* sp. and between 14 and 28 for *Aurelia* sp. in fed treatments (Fig. 1). In contrast, starved treatments, independently of light or nutrients showed very low number of polyps (generally between 0 and 2 per well) throughout the experiment (Fig. 1). This pattern is confirmed by the GLMs which identified presence or absence of prey as the only significant factor impacting polyp number in both species ($p\text{-value}_{\text{prey}} < 0.001$ in both cases). None of the others factors (i.e. light and added nutrients) affected the polyp numbers significantly.

The same general pattern is observed for *Cassiopea* sp. planuloid bud production where fed treatments produced on average between 20 and 50 planuloid buds over the course of the experiment as opposed to no more than 5 in the starved treatments (Fig. 2). GLM indeed found the presence of prey as the only significant between-treatment factor ($p\text{-value}_{\text{prey}} < 0.001$).

A qualitative comparison of treatments' effect on polyp budding in *Cassiopea* sp. and *Aurelia* sp. showed a similar pattern. For both species, prey availability was the most important factor influencing budding while added nutrients and light had no significant effects during the experiment.

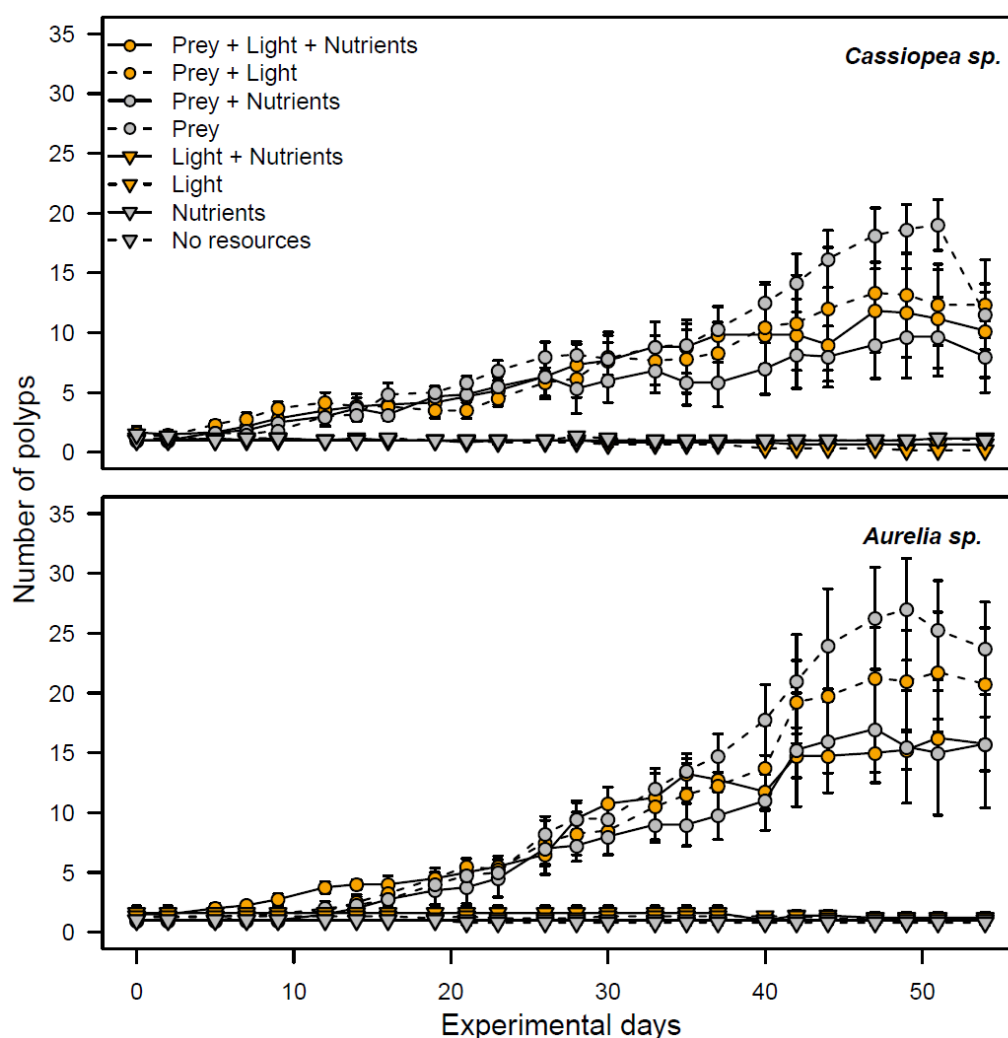


Fig. 1 Changes in the number of *Cassiopea* sp. and *Aurelia* sp. polyps in different treatments (mean \pm SEM) over the 55 days of the experiment. Circles and triangles indicate respectively fed and starved treatments. Orange and grey symbols indicate respectively treatments kept with light or in the dark. And solid and dashed lines indicate respectively treatments with or without added nutrients.

Fig. 1 Changements des nombres de polypes de *Cassiopea* sp. et *Aurelia* sp. dans les différents traitements (moyenne \pm erreur standard) au cours des 55 jours de l'expérience. Les cercles et les triangles indiquent respectivement les traitements avec et sans proies. Les symboles orange et gris indiquent respectivement les traitements avec et sans lumière. Les lignes pleines et pointillées indiquent respectivement les traitements avec ou sans nutriments supplémentaires

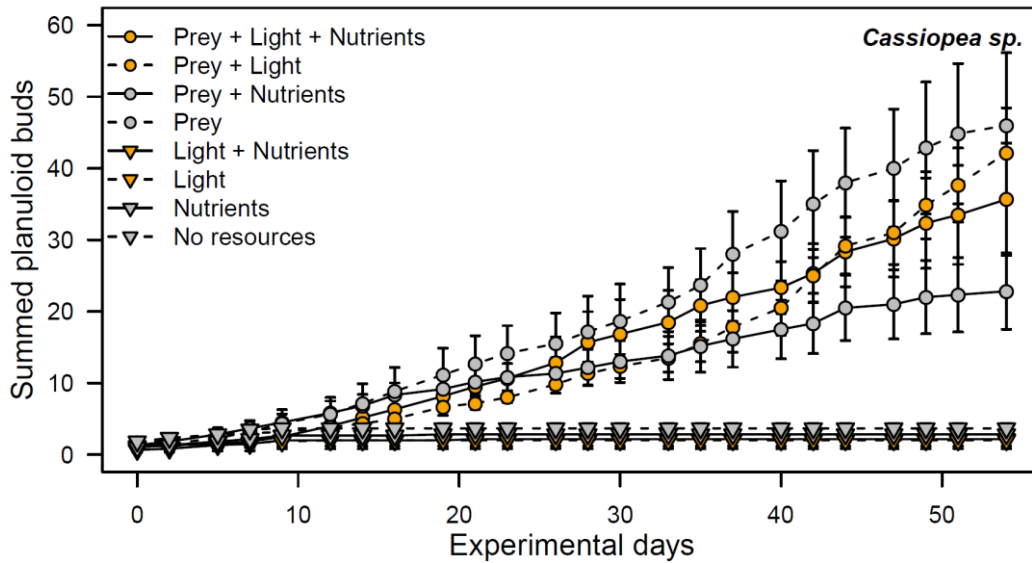


Fig. 2 Summed planuloid buds produced by *Cassiopea* sp. polyps in different treatments (mean \pm SEM) over the 55 days of the experiment. Circles and triangles indicate respectively fed and starved treatments. Orange and grey symbols indicate respectively treatments kept with light or in the dark. And solid and dashed lines indicate respectively treatments with or without added nutrients.

Fig. 2 Production cumulée de bourgeons planuloides par les polypes de *Cassiopea* sp. dans les différents traitements (moyenne \pm erreur standard) au cours des 55 jours de l'expérience. Les cercles et les triangles indiquent respectivement les traitements avec et sans proies. Les symboles orange et gris indiquent respectivement les traitements avec et sans lumière. Les lignes pleines et pointillées indiquent respectivement les traitements avec ou sans nutriments supplémentaires

3.2. Starved Polyp Survival

Starved polyps survived for up to 156 days in *Cassiopea* sp. and 243 days in *Aurelia* sp. (Fig. 3). In the treatment without light and without added nutrients one *Aurelia* sp. polyp died on day 21, much sooner than the other replicates, which might be considered as an outlier. The results of the statistical tests including and excluding this data point are both presented in Fig. 3. The light was identified as the only factor affecting the survival of starved polyps in both *Cassiopea* sp. (Two-way ANOVA, $F_{\text{light}} = 104.6$, $p\text{-value}_{\text{light}} < 0.001$) and *Aurelia* sp. whether the outlier is included (Two-way ANOVA, $F_{\text{light}} = 14.1$, $p\text{-value}_{\text{light}} < 0.01$) or excluded (Two-way ANOVA, $F_{\text{light}} = 42.6$, $p\text{-value}_{\text{light}} < 0.001$). The treatments kept in the dark survived longer than the ones kept with a light cycle. If the outlier is excluded, this pattern is consistent and

significant across all treatments and species (Tukey's Post Hoc test, p -value < 0.05 , Fig. 3). No significant effect of added nutrients on survival time was noted.

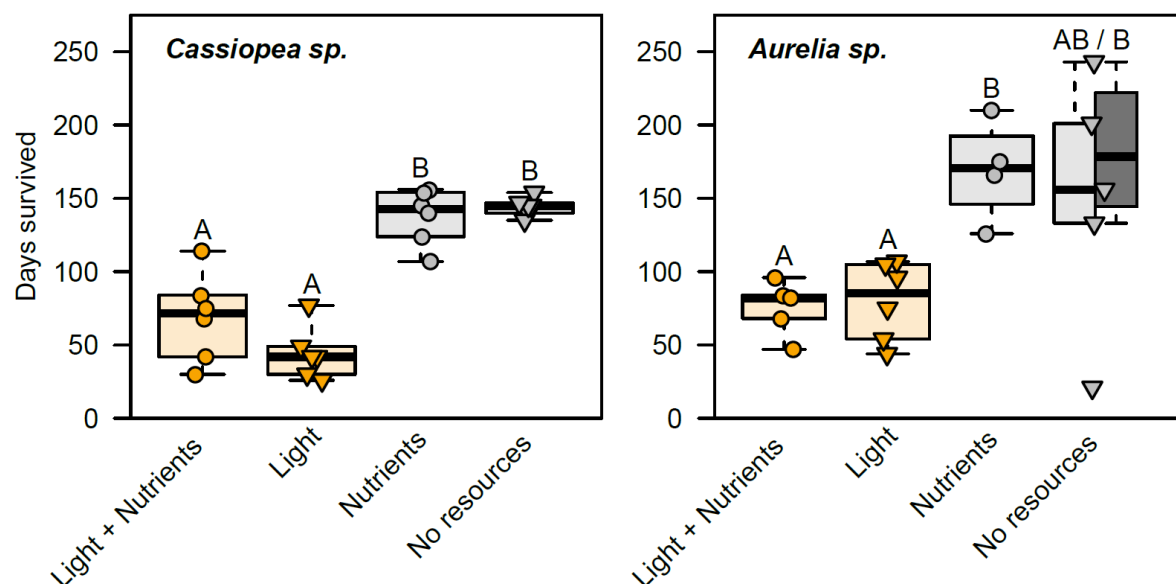


Fig. 3 Survival time of starved *Cassiopea* sp. and *Aurelia* sp. polyps in different treatments. Different letters indicate significant differences (Tukey post hoc test, $\alpha = 0.05$). The dark grey boxplot in *Aurelia* sp. is constructed excluding an outlier (polyp dead at day 21). The two sets of letters associated correspond to the Tukey post hoc tests including and excluding the outlier

Fig. 3 Temps de survie des polypes de *Cassiopea* sp. et *Aurelia* sp. non nourris dans les différents traitements. Des lettres différentes indiquent des différences significatives (test post hoc de Tukey, $\alpha = 0.05$). La boîte gris foncé pour *Aurelia* sp. est construite en excluant une valeur extrême (polype mort après 21 jours). Les deux groupes de lettres associés correspondent aux résultats du tests post hoc de Tukey incluant et excluant la valeur extrême

3.3. Strobilation and Zooxanthellae in Ephyrae

Strobilation was only observed in *Cassiopea* sp. I counted a total of nine monodisc strobilations (Table 2) all of which gave viable ephyra. All strobilations occurred in fed treatments and eight out of nine strobilations occurred in treatments kept with a daily light-dark cycle. In the treatments kept with a daily light-dark cycle zooxanthellae patches were visible in the polyp, strobila and ephyra. In the treatments kept in the dark, zooxanthellae patches were only visible in the forming ephyra (Fig. 4).

Table 2 Strobilation of fed *Cassiopea* sp. polyps as a function of experimental conditions. x/y with x the number of experimental wells in which strobilation occurred and y the total number of wells. No more than one strobilation occurred per well. Only monodisc strobilations were observed. No strobilation occurred in starved treatments which are thus not presented here

Table 2 Strobilation des polypes de *Cassiopea* sp. nourris en fonction des conditions expérimentales. x/y avec x le nombres de puits expérimentaux dans lesquels une strobilation a eu lieu et y le nombre total de puits. Seules des strobilation monodisques ont été observées. Aucune strobilation n'a été observée dans les traitements non-nourris qui ne sont donc pas présentés

| | 12:12 h light:dark cycle | In dark |
|-------------------------|--------------------------|---------|
| With added nutrients | 2/6 | 0/6 |
| Without added nutrients | 6/6 | 1/6 |

The light curve of the ephyra produced in the dark falls among the light curves of the ephyrae produced in light (Model fitted of the shape: $rETR = rETR_{max} [1 - \exp (-I / E_k)]$, with I the light intensity, $rETR_{max}$ the photosynthesis at saturating light and E_k the light saturation parameter. Estimated parameters for the curves are $rETR_{max} = 106.4, 73.0, 91.6, 125.5$ and $E_k = 133.0, 102.7, 106.9, 328.7$ for the ephyrae produced in light and $rETR_{max} = 85.5$ and $E_k = 160.0$ for the ephyra produced in dark, Fig. 5).

4. Discussion

4.1. Budding and Survival Patterns

This study tested the influence of the presence or absence of added nutrients, light, and prey on the budding and survival time of two scyphozoan species' polyps: the zooxanthellate *Cassiopea* sp. and the non-zooxanthellate *Aurelia* sp. While the latter are strictly heterotrophs, *Cassiopea* sp. polyps are able to derive at least one part of their nutrition autotrophically via their zooxanthellae's photosynthesis products (Hofmann and Kremer 1981). Thus, light and

nutrients can only be use by *Cassiopea* sp. for its nutrition via its zooxanthellae whereas prey can be used by both species. Based on these differences in the exploitable resources, differences in budding and survival time responses to the presence or absence of these resources can be expected to arise. Yet the results are qualitatively similar for both species.

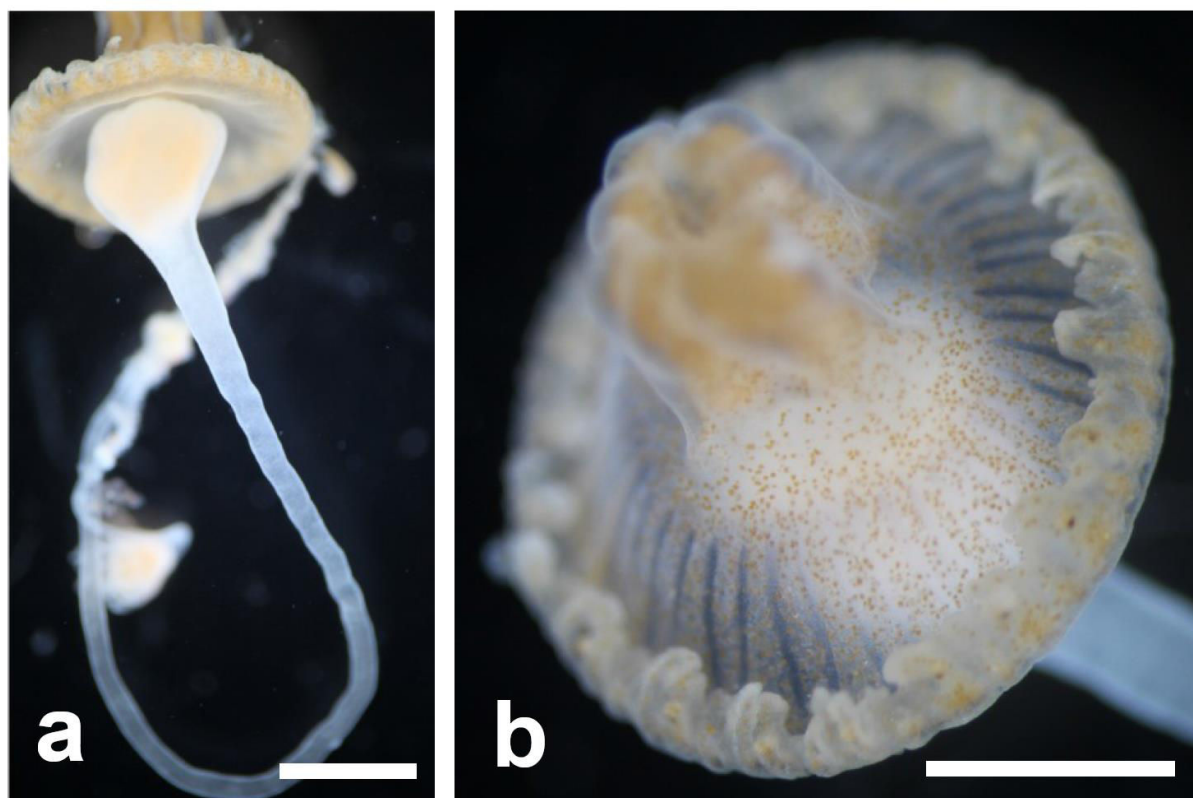


Fig. 4 Development of zooxanthellae clusters in a *Cassiopea* sp. strobila kept in the dark. (a) General view of the strobila. (b) Detail of the forming ephyra. Note that the brownish zooxanthellae clusters are not visible in the peduncle and calyx of the strobila but are visible in the forming ephyra. Scales bars = 1 mm

Fig. 4 Développement de paquets de zooxanthelles dans une strobila de *Cassiopea* sp. gardée au noir . (a) vue générale de la strobila. (b) Détail de l'éphyrule en formation. Noter l'absence de paquets de zooxanthelles dans le pédoncule et le calice de la strobila mais leur présence dans l'éphyrule en formation. Barre d'échelle = 1 mm

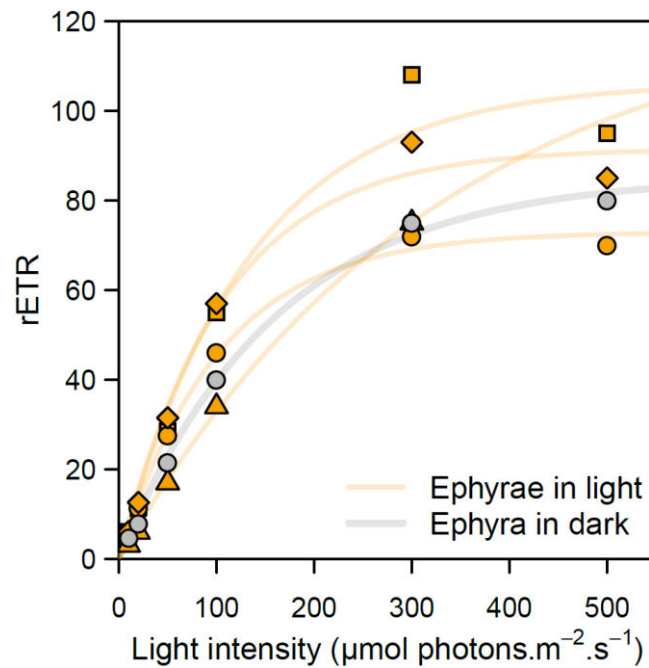


Fig. 5 Light curves of *Cassiopea* sp. ephyrae. The rETR refers to the relative Electron Transport Rates and gives a proxy of photosynthetic activity. Different symbols represent different ephyrae. Orange symbols and curves: ephyrae produced by the polyps kept in a 12:12 h light:dark cycle. Grey symbols and curve: ephyra produced by a polyp kept in dark

Fig. 5 Courbes photosynthèse-lumière d'éphyrules de *Cassiopea* sp. rETR signifie « relative Electron Transport Rates » et est un indicateur de l'activité photosynthétique. Des symboles différents indiquent différentes éphyrules. Symboles et courbes oranges : éphyrules produites par des polypes gardés avec un cycle jour:nuit, 12:12 h. Symboles et courbe gris : éphyrule produite par un polype gardé au noir

Regarding the budding, light appears to have little effect on *Aurelia* spp. (Purcell 2007, Liu et al. 2009, this study) whereas it can increase slightly the budding in *Cassiopea andromeda* (Hofmann et al. 1978). This difference might be due to the ability of *Cassiopea* sp. to use light as nutrition resource. However, in the present study this effect of light on *Cassiopea* sp. budding was not seen. Thus, I noted no clear positive effects of resources linked with autotrophy (i.e. light and added nutrients) in either species budding. The main differences between treatments are related in all cases and in both species to the presence of prey which increased dramatically the budding (Figs. 1, 2). This is consistent with the importance of heterotrophic feeding for budding as has been reported in previous studies for both zooxanthellate and non-zooxanthellate scyphozoan polyps (Hofmann et al. 1978, Schiariti et al. 2014).

In this study, the survival time of starved polyps was negatively influenced by light in both species (Fig. 3). Others studies have not reported such negative effect either in zooxanthellate (Prieto et al. 2010) or non-zooxanthellate (Dong et al. 2015) scyphozoans polyps. This discrepancy might be related to light dependent growth of microalgae during the experiment. Some of the experimental wells were gradually colonized by a biofilm of microalgae, which might have overgrown the polyps, possibly leading to the death of the latter. If this is the case, the finding that light decreases the survival time of polyp may be due to an indirect effect of competition with microalgae (see Lucas et al. 2012). However, active zooxanthellae did not prevent these earlier deaths in *Cassiopea* sp. suggesting that the photosynthates they furnish do not compensate the negative effects of competition with other algae.

Overall the most important pattern observed in this study is that the zooxanthellate *Cassiopea* sp. and the non-zooxanthellate *Aurelia* sp. respond similarly to resource availability (Figs. 1, 3). The budding is mainly controlled by prey availability but not by light or added nutrients. Moreover, this study showed a shortened survival time of starved polyps in both species exposed to light. Thus, these results suggest that in spite of *Cassiopea* sp. ability to acquire a part of its nutrition from zooxanthellae's photosynthesis (Hofmann and Kremer 1981), the polyps react more like the non-zooxanthellate heterotroph *Aurelia* sp. to resource availability in terms of budding and survival.

4.2. Limited Impact of Zooxanthellae on Scyphozoan Polyps' Ecology?

In general, experimental studies suggest that the autotrophic nutrition via zooxanthellae may be of little importance compared to heterotrophy in zooxanthellate scyphozoan polyps budding and survival (Sugiura 1963, Hofmann et al. 1978, Rahat and Adar 1980, Prieto et al. 2010, Schiariti et al. 2014, this study). This low importance of autotrophy during the polyp phase is consistent with the behaviour of zooxanthellate scyphozoan planulae which tend to settle better on the underside of surfaces or in the dark (Kikinger 1992, Fleck and Fitt 1999, Duarte et al. 2012 but see Astorga et al. 2012) inducing the polyp to grow and reproduce in a shaded environment. This contrasts with the fact that the zooxanthellate medusae stage often relies heavily on its zooxanthellae (e.g. Kremer et al. 1990, Verde and McCloskey 1998,

[Mortillaro et al. 2009](#)). This contrast might be explained by the metagenetic life-cycle of most scyphozoans. Medusae and polyps live in distinct environments and the zooxanthellae may not be well suited to these life-style changes. Zooxanthellae may thus only be well adapted to one of the two life stages; the medusae. As a result, their roles in polyps living in shaded environments may be limited. Moreover, scyphozoan polyps lack some features that help access light in other zooxanthellate cnidarians. For instance, scleractinian corals can grow in large and complex colonies that can optimize light exposure (e.g. [Einbinder et al. 2009](#)) which is not the case of scyphozoan polyps.

On the other hand, the low importance of autotrophy for zooxanthellate scyphozoans polyps implies that they are not limited by light. This would allow them to grow and reproduce during winter in seasonally marked environments (see also [Prieto et al. 2010](#)) or in turbid waters. Ultimately this aspect of zooxanthellate scyphozoan life history could help explain their ability to multiply in human-impacted environments ([Stoner et al. 2011](#)) or to invade more seasonally marked waters (e.g. the Mediterranean Sea; [Bayha and Graham 2014](#)).

The similar responses of the zooxanthellate *Cassiopea* sp. and the non-zooxanthellate *Aurelia* sp. to resources in this experiment (Figs. 1, 3) help us to understand the scyphozoan polyp population dynamics. Budding and survival are key components to scyphozoan polyps' population dynamics ([Lucas et al. 2012](#)). They are influenced by a variety of factors both environmental (e.g. temperature, amount of food) and aspect of life history (e.g. budding modes) ([Lucas et al. 2012](#), [Schiariti et al. 2014](#)). The zooxanthellate or non-zooxanthellate state is one important biologic factor. However, these findings suggest that the presence or absence of zooxanthellae in scyphozoan polyps affects little their budding and survival and hence, their population dynamics. Instead, populations of zooxanthellate and non-zooxanthellate scyphozoan polyps may be under the control of similar factors, mainly food and temperature (e.g. [Schiariti et al. 2014](#)).

It is however important to note that the presence of zooxanthellae in scyphozoan polyps may still have important consequences. For instance, zooxanthellae can provide resistance to hypoxia or acidification in *Cassiopea* sp. ([Klein et al. 2017](#)). Moreover, zooxanthellae appear to play an important role for strobilation in zooxanthellate scyphozoans.

4.3. Remarks on the Role of Zooxanthellae During Strobilation

Strobilation in scyphozoans is under the control of a variety of factors. The most important for most species are temperature and available food (Lucas et al. 2012, Helm 2018) but also light (Purcell 2007, Liu et al. 2009). In zooxanthellate species, the symbiont is often considered to play an important role. Its presence helps (Sugiura 1969, Rahat and Adar 1980) or is required for strobilation (Sugiura 1964, Ludwig 1969, Hofmann and Kremer 1981, Kikinger 1992). It is also important to note that even if the zooxanthellae's photosynthetic activity is hindered (e.g. polyps incubated in the dark), their presence alone affects strobilation rates positively (Sugiura 1969, Hofmann and Kremer 1981). Moreover, time to strobilation is independent of zooxanthellae's abundances (Newkirk et al. 2018). This suggests that the role of zooxanthellae in strobilation is more complex than a simple provider of photosynthates.

In this study, I only observed strobilation in *Cassiopea* sp. The absence of strobilation in *Aurelia* sp. is likely related to the need of a seasonal cue such as a temperature change to trigger strobilation in this species (e.g. Fuchs et al. 2014) which was not provided in the experiment. In *Cassiopea* sp., nine monodisc strobilations were counted. This is few, but the pattern observed (Table 2) is consistent with previous observations that zooxanthellae activity helps but is not obligate for strobilation while food is needed (Hofmann and Kremer 1981). An increase in the number of zooxanthellae patches in the polyp before and during strobilation was noted in agreement with previous studies (Sugiura 1964, Ludwig 1969, Sugiura 1969, Hofmann and Kremer 1981, Newkirk et al. 2018). This increase was also observed in the only strobila obtained in the dark but only in the forming ephyra (Fig. 4). The increase of zooxanthellae patches in the dark can only be attributed to heterotrophic nutrition of zooxanthellae (see Steen 1986, Jeong et al. 2012) likely using carbon from their host. Moreover, the light curve measured for the ephyra produced in the dark is similar to those of ephyrae produced with a 12:12 h light:dark cycle (Fig. 5). This suggests that photosynthetic machinery of zooxanthellae produced in the dark is functional and as effective as the one of zooxanthellae produced in light.

Although the role of zooxanthellae in strobilation of zooxanthellate scyphozoan species is admittedly important (Sugiura 1964, Ludwig 1969, Sugiura 1969, Hofmann and Kremer 1981, Kikinger 1992, Newkirk et al. 2018), it remains poorly understood. Jointly with other studies, I also noted that hindered photosynthetic activity does not prevent strobilation (Sugiura 1969,

[Hofmann and Kremer 1981](#)) indicating that the role of zooxanthellae during strobilation goes beyond a simple provider of photosynthates. I argue that zooxanthellae's heterotrophy may also play a role.

4.4. Conclusions

This work was conducted to test the assumption that, in spite of different accessible resources, zooxanthellate and non-zooxanthellate scyphozoan polyps respond similarly to resources availability. I found support for that; the zooxanthellate *Cassiopea* sp. and the non-zooxanthellate *Aurelia* sp. polyps respond in the same way to the presence or absence of light, prey or added dissolved inorganic nutrients. In both species, prey availability alone assured polyps' budding, whereas the survival time of starved polyps was decreased by light possibly as a result of competition with microalgae. The main difference between zooxanthellate and non-zooxanthellate scyphozoan polyps might be linked to strobilation where zooxanthellae become important (e.g. [Sugiura 1964](#), [Ludwig 1969](#)). This role is still unclear and warrants further research, but these observations suggest that zooxanthellae's heterotrophy may be an important factor.

Notwithstanding important differences in medusae ecology ([West et al. 2009](#)), in strobilation ([Sugiura 1964](#)) or in the response to stress ([Klein et al. 2017](#)), similar budding and survival responses of *Cassiopea* sp. and *Aurelia* sp. to resources availability suggests that the state zooxanthellate or non-zooxanthellate may have little impact on scyphozoan polyps' population dynamics.

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Chapter III: $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratios as nutrition indicators of zooxanthellate jellyfishes: insights from an experimental approach

Chapitre III : $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, et ratios C:N en tant qu'indicateurs de la nutrition des méduses à zooxanthelles : apports d'une approche expérimentale

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Abstract

Some jellyfish host zooxanthellae in their tissues (mostly from the family Symbiodiniaceae; Dinophyceae) and supplement their heterotrophic nutrition with their symbiont's photosynthates. The mixotrophy of zooxanthellate jellyfishes (as holobionts) renders the study of their nutrition, growth, and population dynamics complicated. Here, we used an experimental approach to assess how carbon and nitrogen stable isotopes signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) as well as the elemental composition (C:N ratios) of zooxanthellate jellyfishes are affected by variations in nutrition sources: i.e. predation (heterotrophic) versus photosynthesis (autotrophic). Our laboratory experiment, conducted on the zooxanthellate jellyfish *Cassiopea* sp. medusae (including symbionts) in the presence or absence of light and prey during 24 days, showed conclusive results. Presence of light decreased $\delta^{15}\text{N}$, increased $\delta^{13}\text{C}$ and C:N ratios, whereas presence of prey increased $\delta^{15}\text{N}$, and decreased $\delta^{13}\text{C}$ and C:N ratios. The medusae incubated with both light and prey had intermediate $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and C:N ratios. Variations in zooxanthellate jellyfishes' nutrition sources (autotrophy vs. heterotrophy) are thus reflected by their isotopic and elemental composition. These results provide a baseline for interpreting the values of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N ratios that can be observed on these organisms in fieldwork studies.

Résumé

Certaines méduses hébergent des zooxanthelles dans leur tissus (principalement de la famille Symbiodiniaceae ; Dinophyceae) et complètent leur nutrition hétérotrophe par les photosynthates de leurs symbiontes. La mixotrophie de ces méduses à zooxanthelles (en tant que holobiontes) rends l'étude de leur nutrition, croissance et dynamiques de populations compliquée. Ici, nous employons une approche expérimentale pour observer comment les signatures en isotopes stables du carbone et de l'azote ($\delta^{13}\text{C}$ et $\delta^{15}\text{N}$) ainsi que la composition élémentaire (ratios C:N) des méduses à zooxanthelles sont affectées par des variations des sources de nutrition : i.e. predation (hétérotrophie) et photosynthèse (autotrophie). Notre expérience de laboratoire, conduite sur la méduse à zooxanthelles *Cassiopea* sp. (symbiontes inclus) en présence ou absence de lumière et de proies pendant 24 jours, a montré des résultats concluants. La présence de lumière a diminué le $\delta^{15}\text{N}$, et augmenté le $\delta^{13}\text{C}$ et les ratios C:N, tandis que la présence de proies a augmenté le $\delta^{15}\text{N}$, et diminué le $\delta^{13}\text{C}$ et les ratios C:N. Les méduses incubées en présence de lumière et de proies ensemble présentaient des $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ et ratios C:N intermédiaires. Les variations dans la nutrition des méduses à zooxanthelles sont donc reflétées dans leur compositions isotopique et élémentaire. Ces résultats posent une base pour l'interprétation des $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ et ratios C:N observés chez ces organismes sur le terrain.

1. Introduction

Jellyfishes are increasingly acknowledged as an important component of marine ecosystems. Population dynamics of the pelagic life stages are often characterized by important fluctuations with dramatic biomass increases followed by sudden collapses (Lucas and Dawson 2014, Pitt et al. 2014). These fluctuations can have important consequences for pelagic community dynamics and nutrient cycling (Pitt et al. 2009a), or for human activities (Purcell et al. 2007). One of the key factors controlling jellyfish population dynamics, is nutrition (e.g. Lucas and Dawson 2014, Pitt et al. 2014). One way to study jellyfish nutrition is to use their stable isotopes signatures (mainly $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, see Pitt et al. 2009b). Many recent studies have focused on jellyfish stable isotopes and have provided insights of their diets as well as competition relationships (e.g. Fleming et al. 2015, Javidpour et al. 2016, Vansteenbrugge et al. 2016, D'Ambra et al. 2018, Milisenda et al. 2018). Most of these studies have strictly focused on heterotrophic jellyfishes. However, some jellyfishes are known to live in symbiosis with zooxanthellae. Comparatively, the zooxanthellate jellyfishes have received less interest (see however Freeman et al. 2017, Zeman et al. 2018).

Zooxanthellate jellyfishes (mostly Rhizostomeae, Scyphozoa) are characterized by their photosymbiotic relationship with zooxanthellae (generally from the family Symbiodiniaceae, Dinophyceae; LaJeunesse 2001, LaJeunesse et al. 2018). This symbiotic relationship is thought to be similar to the one well known in corals with the zooxanthellae providing their host with photosynthates while recycling the host's respiration and excretion products (see Davy et al. 2012). In such symbiosis, both carbon and nitrogen can be obtained via heterotrophy and autotrophy and are recycled between the host and its zooxanthellae. Carbon dioxide (CO_2) and nitrogen from host respiration and excretion are used and metabolized by the zooxanthellae. Simultaneously, complex molecules (including carbohydrates, lipids and amino acids) are transferred from one partner to the other (Davy et al. 2012). Zooxanthellate jellyfishes, as holobionts (host + symbionts), are thus mixotrophs, deriving their nutrition from both predation and zooxanthellae's photosynthesis (Kremer 2005, Welsh et al. 2009). Generally, the symbiosis provides most if not all of the carbon needed for respiration (Kremer et al. 1990, Kikinger 1992, McCloskey et al. 1994, Verde and McCloskey 1998) while predation is still needed to meet nitrogen and phosphorus requirements (Kremer 2005, Welsh et al. 2009). However, the relative contribution to nutrition of the predation versus the

photosynthesis might be variable across species, populations, environments, or during growth (see e.g. [Sugiura 1969](#), [McCloskey et al. 1994](#), [Verde and McCloskey 1998](#), [Bolton and Graham 2004](#)). Studies using stable isotopes, in this context might be valuable tools to understand these variations.

Numerous studies on other photosymbiotic cnidarians (mainly corals), have shown that variations of nutrition affect the isotopic and elemental composition (see e.g. [Muscatine et al. 1989a](#), [Muscatine and Kaplan 1994](#), [Alamaru et al. 2009](#), [Reynaud et al. 2009](#), [Ferrier-Pagès et al. 2011](#), reviewed by [Ferrier-Pagès and Leal 2018](#)). Similar effects can be expected in zooxanthellate jellyfishes. To date, only few fieldwork studies have focused on the isotopic composition of zooxanthellate jellyfishes (see [Freeman et al. 2017](#), [Zeman et al. 2018](#)). The conclusions of these studies have, however, been limited due to the lack of data on the interplay between autotrophy and heterotrophy of zooxanthellate jellyfishes as reflected in their isotopic and elemental composition ([Zeman et al. 2018](#)). To better understand this, controlled experiments are needed where the resources for heterotrophy (prey), and for autotrophy (light) can be manipulated and their effect on stable isotopes signatures and elemental composition can be assessed. In this study, we aim to provide experimental insights on how isotopic and elemental composition of zooxanthellate jellyfishes are affected by relative variations of autotrophy and heterotrophy. In order to achieve this, we assessed the changes in the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratios in young specimens of zooxanthellate *Cassiopea* sp. medusae (Scyphozoa: Rhizostomeae) over a period of 24 days and in the presence or absence of prey and light.

2. Materials and Methods

2.1. Experimental Setup

Small specimens of *Cassiopea* sp. medusae (ca. 6 mm in bell diameter and 1 month old) were acquired from the Trocadéro Aquarium (Paris, France). In this aquarium, the medusae were kept at 25 °C with a daily light cycle, and were fed *Artemia* sp. nauplii twice a day. After their arrival to the laboratory the medusae were acclimatized to local heated (25-26 °C) filtered (1 μm pore size) sea-water during one day. In the following day, five medusae were randomly sampled to represent the initial state and then, the experiment was set up. A total of 72

medusae specimens were individually placed in 75 ml glass flasks filled with 50 mL of filtered sea-water. The flasks were then randomly assigned to one of the four experimental treatments (18 medusae per treatment). The experimental treatments were as follows: (1) fed and in light, (2) fed and in the dark, (3) starved and in light, and (4) starved and in the dark. The goal of these different treatments was to target respectively: mixotrophy, heterotrophy, and autotrophy, the fourth treatment being a control. The flasks containing the medusae were kept in water baths, which regulated a fixed temperature (25-26 °C). Two water baths were used, one for the medusae kept in light, and the other for the medusae kept in the dark. The temperature changed little during the experiment and between the two water baths (25.6 ± 0.4 °C and 25.3 ± 0.4 °C respectively in the lit and darkened water baths; mean \pm s.d.). The light was provided by a fluorescent lamp on a 12:12 hours day:night cycle at an intensity of ca. $110 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$. Food consisted of 2 h of feeding *ad libitum* every two days on *Artemia* sp. young nauplii (< 24 h after hatching). The medusae's full guts and numerous remaining prey in the flasks confirmed a proper *ad libitum* feeding. Every two days, and after the feeding, the incubation water of the medusae was entirely changed. During the latter process, the flasks were also washed to prevent fouling.

Every four days, and before the feeding (ensuring empty guts), three medusae were randomly sampled from each treatment. Thus the sampling occurred at the following days: 4, 8, 12, 16, 20 and 24; plus the initial state being represented by the five medusae sampled before setting the treatments.

2.2. Processing of Medusae

Immediately after sampling, presence and physiological state of the symbionts were assessed. For this purpose, the medusae were put in the dark for at least 20 minutes allowing the opening of the photosystem reactive centers of zooxanthellae. The photosynthetic parameters of the zooxanthellae were assessed by pulse amplitude modulatory (PAM) fluorometry using the "OJIP protocol" of an AquaPen-C-AP-C100 (®Photon Systems Instruments, PSI, Brno, Czech Republic), at a 450 nm excitation wavelength. This protocol measures the fluorescence emitted after a flash of saturating light. This yields the estimation of several variables among which the maximum photosystem II quantum yield (F_v/F_m), which

is a proxy of the photosystem II efficiency. With F_m the maximum fluorescence under saturating light, and $F_v = F_m - F_0$ with F_0 the initial fluorescence (Strasser et al. 2000). The measures were performed on whole medusae specimens. Prior to the measurement, it was ensured that the medusae were settled at the cuvette bottom to insure proper exposition to the saturating flash. Additionally, along each medusae measure, a blank was realized by using the “OJIP protocol” on incubation water without medusae (75 blanks total).

Preparation for elemental and isotopic analysis started first by quickly rinsing the medusae in deionized water in order to remove the sea salt. The whole medusae were then placed in pre-weighted tin capsules (10.5 × 9 mm EMAL technology, United Kingdom) and oven-dried at 60 °C for ca. 48 h. After drying, the tin capsules containing the medusae were locked and weighed again to obtain the medusae dry mass, which varied between 0.2 and 3.2 mg (0.9 ± 0.6 mg; mean \pm s.d.). In the preparation procedure, the medusae were unpreserved before the isotopic analysis, following the recommendations of Fleming et al. (2011). Due to the small size of the medusae specimens, it was not possible in this experiment to follow the recommendations of MacKenzie et al. (2017) by dissecting, washing and rubbing the mesoglea before conservation. In addition, due to their small size, it was not possible to separate the animal tissue from the zooxanthellae. This would have resulted in acquiring an insufficient biomass for the isotopic analysis. Thus, the results presented here are measures on the holobiont (animal host + zooxanthellae).

2.3. Processing of Prey Nauplii

In order to assess the isotopic and elemental composition of the *Artemia* sp. nauplii given as food for the medusae, we sampled them three times during the experiment (at days 8, 14 and 28). At each sampling, concentrated nauplii were divided in five aliquots, and oven-dried at 60 °C for ca. 48 h in clean glass flasks. The dried nauplii were then scratched from the flasks and ground into a powder. Finally, between 0.5 and 1.5 mg of the powder were inserted and locked in tin capsules (10.5 × 9 mm EMAL technology, United Kingdom).

2.4. Elemental and Stable Isotopes Composition

The analyses of medusae and nauplii samples were performed using an Elemental analyzer (Thermo Scientific EA Flash 2000), coupled to a Mass Spectrometer (Thermo Scientific DELTA V Plus) at the Stable Isotopes Laboratory of the “Pôle Spectrométrie Océan” (PSO-IUEM, Plouzané, France). The nitrogen and carbon mass of medusae samples ranged respectively from 15 to 109 μgN ($35 \pm 22 \mu\text{gN}$; mean \pm s.d.) and from 60 to 543 μgC ($175 \pm 120 \mu\text{gC}$; mean \pm s.d.). As the whole medusae were inserted in the tin capsules, these values are representative of their total weights. The nitrogen and carbon mass of nauplii samples ranged respectively from 40 to 105 μgN ($64 \pm 18 \mu\text{gN}$; mean \pm s.d.) and from 200 to 522 μgC ($326 \pm 90 \mu\text{gC}$; mean \pm s.d.). The samples were calibrated for mass bias using casein (IVA-33802155, Analysentechnik, Germany) as the elemental standard (range: 5-108 μgN ; 16-377 μgC). Some material-rich samples were automatically diluted during the analysis process (Thermo Scientific ConFlo IV).

Stable isotopes values are expressed as permil (‰) using the δ notation (normalized to Vienna Pee Dee Belemnite and atmospheric N_2 for respectively carbon and nitrogen):

$$\delta X = \left(\frac{X_{\text{sample}}^H / X_{\text{sample}}^L}{X_{\text{std}}^H / X_{\text{std}}^L} - 1 \right) \times 1000$$

With X the element measured, X^H the amount of the heavy isotope and X^L the amount of the light isotope from the samples (X_{sample}) and the standard (X_{std}).

As some of our samples had a low ($< 20 \mu\text{gN}$) nitrogen mass, we analyzed five replicates of casein standards with a low nitrogen mass ($13.4 \pm 1.9 \mu\text{gN}$; mean \pm s.d.) to check whether this low mass may have led to uncertainties in our measures. We found only a low variability on the obtained $\delta^{15}\text{N}$ measures (0.04 ‰ s.d., $n=5$) indicating that our measures were consistent even at low biomass levels.

Unless indicated otherwise, all C:N ratios are expressed by mass (following Ikeda 2014 and Molina-Ramírez et al. 2015). As the C:N ratios of both the medusae and their prey were higher than 3.5, a normalization of the $\delta^{13}\text{C}$ for lipid content was advisable (Post et al. 2007). For the nauplii, we used the general normalization for aquatic animals given by Post et al. (2007) and for the medusae, we used the normalization specific to scyphozoans proposed by D’Ambra et

al. (2014). For comparison, raw data is still presented as supplementary material (see discussion).

2.5. Statistics

The data collected during the experiment (carbon masses, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C:N ratios and F_v/F_m) were analyzed using linear mixed-effects models (LME) (e.g. Crawley 2012). The presence or absence of prey and light were considered as fixed effects while time was considered as random effect. Model assumptions (mean of residuals = 0, linearity and normality) were checked using model-checking plots. If the model assumptions were not met, the data were log transformed. If the fixed effects affected significantly the results (if p-value < 0.05), subsequent Tukey post-hoc tests were performed on least-square means (i.e. means adjusted for the effect of time) to assess which combination of the fixed effects (light and prey) led to different responses.

One-way ANOVAs were used to assess possible variations in prey $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratios over time. Normality and homoscedasticity assumptions were checked using the Shapiro-Wilk normality test and Bartlett homogeneity of variance test (threshold: $\alpha = 0.05$). If the assumptions were not met, the data were Box-Cox transformed. All statistical analyses were performed in R (R Core Team 2017).

3. Results

3.1. Mass Variation in Medusae

The carbon mass of the medusae (Fig. 1) was significantly affected by light alone (LME, t-value = 7.5, p-value < 0.001) and by the interaction between light and prey (LME, t-value = 3.4, p-value < 0.01). At the beginning of the experiment, the carbon mass of *Cassiopea* sp. medusae was of $115.1 \pm 27.0 \mu\text{g C}$ (mean \pm s.d.). Only the medusae in the treatment with both light and prey did noticeably grow, reaching $449.3 \pm 68.2 \mu\text{g C}$ (mean \pm s.d.) at the end of the experiment. The medusae in the treatment with only light did not grow significantly reaching a carbon mass value of $133.9 \pm 27.9 \mu\text{g C}$ (mean \pm s.d.) at the end of the experiment. The medusae in the treatments with only prey or without resources tended to shrink, with carbon mass values of respectively $88.0 \pm 26.1 \mu\text{g C}$ and $71.1 \pm 15.7 \mu\text{g C}$ (mean \pm s.d.) at the end of

the experiment. In the treatment without resources, one medusae specimen died. Thus for this treatment, there remained two replicates instead of three at day 24.

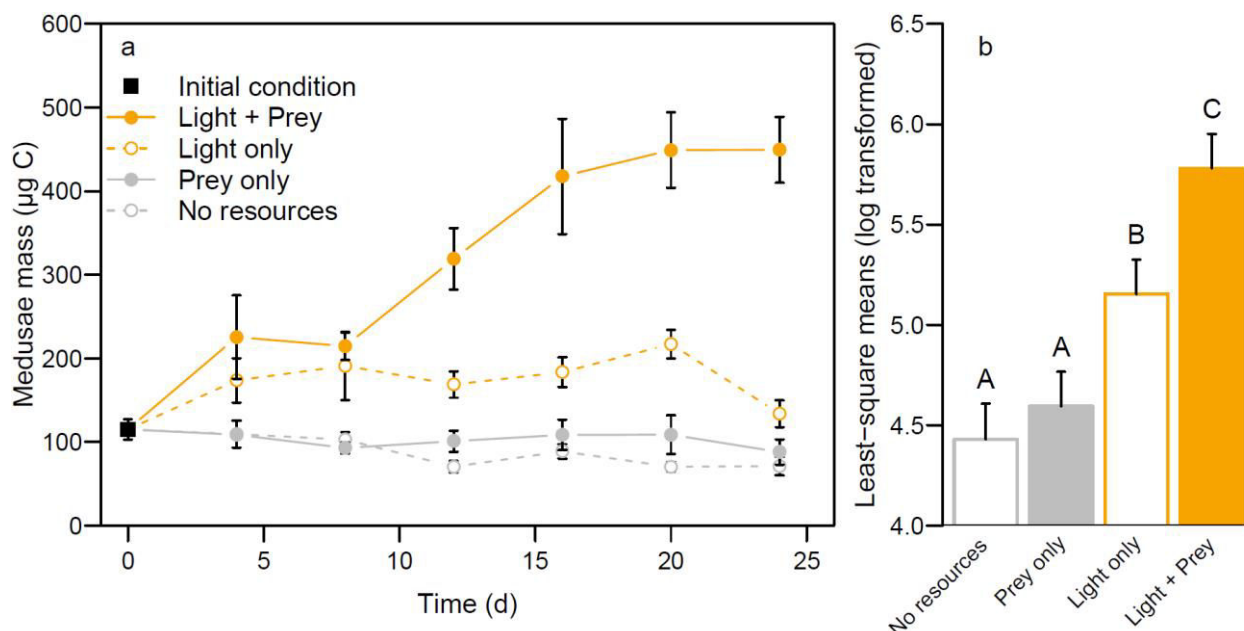


Fig. 1 (a) Changes in the *Cassiopea* sp. medusae carbon mass ($\mu\text{g C}$; means \pm s.e.m.) over the course of the experiment as a function of the experimental conditions. (b) Comparison of least-square means obtained from each treatment (\pm 95 % C. I.). The letters (A, B, and C) indicate statistically different treatments (Tukey post hoc test, p -value < 0.05)

Fig. 1 (a) Changements de la masse carbonée des méduses *Cassiopea* sp. ($\mu\text{g C}$; moyenne \pm erreur standard) durant l'expérience et en fonction des conditions expérimentales. (b) Comparaison des moyennes aux moindres carrés obtenues pour chaque traitement (\pm 95 % I. C.). Les lettres (A, B, et C) indiquent les traitements statistiquement différents (test post hoc de Tukey, valeur- $p < 0.05$)

3.2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

The medusae $\delta^{13}\text{C}$ values (Fig. 2a and b) were significantly affected by light (LME, t -value = 22.5, p -value < 0.001) and by the interaction of light and prey (LME, t -value = -6.2, p -value < 0.001). At the beginning of the experiment $\delta^{13}\text{C}$ values of the medusae were -18.7 ± 0.9 ‰ (mean \pm s.d.). In the treatment with both light and prey the $\delta^{13}\text{C}$ values of the medusae increased quickly (in less than four days) reaching ca. -15 ‰. This trend was even more pronounced in the treatment with only light where the $\delta^{13}\text{C}$ values of the medusae reached

ca. -13 ‰. Conversely, in the treatments with only prey or without resources the $\delta^{13}\text{C}$ values of the medusae remained similar or decreased slightly throughout the experiment (generally comprised between -19 ‰ and -21 ‰). It should be noted that the distinction between the medusae from the treatments with light alone and with light and prey is not as distinct with data not normalized for lipids (Fig. S1).

The medusae $\delta^{15}\text{N}$ values (Fig. 2c and d) were significantly affected by both light (LME, t-value = -8.7, p-value < 0.001) and prey (LME, t-value = 2.2, p-value < 0.05), but not by their interaction. At the beginning of the experiment, the $\delta^{15}\text{N}$ values of medusae was of 8.9 ± 1.1 ‰ (mean \pm s.d.). These values decreased slightly in the treatment with light and prey reaching 8.0 ± 0.3 ‰ (mean \pm s.d.) at the end of the experiment. The decrease was more pronounced in the treatment with light only which reached 5.5 ± 0.4 ‰ at the end of the experiment. Finally, $\delta^{15}\text{N}$ values did not change in the treatments with only prey or without resources (values at the end of the experiment of 9.0 ± 0.3 ‰ and 8.8 ± 1.0 ‰ respectively; mean \pm s.d.).

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ obtained in *Artemia* sp. nauplii prey did not vary significantly during the experiment (ANOVAs, p-values > 0.05) averaging respectively -19.4 ± 0.2 ‰ and 10.7 ± 0.5 ‰ (mean \pm s.d.) (Fig. 2a and c).

3.3. C:N Ratios

The C:N ratios obtained in the medusae (Fig. 3) were significantly affected by light (LME, t-value = 27.5, p-value < 0.001) and by the interaction of light and prey (LME, t-value = -6.6, p-value < 0.001). At the beginning of the experiment, the C:N ratios of medusae were of 4.5 ± 0.1 . These values increased in the treatment with both light and prey (4.8 ± 0.1 at the end of experiment; mean \pm s.d.). A similar, but more pronounced increase was seen in the C:N ratios of the medusae exposed to only light (5.9 ± 0.2 at the end of experiment; mean \pm s.d.). By opposition, C:N ratios decreased slightly in the treatments with only prey or without resources (respectively reaching 4.1 ± 0.1 and 3.9 ± 0.1 at the end of the experiment; mean \pm s.d.).

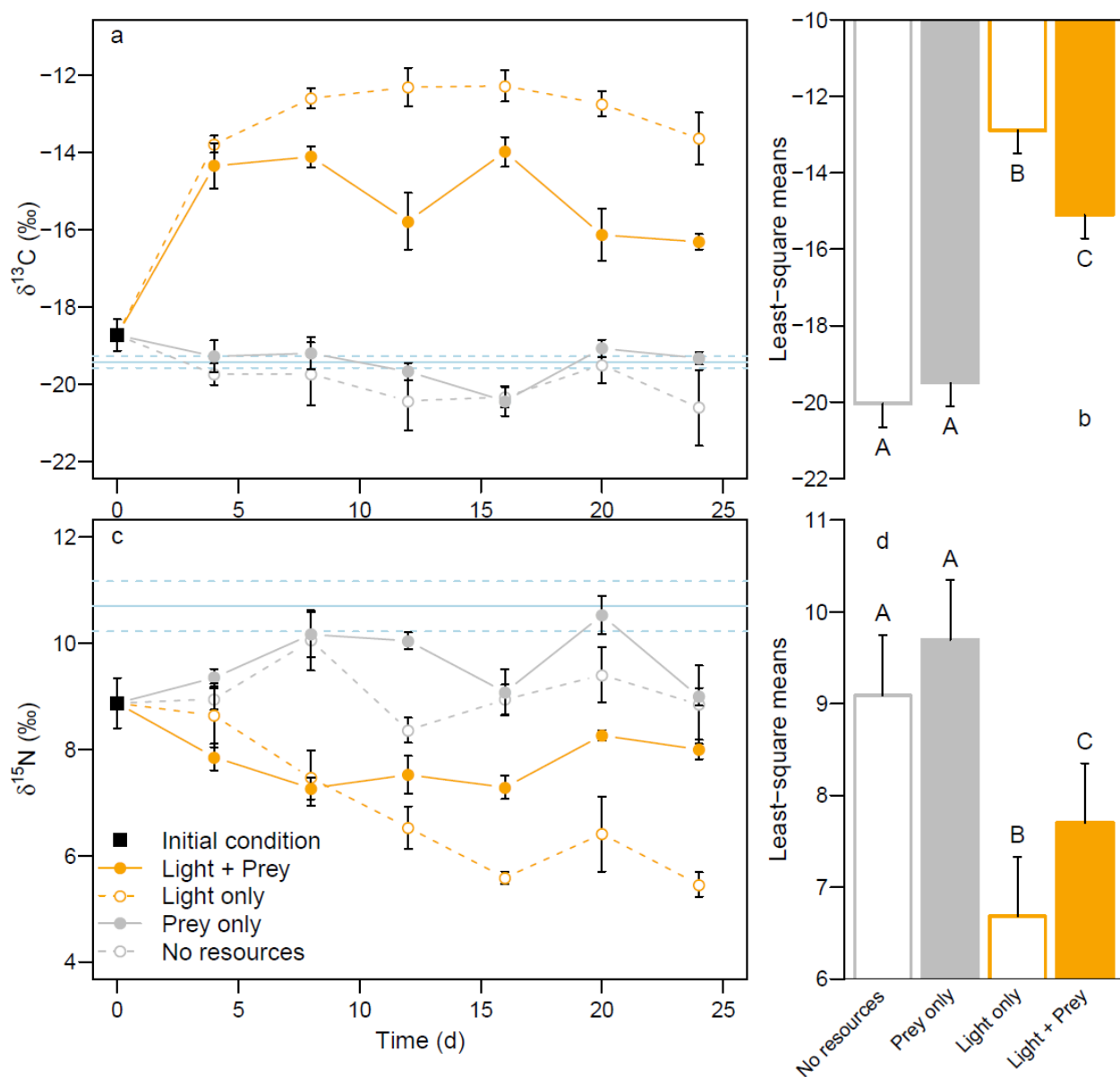


Fig. 2 Changes in the *Cassiopea* sp. medusae $\delta^{13}\text{C}$ (‰) (a) and $\delta^{15}\text{N}$ (‰) (c) (means \pm s.e.m.) over the course of the experiment as a function of the experimental conditions. Solid and dashed blue lines represent the mean \pm s.d. of the isotopic signatures of *Artemia* sp. nauplii used as prey in fed treatments. (b and d) Comparison of least-square means obtained for each treatment (\pm 95 % C. I.). The letters (A, B, and C) indicate statistically different treatments (Tukey post hoc test, p-value < 0.05). $\delta^{13}\text{C}$ values of have been normalized for lipid content according to [Post et al. \(2007\)](#) for nauplii, and [D'Ambra et al. \(2014\)](#) for *Cassiopea* sp. medusae

Fig. 2 ← Changements des $\delta^{13}\text{C}$ (‰) (a) et $\delta^{15}\text{N}$ (‰) (c) des méduses *Cassiopea* sp. (moyenne \pm erreur standard) durant l'expérience et en fonction des conditions expérimentales. Les lignes bleues continues et pointillées représentent la moyenne \pm écart-type des signatures isotopiques des nauplies d'*Artemia* sp. utilisées comme proies dans les traitements nourris. (b et d) Comparaison des moyennes aux moindres carrés obtenues pour chaque traitement (\pm 95 % I. C.). Les lettres (A, B, et C) indiquent les traitements statistiquement différents (test post hoc de Tukey, valeur-p < 0.05). Les valeurs de $\delta^{13}\text{C}$ ont été normalisées pour le contenu en lipides suivant [Post et al. \(2007\)](#) pour les nauplies, et [D'Ambra et al. \(2014\)](#) pour les méduses *Cassiopea* sp.

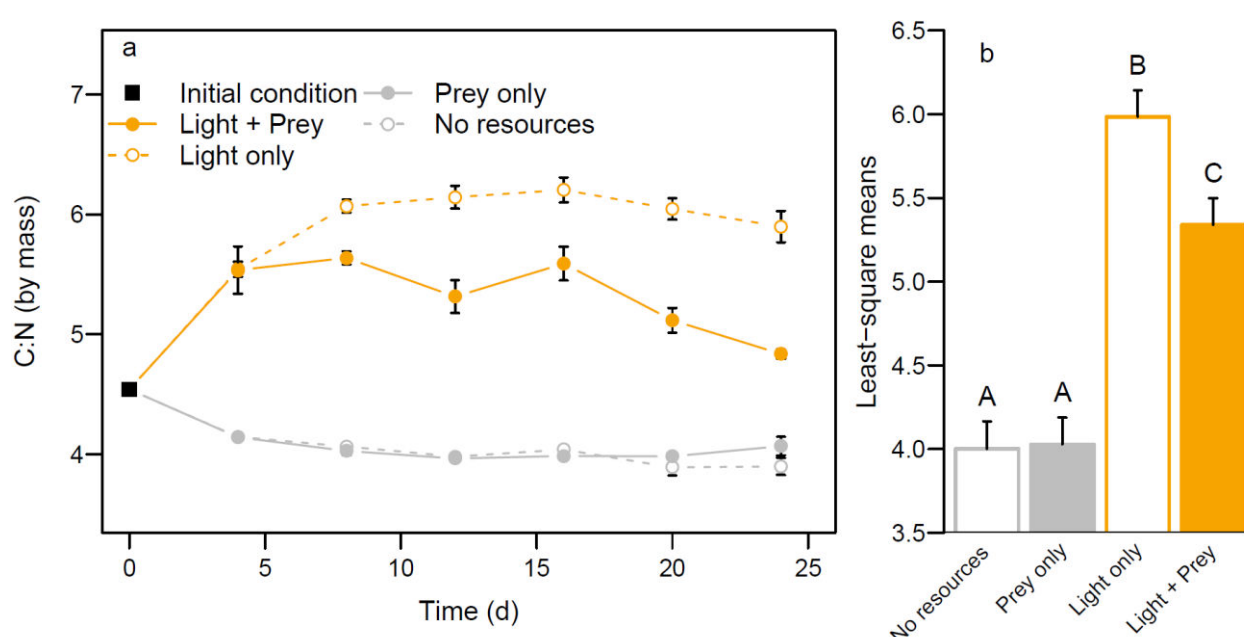


Fig. 3 (a) Changes in the *Cassiopea* sp. medusae mass C:N ratios (means \pm s.e.m.) over the course of the experiment as a function of the experimental conditions. (b) Comparison of the least-square means obtained for each treatment (\pm 95 % C. I.). The letters (A, B, and C) indicate statistically different treatments (Tukey post hoc test, p-value < 0.05)

Fig. 3 (a) Changements des ratios C:N massiques des méduses *Cassiopea* sp. (moyenne \pm erreur standard) durant l'expérience et en fonction des conditions expérimentales. (b) Comparaison des moyennes aux moindres carrés obtenues pour chaque traitement (\pm 95 % I. C.). Les lettres (A, B, et C) indiquent les traitements statistiquement différents (test post hoc de Tukey, valeur-p < 0.05)

The C:N ratios obtained in *Artemia* sp. nauplii did vary significantly during the experiment (ANOVA, $F = 25.9$, $p\text{-value} < 0.001$). However, these variations were of small amplitudes (minimum: 4.97, maximum: 5.20) compared to the variations obtained in *Cassiopea* sp. medusae (Fig. 3a). Throughout the experiment, C:N ratios in *Artemia* sp. nauplii averaged 5.1 ± 0.1 (mean \pm s.d.).

3.4. PAM Parameters of Zooxanthellae

The blanks always yielded low values of F_0 (90 ± 9 ; mean \pm s.e.m.) as compared to the F_0 values of the medusae (5270 ± 630 ; mean \pm s.e.m.). This equates to a signal-to-noise ratio of ca. 60, which is sufficient to have a reliable estimate of photosynthetic activity. Two outliers were removed from the medusae's PAM data (F_v/F_m below 0.4, similar to a blank, most likely due to a lack of exposition of the medusae to the saturating flash). The F_v/F_m of medusae remained very stable in all conditions and during the whole experiment averaging an overall value of 0.70 ± 0.06 (mean \pm s.d.; Fig. S2). The LME models did not indicate any effect of presence or absence of prey and light on the zooxanthellae F_v/F_m .

4. Discussion

4.1. Experimental Culturing Conditions, Growth, and PAM Parameters

The experimental culturing conditions in this experiment were generally satisfying as the medusae from the treatment with light and prey showed positive growth, as shown by the positive evolution of their total carbon mass (Fig. 1) and the survival rate was high (only 1 out of 72 medusae died). An absence of biomass increase in the other treatments confirmed previous findings stipulating that both predation and zooxanthellae's photosynthates are necessary for some zooxanthellate jellyfishes (Kremer 2005, Welsh et al. 2009). The F_v/F_m ratio can be used as a proxy of photosynthetic organism's performance (e.g. Strasser et al. 2000, Long et al. 2018), and was constantly high (0.70, higher or equal to values typically reported for coral zooxanthellae: e.g. Iglesias-Prieto et al. 2004, Roth et al. 2012) in our experiment, independently of treatments (Fig. S2). The absence of decreasing F_v/F_m ratio in the treatments kept in the dark, suggests that zooxanthellae within their *Cassiopea* sp. host stayed

photochemically competent for several days without light. We hypothesize that, in such conditions, as their nutrition can only be provided by the host (e.g. in the form of fatty acids; [Imbs et al. 2014](#)), zooxanthellae were heterotrophic (see [Steen 1986](#), [Jeong et al. 2012](#)).

4.2. Isotopic Composition

The use of stable isotopes as tracers for trophic sources in marine photosymbioses—mainly scleractinian corals—has recently been reviewed by [Ferrier-Pagès and Leal \(2018\)](#). In photosymbioses, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can be affected by autotrophy and heterotrophy through two main processes:

The first process is the mixing of carbon or nitrogen coming from two contrasted sources; autotrophic uptake of dissolved inorganic nutrients, on one hand, and heterotrophic predation—mainly on zooplankton—on the other hand ([Reynaud et al. 2002](#), [Alamaru et al. 2009](#), [Ferrier-Pagès et al. 2011](#), [Ferrier-Pagès and Leal 2018](#)). The uptake of dissolved inorganic carbon by zooxanthellae generally leads to higher $\delta^{13}\text{C}$ values (typically -10 ‰ to -14 ‰) than those of typical oceanic particulate organic matter and plankton (ca. -20 ‰; [Muscatine et al. 1989a](#), [Ferrier-Pagès et al. 2011](#), [Ferrier-Pagès and Leal 2018](#)). Thus, $\delta^{13}\text{C}$ values obtained through zooxanthellae's autotrophy would be higher than those obtained through predation on zooplankton (Fig. 4a). For nitrogen, the pattern is reversed; zooxanthellae take up dissolved inorganic nitrogen with a low $\delta^{15}\text{N}$ value (ca. 5 ‰ [Ferrier-Pagès and Leal 2018](#)) while predation leads the uptake of nitrogen with higher $\delta^{15}\text{N}$ values due to fractionation through the food web ([Post 2002](#), [Ferrier-Pagès et al. 2011](#), [Ferrier-Pagès and Leal 2018](#), Fig. 4b). For both carbon and nitrogen, the isotopic signature of the two sources (inorganic nutrient uptake, and predation) is then exchanged and recycled between the zooxanthellae and the host (e.g. [Reynaud et al. 2009](#)).

The second process involves the depletion of *in-hospite* nutrient pools due to photosynthesis. Zooxanthellae tend to preferentially take up inorganic nutrients with light isotopes resulting in fractionation ([Ferrier-Pagès and Leal 2018](#)). However, at high photosynthesis rates, the host's pool of inorganic nutrients can get depleted. Thus, to meet their photosynthetic requirements, zooxanthellae take up more heavy isotopes, reducing fractionation ("depletion-diffusion hypothesis", see [Muscatine et al. 1989a](#), Fig. 4a and b). This

results in a tendency for isotopic signature to correlate with photosynthesis levels. The higher the photosynthesis, the higher the $\delta^{13}\text{C}$ (Muscatine et al. 1989a, Swart et al. 2005, Alamaru et al. 2009) or the $\delta^{15}\text{N}$ values (Muscatine and Kaplan 1994, Baker et al. 2011; reviewed in Ferrier-Pagès and Leal 2018).

It is important to notice that these two processes—mixing of the heterotrophic and autotrophic sources, and reduced fractionation at high photosynthesis levels—would have similar consequences on $\delta^{13}\text{C}$, but not on $\delta^{15}\text{N}$. For $\delta^{13}\text{C}$, a predominantly autotrophic nutrition would imply a decreased fractionation *in-hospite* due to high photosynthesis and a predominant uptake of dissolved inorganic nutrients. Both of these processes result in high $\delta^{13}\text{C}$ (and *vice-versa* for a predominantly heterotrophic nutrition, Fig. 4a). By opposition, for $\delta^{15}\text{N}$, reduced fractionation due to high photosynthesis levels would result in high $\delta^{15}\text{N}$, but high uptake of dissolved inorganic nutrients would result in low $\delta^{15}\text{N}$ (and *vice-versa* for a predominantly heterotrophic nutrition, Fig. 4b). Thus, to understand how $\delta^{15}\text{N}$ would react to change in holobiont nutrition, it is important to know which of the above-mentioned processes controls its dynamics.

In this study, the $\delta^{13}\text{C}$ values obtained in the medusae were the highest in the treatment with light only, lowest in the treatments with only prey and without resources, and intermediate in the treatment with both prey and light (Fig. 2a and b). Similar effects of heterotrophic feeding on $\delta^{13}\text{C}$ values have been reported for corals (e.g. Reynaud et al. 2002, Ferrier-Pagès et al. 2011). As in this study, the $\delta^{13}\text{C}$ of the predator tended towards the $\delta^{13}\text{C}$ of the prey when fed. However, some caution should be taken when interpreting the results of this study concerning the effects of heterotrophic feeding on zooxanthellate jellyfish's $\delta^{13}\text{C}$. Indeed, it is unsure that the lipid normalization used here can be applied to a photosymbiotic holobiont as it has been derived from the heterotrophic *Aurelia* sp. (D'Ambra et al. 2014). Without this normalization, the effect of heterotrophic feeding on $\delta^{13}\text{C}$ is less clear (Fig. S1). Thus, albeit an effect of heterotrophic feeding on zooxanthellate jellyfishes' $\delta^{13}\text{C}$ is likely, our results should be taken with caution regarding this point. In contrast, light had a clear positive effect on $\delta^{13}\text{C}$ of zooxanthellate jellyfishes whether the normalization for lipid content is made or not (Fig. 2a and b; Fig. S1). These conclusions are consistent with the previous findings on corals (e.g. Muscatine et al. 1989a, Swart et al. 2005, Alamaru et al. 2009, Ferrier-Pagès et al. 2011) and

could be explained by both increased uptake of CO_2 and reduced fractionation at higher photosynthesis (Fig. 4a).

In the experiment, $\delta^{15}\text{N}$ values were the lowest in the medusae exposed to only light, compared to the other treatments (Fig. 2c and d). This suggests that, in zooxanthellate jellyfishes, of the two processes above-mentioned—mixing of autotrophic and heterotrophic sources, and reduced fractionation at high photosynthesis—the former was the dominant one (Fig. 4b). This is different from what is known in tropical scleractinian corals in which photosynthesis tend to increase, or have little effect on $\delta^{15}\text{N}$ rather than decrease it, as seen here (see [Muscatine and Kaplan 1994](#), [Alamaru et al. 2009](#), [Reynaud et al. 2009](#)). Our results are more comparable to what is observed in more heterotrophic temperate corals ([Ferrier-Pagès et al. 2011](#)). The treatment with no resources and the treatment with only prey presented the same $\delta^{15}\text{N}$ (Fig. 2c and d). However, the effect of predation is clear as the $\delta^{15}\text{N}$ values in the treatment with prey and light were intermediate between those of the treatment with only light, and the treatment with only prey (Fig. 2c and d). The similarity between the treatment without resources and the treatment with only prey would thus be explained by the initial condition (i.e. at day 0, medusae already had high $\delta^{15}\text{N}$). Thus, overall, predation would have led to higher $\delta^{15}\text{N}$ of medusae (Fig. 2c and d). Interestingly however, the $\delta^{15}\text{N}$ values in medusae were never higher than those obtained on prey (Fig. 2c), which suggests that no measurable fractionation occurred between the holobiont and their prey. This is most likely due to high recycling of nitrogen between the host and its symbionts (see also [Reynaud et al. 2009](#)). Hence, in zooxanthellate jellyfishes, a predominantly autotrophic nutrition would imply that most nitrogen comes from the fixation of dissolved inorganic nitrogen (see e.g. [Muscatine and Marian 1982](#), [Wilkerson and Kremer 1992](#), [Freeman et al. 2016](#)), and would result in low $\delta^{15}\text{N}$. On the contrary, a predominantly heterotrophic nutrition would imply that more nitrogen comes from predation (mainly on zooplankton) resulting in a comparatively higher $\delta^{15}\text{N}$. Thus, values of $\delta^{15}\text{N}$ can be considered as a good indicator of the relative importance of autotrophy and heterotrophy in zooxanthellate jellyfishes.

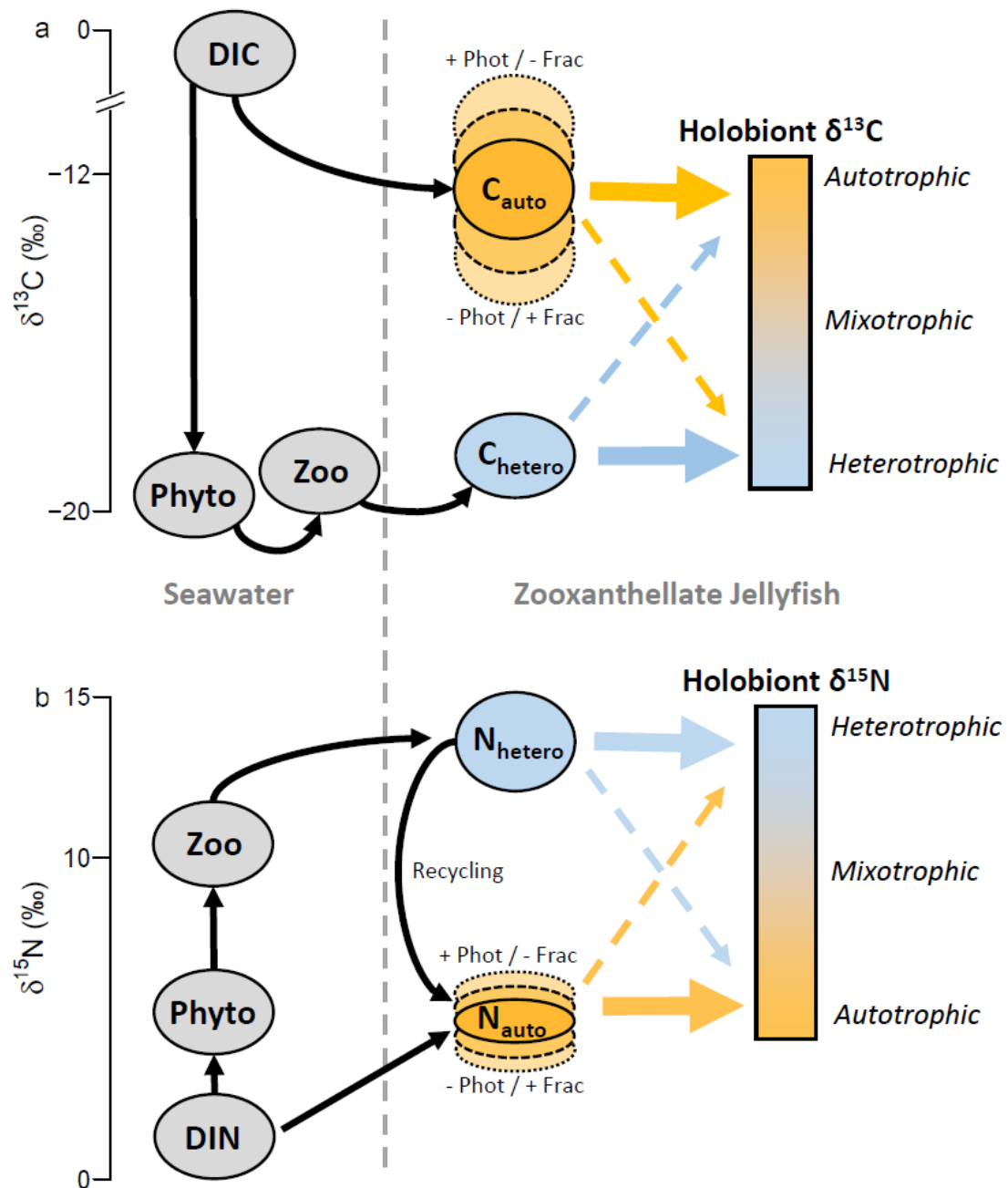


Fig. 4 Conceptual diagram illustrating how $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) are affected by the relative importance of heterotrophic (blue circles and arrows, C_{hetero} and N_{hetero}) and autotrophic (orange circles and arrows, C_{auto} and N_{auto}) nutrition pathways in zooxanthellate jellyfishes. DIC = dissolved inorganic carbon, DIN = dissolved inorganic nitrogen, Phyto = phytoplankton, Zoo = zooplankton, Phot = photosynthesis, Frac = fractionation (more photosynthesis tend to decrease fractionation; this effect, in zooxanthellate jellyfishes, is unlikely to be significant for nitrogen, see text). Values on the axes are provided for illustrative purpose only. See also [Ferrier-Pagès and Leal \(2018\)](#)

Fig. 4 ← Diagramme conceptuel illustrant comment les $\delta^{13}\text{C}$ (a) et $\delta^{15}\text{N}$ (b) sont affectés par l'importance relative de l'hétérotrophie (cercles et flèches bleus, C_{hetero} et N_{hetero}) et de l'autotrophie (cercles et flèches orange, C_{auto} et N_{auto}) chez les méduses à zooxanthelles. DIC = carbone inorganique dissous, DIN = azote inorganique dissous, Phyto = phytoplancton, Zoo = zooplancton, Phot = photosynthesis, Frac = fractionnement (plus de photosynthèse tend à diminuer le fractionnement ; cet effet, chez les méduses à zooxanthelles, est probablement peu important pour l'azote, voir texte). Les valeurs sur les axes ne sont données que dans un but illustratif. Voir aussi [Ferrier-Pagès et Leal \(2018\)](#)

4.3. C:N Ratios

C:N ratios varied in our experiment. Since our measures were performed on the holobionts, we cannot conclusively attribute these variations to the zooxanthellae and/or to the medusae partners. However, variations of C:N ratios due to changes in available resources have already been documented in zooxanthellae (e.g. [Cook et al. 1988](#), [Belda et al. 1993](#), [Hoegh-Guldberg et al. 2004](#)), and also in their host tissues ([Muscattine et al. 1989b](#), [Belda et al. 1993](#), [Alamaru et al. 2009](#)). Moreover, variations in C:N ratios have also been documented in non-zooxanthellate medusae ([Javidpour et al. 2016](#)). We hypothesize that the variations in C:N ratios obtained in our experiment were not only due to zooxanthellae but also to the medusae host, which question a strict homeostasis in these organisms (see also [Persson et al. 2010](#)).

In our experiment, light increased C:N ratios whereas prey generally decreased it, with intermediate C:N ratios in the treatment with both light and prey (Fig. 3a and b). As with $\delta^{15}\text{N}$, C:N ratios of the treatment with no resources and with prey only were similar. This similarity may be again explained by the initial condition. Another potential explanation would be that starved zooxanthellate jellyfishes use first reserves accumulated by photosynthesis (generally carbon rich, e.g. [Muller-Parker et al. 1996](#)). Such preferential degradation would make their C:N ratios decrease and get similar to the ones typically reported for non-zooxanthellate jellyfishes ([Ikeda 2014](#), [Molina-Ramírez et al. 2015](#)). Independently of the treatment with no resources, our results suggest that predation would tend to decrease C:N ratios (Fig. 3a and b). Such a decrease of C:N ratios due to predation have already been reported for the zooxanthellae of a sea anemone ([Cook et al. 1988](#)). However, other studies have pointed out that a similar decrease of C:N ratios can also be due to an enrichment by dissolved inorganic nitrogen (e.g. [Muscattine et al. 1989b](#), [Belda et al. 1993](#)). As zooxanthellate jellyfishes are able

to take up dissolved inorganic nutrients via their symbionts (see e.g. [Muscatine and Marian 1982](#), [Wilkerson and Kremer 1992](#), [Pitt et al. 2005](#), [Welsh et al. 2009](#), [Freeman et al. 2016](#), see [Pitt et al. 2009a](#) for a review), it is likely that their C:N ratios would react to nutrient enrichment too. This suggests that C:N ratios of zooxanthellate jellyfishes might be impacted by nitrogen availability (either as prey or as dissolved inorganic nitrogen).

4.4. Remarks on Tissue Turnover

One of the advantages of the study of the elemental and isotopic composition over e.g. gut content analyses, is that it provides a more time-integrated information ([Pitt et al. 2009b](#)). This is due to the tissue turnover of organisms. In another scyphozoan jellyfish, *Aurelia* sp., the isotopic half-life was determined to be ca. 10 days for both carbon and nitrogen ([D'Ambra et al. 2014](#)). In the present experiment, changes of $\delta^{13}\text{C}$ and C:N ratios occurred very fast (within the first four days, Figs. 2a, 3). This was apparently less true for the $\delta^{15}\text{N}$ values which may have experienced slower changes (Fig. 2b). These fast changes may have several explanations: First, the medusae used here were of small size which can explain the fast changes ([Thomas and Crowther 2015](#)). In its natural environment, *Cassiopea* sp. can grow up to ca. 20-25 cm in bell diameter (see e.g. [Morandini et al. 2017](#)). It is unlikely that such large specimens would display such fast change in composition. Another aspect that could explain the fast change in elemental and isotopic composition observed here, is that *Cassiopea* sp. is zooxanthellate. The zooxanthellae are also likely to impact residence time of elements within the holobiont, possibly differently for nitrogen and carbon, due to recycling ([Reynaud et al. 2009](#)).

4.5. Implications for Fieldwork Studies

One of the challenges to understand the nutrition of zooxanthellate jellyfishes in their natural environments relates to their mixotrophy. As zooxanthellate jellyfishes obtain their nutrition from predation and photosynthesis ([Kremer 2005](#), [Welsh et al. 2009](#)), both processes must be investigated. Ideally, predation, photosynthesis, respiration, nutrient uptake and excretion have all to be measured which may represent an intensive amount of work rarely carried out in its entirety (see however, [Kremer et al. 1990](#), [Kremer 2005](#)). Studies of stable isotopes and

elemental composition are comparatively easier and have the advantage of providing more time-integrated information (Pitt et al. 2009b). The findings of this study provide baseline information on how C:N ratios, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can be interpreted in fieldwork studies focusing on the nutrition sources of zooxanthellate jellyfishes.

To summarize, our results suggest that both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ vary with the relative balance of autotrophy vs. heterotrophy (although, the effect of heterotrophy on $\delta^{13}\text{C}$ is less well supported by our results). It would be expected that, if dominantly heterotrophic, zooxanthellate jellyfishes would have $\delta^{15}\text{N}$ values close to those of their prey (see e.g. Zeman et al. 2018). By opposition, if dominantly autotrophic, zooxanthellate jellyfishes would have $\delta^{15}\text{N}$ values close (or lower) than those of primary producers (see e.g. Freeman et al. 2017). The $\delta^{13}\text{C}$ values would display opposite trends. Finally, C:N ratios may be indicators of the efficiency of nitrogen supplies. Future fieldwork studies would be able to build on these results to better characterize zooxanthellate jellyfishes' nutrition.

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Supplementary Material:

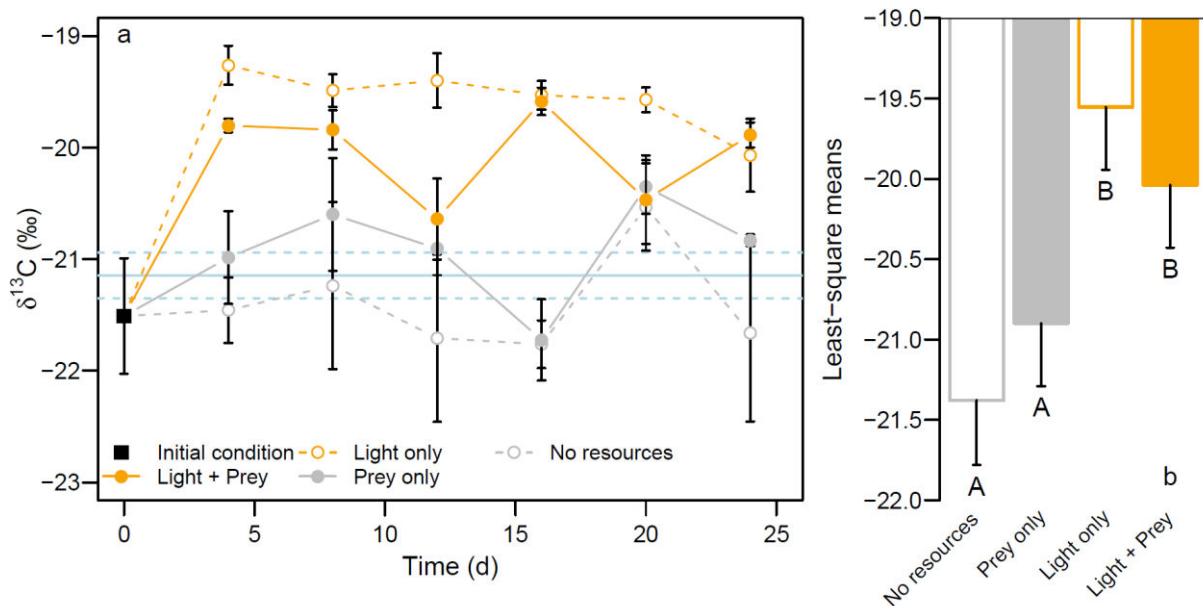


Fig. S1 (a) Changes in the *Cassiopea* sp. medusae $\delta^{13}\text{C}$ (‰) without normalization for lipids (means \pm s.e.m.) over the course of the experiment as a function of the experimental conditions. Solid and dashed blue lines represent the mean \pm s.d. of the isotopic signatures of *Artemia* sp. nauplii used as prey in fed treatments. (b) Comparison of least-square means obtained for each treatment (\pm 95 % C. I.). The letters (A and B) indicate statistically different treatments (Tukey post hoc test, p-value < 0.05)

Fig. S1 (a) Changement dans les $\delta^{13}\text{C}$ (‰) des méduses *Cassiopea* sp. sans normalisation pour les lipides (moyennes \pm erreur standard) durant l'expérience et en fonction des conditions expérimentales. Les lignes bleues continues et pointillées représentent la moyenne \pm écart-type des signatures isotopiques des nauplies d'*Artemia* sp. utilisées comme proies dans les traitements nourris. (b) Comparaison des moyennes aux moindres carrés (\pm 95 % I. C.). Les lettres (A et B) indiquent les traitements significativement différents (test post hoc de Tukey, valeur-p < 0.05)

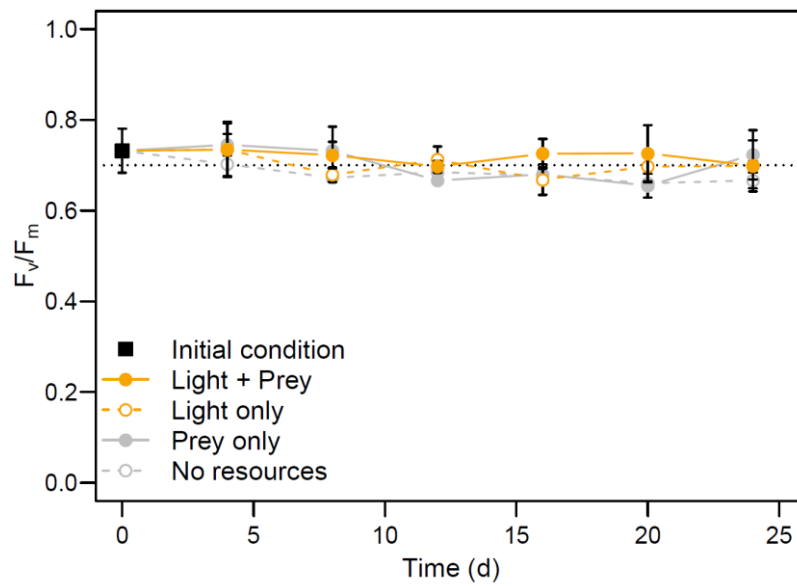


Fig. S2 Absence of evolution in the F_v/F_m ratios of the whole *Cassiopea* sp. medusae (including symbionts) over the course of the experiment (means \pm s.e.m.). The dotted line represents the mean of all points

Fig. S2 Absence de changements des ratios F_v/F_m des méduses *Cassiopea* sp. entières (symbiontes inclus) durant l'expérience (moyennes \pm erreur type). La ligne en pointillés représente la moyenne de tous les points

Chapter IV: Photosynthesis or predation? Nutritional plasticity of Palau's *Mastigias papua* (Scyphozoa: Rhizostomeae) using stable isotopes and fatty acids

Chapitre IV : Photosynthèse ou prédation ? Plasticité nutritionnelle des *Mastigias papua* (Scyphozoa : Rhizostomeae) de Palaos à l'aide d'isotopes stables et d'acides gras

This chapter describes results obtained from the field in Palau and in particular, from marine lakes. Prior to the description of the results, a foreword gives a short description of these marine lakes

Ce chapitre décrit des résultats obtenus sur le terrain à Palaos et en particulier, dans des lacs marins. Avant la description des résultats, un avant-propos donne une rapide description de ces lacs marins.



Foreword: The Marine Lakes of Palau

Geology of Palau and the Formation of the Marine Lakes

The Palau archipelago is located in the Western Pacific Ocean (7° N, 134° E). Palau was formed through the accumulation of basalt from volcanic origin and limestone formed by bioaccumulation in ancient coral reefs (Colin 2009). The geology of the different islands can vary. For instance, the main island of Palau, Babeldaob (Fig. F1), is essentially basaltic. In contrast, the Rock Islands (Fig. F1), south of Babeldaob, are ancient (Miocene, ca. 25 Ma BP), uplifted, coral reefs and are thus constituted mainly of limestone (Colin 2009). That is on these islands that the marine lakes of Palau are found.

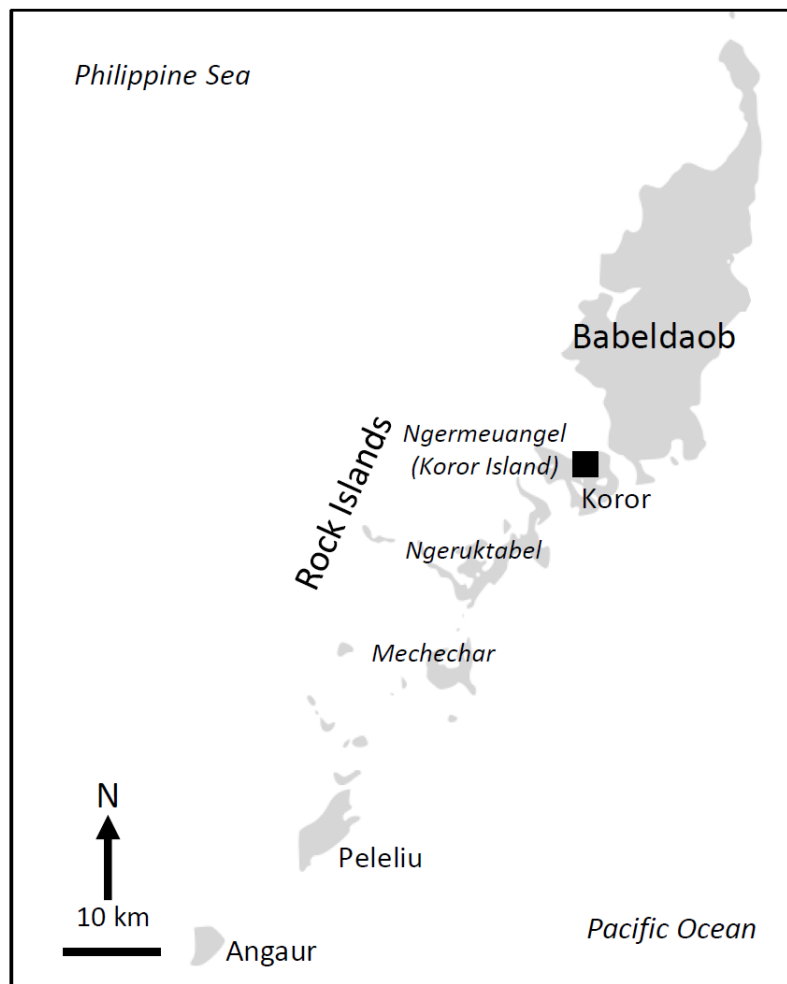


Fig. F1 Map of Palau. Koror = main city. Only the names of the main islands are reported

Fig. F1 Carte de Palaos. Koror = principale ville. Seuls les noms des îles les plus grandes sont indiqués

During the last glacial maximum (ca. 20 000 years ago) the sea level was 120 m below its actual level (Colin 2009, Fig. F2a). Limestone is easily dissolved in water. Hence the Rock Islands are riddled with cracks, crevices, and even cave complexes. When the sea level started to rise again after the glacial maximum, it eventually went above the lowest valley of Palau's Rock Islands. The sea-water reached the valleys through infiltration (or even through tunnels) and, eventually, filled them (Colin 2009, Fig. F2b). This resulted in the formation of the marine lakes between 12 000 and 5000 years ago (Colin 2009) and in the present landscape of Palau (Fig. F2c). Interestingly, this mode of formation of lakes implies that the deepest lakes are also the oldest.

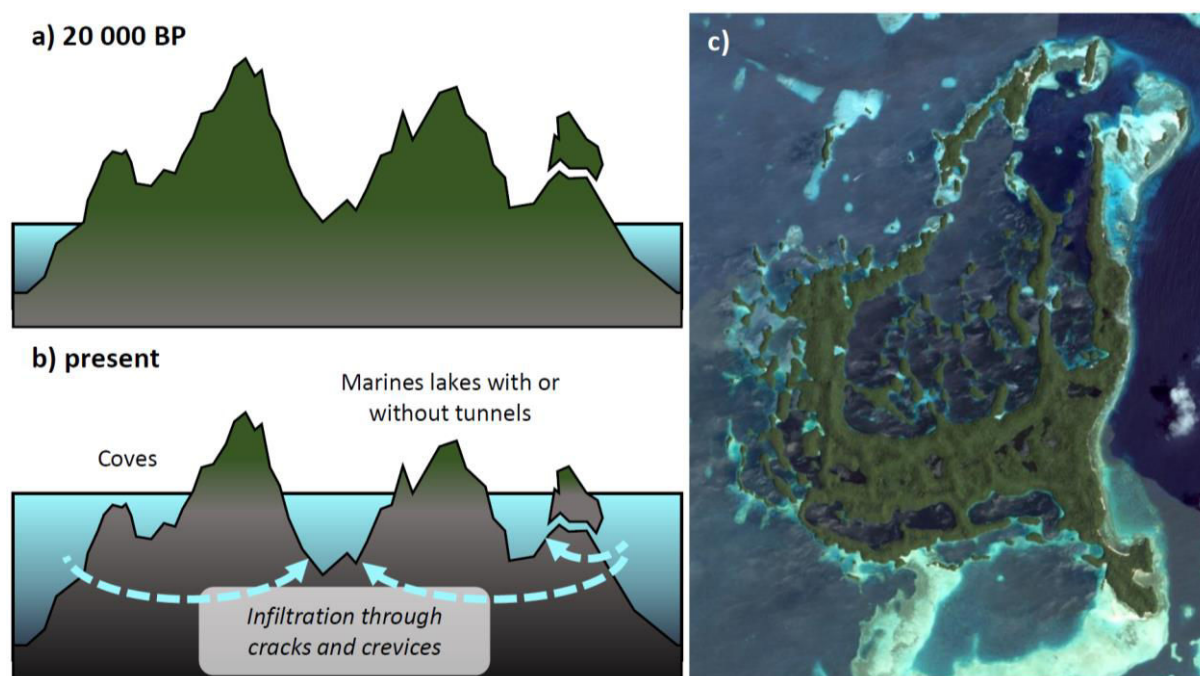


Fig. F2 Formation of the marine lakes of Palau. (a) 20 000 years before present (BP) the sea level was 120 m lower than nowadays. (b) Marine lakes form with rising sea level. (c) Present landscape of Palau (Mechechar Island). Aerial photograph courtesy of Patrick L Colin

Fig. F2 Formation des lacs marins de Palaos. (a) 20 000 avant le présent (BP) le niveau de la mer était 120 m inférieur à ce qu'il est aujourd'hui. (b) Les lacs marins se forment avec la remontée du niveau de la mer. (c) Paysage actuel de Palaos (île de Mechechar). Photographie aérienne fournie par Patrick L Colin

Physical and Chemical Characteristics of the Marine Lakes

The marine lakes of Palau can be classified as holomictic (mixed) or meromictic (stratified, [Hamner and Hamner 1998](#)). Several factors such as depth, exposure to wind, or to tidal currents (through a tunnel for instance) or the residence time of sea-water determine whether a lake is holomictic or meromictic ([Hamner and Hamner 1998](#), [Colin 2009](#)). Additionally, the distance of a lake from the sea affects the balance between freshwater (from rain) and salt-water inputs. Therefore, the specific topography and location of each lake has an important impact on the characteristics of its water column. This results in different profiles of salinity and temperatures which in turn imply differences in the biology from one lake to another (Fig. F3).

In many of the meromictic lakes of Palau the pycnocline is also a chemocline ([Hamner et al. 1982](#), [Hamner and Hamner 1998](#)). Above the pycnocline (in the mixolimnion) the water is well oxygenated but below the pycnocline (in the monimolimnion), it is anoxic. In between these two layers of waters is often found a thick layer of purple sulfur bacteria ([Hamner et al. 1982](#), [Hamner and Hamner 1998](#), [Meyerhof et al. 2016](#)).

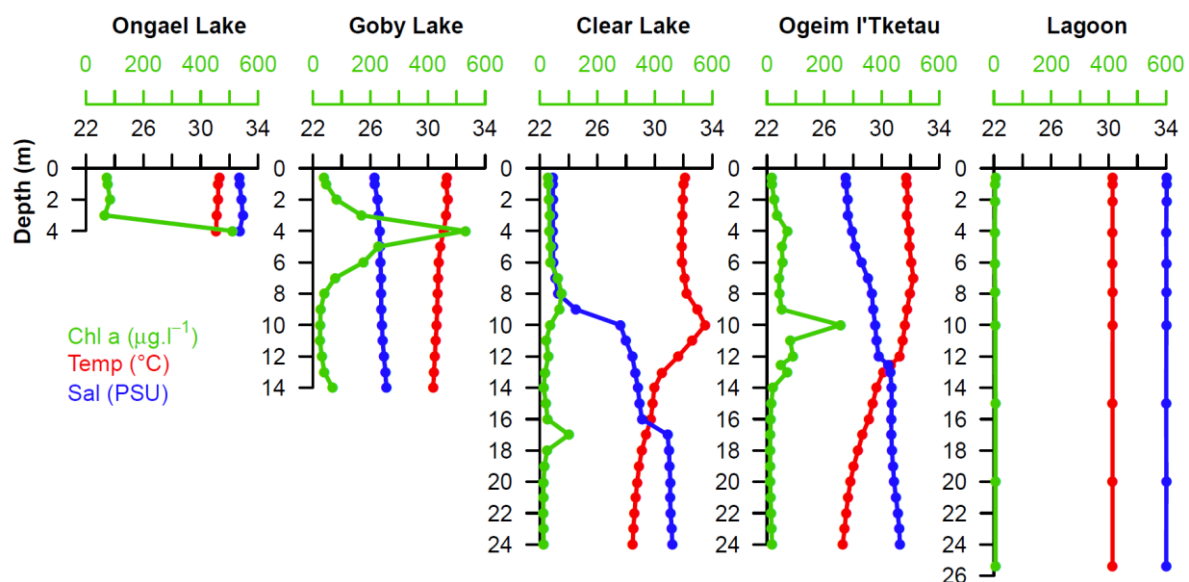


Fig. F3 Temperature (red), salinity (blue) and chlorophyll a (green) profiles in four Palauan marine lakes and in the lagoon. Data courtesy of Gerda Ucharm and Sharon Patris (CRRF)

Fig. F3 Profils de température (rouge), salinité (bleu) et chlorophylle a (vert) dans quatre lacs marins et dans le lagon paluan. Données fournies par Gerda Ucharm et Sharon Patris (CRRF)

Biological Diversity in the Marine Lakes

The diversity of species found in the marine lakes primarily depends on their distance and connection to the sea (Colin 2009). The more a lake is connected to the sea, the more diverse its biological communities. For instance, in lakes connected through a tunnel to a lagoon, species of scleractinian corals can be found (e.g. the solitary coral *Heliofungia* gives its name to one Palauan lake, Colin 2009). By contrast, in more isolated lakes, the biological communities are less diverse. The distance from a lake to the sea acts thus as a filter (through diverse mechanisms: filter-feeding communities in tunnels, filtering during the infiltration of seawater, temperature and salinity differences, Colin 2009). Then, selection pressures within the lake (e.g. anoxic layer in meromictic lakes) may further shape the biological communities.

This also implies that the more a lake is isolated, the more it depends on the surrounding tropical rainforest for nutrients inputs (Herwig Stibor, unpublished isotopic data). Trees around the lakes also furnish an important habitat for benthic species (especially relevant in lakes with an anoxic deep layer). Dead branches and trunks are indeed colonized by sponges, algae or mussels (Colin 2009). Another habitat provided by the trees, is the roots of mangrove trees (*Rhizophora* sp.) which surrounds the lakes (Fig. F4a).

One aspect of the Palauan marine lakes particularly relevant to this thesis is that some of them host populations of the zooxanthellate jellyfish *Mastigias papua* (Colin 2009). The polyps live in the benthic compartment, attached to roots, rocks or decaying wood and leaves. The medusae live in the pelagic compartment and display behavioral adaptation to avoid the edges of the lake (Dawson and Hamner 2003). These medusae populations can reach important densities (Fig. F4b). This is due to the closed nature of the lake which avoids export of individuals and to low predation pressure (Hamner et al. 1982). In fact, the only predator of the medusae known in Palauan lakes is the anemone *Entacmaea medusivora* (Fautin and Fitt 1991, Fig. F4c).

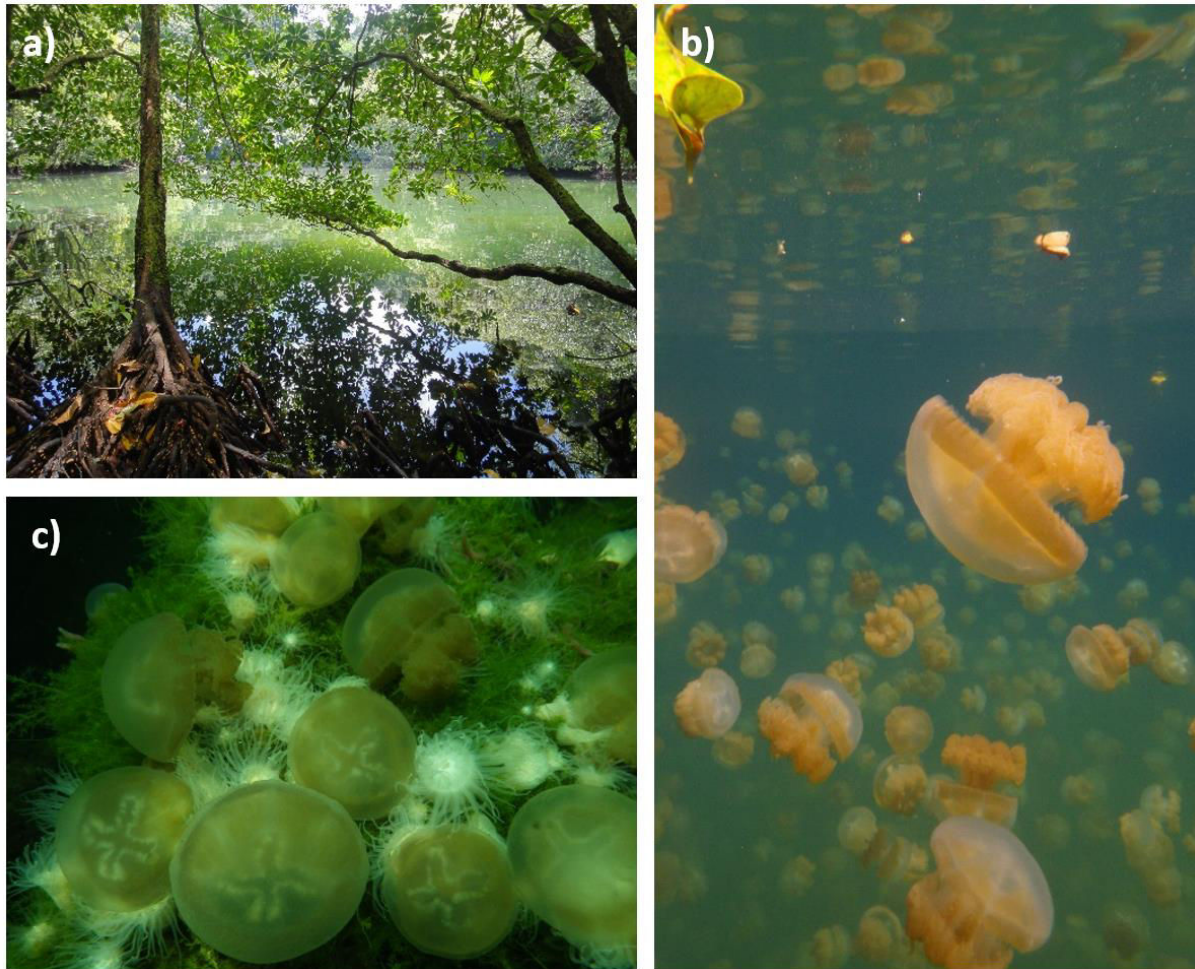


Fig. F4 The marine lakes of Palau as habitat for *Mastigias papua*. (a) Mangrove vegetation surrounding Goby Lake. (b) *Mastigias papua etpisoni* medusae population and (c) its predator (the anemone *Entacmaea medusivora*) in Ongeim'l Tketau. Photographs (a) and (b) by Philippe Pondaven and photograph (c) by Herwig Stibor

Fig. F4 Les lacs marins de Palaos en tant qu'habitat pour *Mastigias papua*. (a) Végétation de type mangrove autour de Goby Lake. (b) Population de méduses *Mastigias papua etpisoni* et (c) leurs prédateurs (l'anémone *Entacmaea medusivora*) dans Ongeim'l Tketau. Photographies (a) et (b) prises par Philippe Pondaven et photographie (c) par Herwig Stibor

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Photosynthesis or predation? Nutritional plasticity of Palau's *Mastigias papua* (Scyphozoa: Rhizostomeae) using stable isotopes and fatty acids

Photosynthèse ou prédation ? Plasticité nutritionnelle des *Mastigias papua* (Scyphozoa : Rhizostomeae) de Palaos à l'aide d'isotopes stables et d'acides gras

It is planned to submit the results of this section in two articles, one focused on nutritional plasticity of *Mastigias papua*, the other describing a density-dependent mechanism affecting the nutrition

Il est prévu de soumettre cette section en en deux articles, le premier centré sur la plasticité nutritionnelle de *Mastigias papua*, le second décrivant un mécanisme densité-dépendant affectant la nutrition

Djeghri N¹, Pondaven P¹, Le Grand F¹, Bideau A¹, Duquesne N¹, Stockenreiter M², Behl S², Huang Y-T², Hansen T³, Patris S⁴, Ucharm G⁴, Stibor H² (in preparation) Photosynthesis or predation? Nutritional plasticity of Palau's *Mastigias papua* (Scyphozoa: Rhizostomeae) using stable isotopes and fatty acids

Djeghri N¹, Pondaven P¹, Stockenreiter M², Behl S², Huang Y-T², Hansen T³, Patris S⁴, Ucharm G⁴, Stibor H² (in preparation) Density-dependent nutrition in a mixotrophic jellyfish holobiont

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Abstract

Jellyfishes (i.e. pelagic cnidarians) are recognized as important pelagic predators. However, many species use an additional nutrition source through symbiosis with Symbiodiniaceae (zooxanthellae). These zooxanthellate jellyfishes are thus mixotrophs as holobionts (i.e. symbionts and host considered as one organism). Extreme cases of zooxanthellae presence or absence in their jellyfishes' host suggest that this mixotrophy is, however, highly plastic. In this study, we take advantage of the marine lakes and the lagoon of Palau (Micronesia) to attempt to characterize this plasticity of the nutrition in the zooxanthellate jellyfish *Mastigias papua*. We sampled *Mastigias papua* medusae in five different locations from the Palau archipelago in 2018 and characterized their nutrition using isotopic (bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), elemental (C:N ratios), and fatty acid compositions, as indicators of their nutrition. We found that the different indicators used were well correlated, allowing for a robust characterization of the nutrition of *Mastigias papua*. We observed a high variability in their nutrition, from pure heterotrophy to dominant autotrophy, between the different populations of *Mastigias papua*. In addition, some observations from previous years suggest that the nutrition of *Mastigias papua* medusae is not fixed in a given population and can vary over time. This evidences that the variability documented here is due to a plasticity of the nutrition rather than to genetic differences between populations. Some possible environmental drivers of the plasticity of *Mastigias papua* nutrition are explored and, using a data-set from 2010 to 2018, the role of density-dependent processes is demonstrated in one of the populations.

Résumé

Les méduses (i.e. cnidaires pélagiques) sont aujourd'hui reconnues comme prédateurs pélagiques importants. Toutefois, de nombreuses espèces utilisent une ressource additionnelle dans leur nutrition *via* la symbiose avec des Symbiodiniaceae (zooxanthelles). Ces méduses à zooxanthelles sont donc mixotrophes en tant qu'holobiontes (i.e. symbiontes et hôte considérés comme un seul organisme). Des cas extrêmes de présence ou d'absence de zooxanthelles dans les méduses hôtes suggèrent que cette mixotrophie est toutefois, hautement plastique. Dans cette étude, nous tentons de caractériser la plasticité de la nutrition de la méduse à zooxanthelles *Mastigias papua*. Pour cela, nous avons échantillonné les méduses de l'espèce *Mastigias papua* dans cinq sites de l'archipel de Palaos (lacs marins et lagon) en 2018 et caractérisons leur nutrition à l'aide de leurs compositions isotopique ($\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ totaux), élémentaire (ratios C:N), et en acides gras, utilisées comme indicateurs de la nutrition. Les différents indicateurs utilisés étaient fortement corrélés ce qui a permis une caractérisation robuste de la nutrition de *Mastigias papua*. Nous avons observé une importante variabilité dans la nutrition entre les différentes populations de *Mastigias papua* qui peut aller de la pure hétérotrophie à une nutrition dominée par l'autotrophie. De plus, certaines observations antérieures montrent que la nutrition de *Mastigias papua* n'est pas fixée dans une population mais peut varier dans le temps. Cela démontre que la variabilité documentée ici est due à une plasticité de la nutrition plutôt qu'à des différences entre les populations. Quelques déterminants environnementaux possibles pour cette plasticité sont explorés et, en utilisant un jeu de données de 2010 à 2018, le rôle de processus densité-dépendants est démontré dans l'une des populations.

1. Introduction

Metazoan-dinoflagellate photosymbioses are important relationships in shallow tropical seas (Venn et al. 2008). Scleractinian corals, because of the ecological significance of the reefs they build, have been the most studied. However, a diversity of other hosts exists, displaying a wide variety of traits, life-histories, and ecologies. Studying such different hosts, with contrasting ecologies, has the potential to illuminate our understanding of what are the conditions in which photosymbiosis is advantageous or not. Zooxanthellate jellyfishes are an example of a host contrasting with the more traditionally studied scleractinian corals. Their ecology is intriguing; they contrast with scleractinian corals by being, at the medusae phase, pelagic, annual, and fast growing (Djeghri et al. 2019). They also contrast with other photosymbiotic, or more generally, mixotrophic plankters (see Stoecker et al. 2017) by their larger size. However—with the exception of the unusual benthic jellyfish *Cassiopea* spp. (see Ohdera et al. 2018)—zooxanthellate jellyfishes remain little studied.

Zooxanthellate jellyfishes derive their nutrition from both predation and their symbiont's photosynthesis (Kremer 2005, Welsh et al. 2009), making them mixotrophs as holobionts (i.e. symbionts and host considered as one organism). This general picture may hide many variations in the nutrition of these organisms. For instance, within the same species, some populations or individuals can be non-zooxanthellate while other are zooxanthellate (Dawson et al. 2001, Bolton and Graham 2004). These extreme cases suggest that zooxanthellate jellyfishes can display important plasticity (sensu Levis and Pfennig 2016) in their nutrition; i.e. from complete heterotrophy to mostly autotrophy (Djeghri et al. 2019). Similarly, variations in the symbiont contribution to nutrition have been documented along ontogeny and size gradients (Sugiura 1969, McCloskey et al. 1994). Such high plasticity in nutrition is known in scleractinian corals (e.g. Teece et al. 2011, Fox et al. 2019) but, stays poorly characterized in zooxanthellate jellyfishes due to a small number of comparative studies available (see however McCloskey et al. 1994). A high plasticity in nutrition has important ecological consequences. In scleractinian corals, nutritional plasticity plays an important role in resistance to, or recovery after, bleaching events (Grottoli et al. 2006). In zooxanthellate jellyfishes, the nutritional plasticity could impact their reactions to environmental perturbations (e.g. eutrophication, Stoner et al. 2011; temperature fluctuations, Dawson et al. 2001), their blooming ability (Dawson and Hamner 2009) or their invasiveness (Bayha and

Graham 2014). In order to characterize and understand the nutritional plasticity of zooxanthellate jellyfishes, comparative studies assessing the nutrition of zooxanthellate jellyfishes in contrasted environments, are thus needed.

In this study, we compare the nutrition of different populations of the zooxanthellate jellyfish *Mastigias papua* (Lesson 1830) medusae from different locations from Palau (Micronesia) (see Souza and Dawson 2018 for a recent redescription of the species). Palau is characterized by the presence of many marine lakes formed between 12 000 and 5 000 years ago as a consequence of rising sea levels after the last glacial maximum (Dawson and Hamner 2005). This has led to the isolation of several *Mastigias papua* populations in different lakes from the original lagoon population (Swift et al. 2016). The lagoon and lake jellyfishes are considered as different ecotypes (Swift et al. 2016), and the populations of five marine lakes are described as distinct sub-species (Dawson 2005, Table 1). In these “jellyfish” lakes, the *Mastigias papua* populations can reach millions of individuals (Hamner et al. 1982, Cimino et al. 2018). The different locations inhabited by *Mastigias papua* in Palau represent different environments (Hamner and Hamner 1998), submitting the jellyfishes to different access to resources (i.e. light, dissolved inorganic nutrients, prey), and to different stresses (e.g. temperature, see Dawson et al. 2001). The combination of a recent genetic isolation and contrasted ecological constraints makes Palau’s *Mastigias papua* populations a promising case-study to better understand zooxanthellate jellyfishes’ nutritional plasticity.

In order to investigate the nutrition of *Mastigias papua* medusae, and the relative contribution of autotrophy and heterotrophy, we used stable isotopes and fatty acids analyses. Both stable isotopes (mainly bulk $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, reviewed in Ferrier-Pagès and Leal 2018) and fatty acids (e.g. Treignier et al. 2008, Mortillaro et al. 2009) can be affected by autotrophic and heterotrophic processes, making them indicators of variations of the nutrition of photosymbiotic organisms (Imbs et al. 2010, Seeman et al. 2013, Ferrier-Pagès and Leal 2018, Mies et al. 2018, Radice et al. 2019). These two types of tracers have also been emphasized as valuable tools for the study of jellyfish nutrition (Pitt et al. 2009). In this study, the simultaneous use of these two tracers has the potential to provide complementary information and independent confirmation to our conclusions. By studying the stable isotope signatures and fatty acid profiles of *Mastigias papua* in contrasted habitats from Palau, we aim to evaluate the plasticity of its nutrition and investigate its potential causes.

2. Material and Methods

2.1. Sampling

In late August and early September 2018, sampling for *Mastigias papua* medusae was conducted in four different marine lakes (lake nomenclature follows Dawson 2005, Table 1) and three lagoon-connected sites in the Koror state, Palau (Micronesia, Fig. 1). The medusae from the three lagoon-connected sampling sites were considered representative of the lagoon population and were thus pooled together for analysis (hereafter referred to as “Lagoon”). In addition to this main sampling, we present isotopic and elemental data (bulk $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and, C:N ratios) from medusae collected in Ongeim’l Tketau (OTM) during five, earlier, field sampling programs: August–September 2010, July–August 2011, August 2013, April 2015 and April 2016.

Mastigias papua medusae were either sampled directly by hand while snorkeling (mainly for medusae sampled in lakes) or using a dip net from a boat (mainly for medusae sampled in lagoon-connected sites). A total of 146 individuals, of varied sizes, were collected. The number sampled varied from one sampling site to another (Table 1). Once sampled, the medusae were then brought back to the laboratory within 2 h in a container filled with local sea-water. Upon arrival in the laboratory, the medusae were measured (umbrella diameter), weighed and dissected.

The zooplankton communities of marine lakes and lagoon were sampled using different plankton nets as a function of material availability and years (mesh sizes comprised between 63 and 200 μm).

Table 1 Names, location, and sampling effort of the populations and sub-species of *Mastigias papua* from Palau (see Dawson 2005, Souza and Dawson 2018)

Table 1 Noms, position, et effort d’échantillonnage des différentes populations et sous-espèces de *Mastigias papua* de Palau (voir Dawson 2005, Souza and Dawson 2018)

| Populations and sub-species | Location | Number sampled |
|--|--|----------------|
| <i>M. papua</i> (ancestral population) | Lagoon, coves | 13 |
| <i>M. papua remeliiki</i> | Uet era Ngermeuangel, Koror Island (NLK) | 41 |
| <i>M. papua nakamurai</i> | Goby Lake, Koror Island (GLK) | 37 |
| <i>M. papua etpisoni</i> | Ongeim’l Tketau, Mechechar Island (OTM) | 35 |
| <i>M. papua saliii</i> | Clear Lake, Mechechar Island (CLM) | 20 |

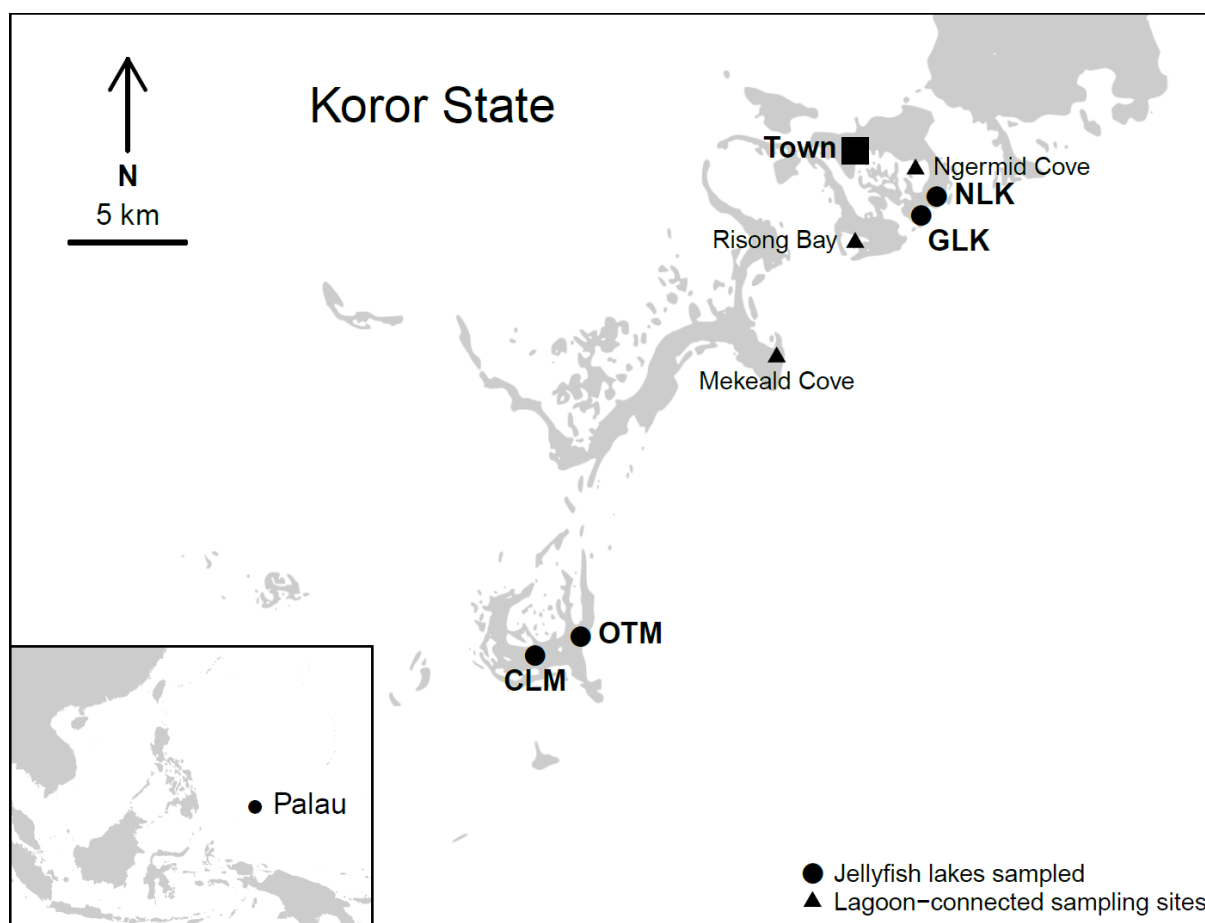


Fig. 1 Map of sampling sites in the Koror state, Palau. NLK = Uet era Ngermeuangel, GLK= Goby Lake, OTM = Ongeim'l Tketau, CLM = Clear Lake

Fig. 1 Carte des sites d'échantillonnage dans l'état de Koror à Palaos. NLK = Uet era Ngermeuangel, GLK= Goby Lake, OTM = Ongeim'l Tketau, CLM = Clear Lake

2.2. Elemental and Isotopic Analyses

2.2.1. Preparation of samples

Pieces of medusae umbrella were placed in aluminum foil and oven-dried at 60 °C for 24 h. For the smallest medusae, organs from two to five different individuals were pooled to ensure enough biomass was collected. After drying, the aluminum foil was folded and samples were stored. Between 820 and 1850 µg of dry medusa tissues (1160 ± 160 µg, mean \pm s.e.) was scratched from the aluminum foil and transferred to tin capsules (8 × 5 mm EMAL technology, United Kingdom) for elemental and isotopic analyses (carbon and nitrogen mass, bulk $\delta^{13}\text{C}$

and $\delta^{15}\text{N}$). During this process, the samples were only dried (i.e. no congelation or preservation in fixatives) following the recommendations of [Fleming et al. \(2011\)](#). In order to keep our methodology consistent across the years, we did not follow the more recent recommendations for the preparation of jellyfish stable isotopes samples ([Kogovšek et al. 2014](#), [MacKenzie et al. 2017](#)).

Individual zooplankters were sorted by taxon (e.g. copepods, chaetognaths, decapod larvae) using pliers under a dissecting microscope. The zooplankters belonging to different groups were then placed in different tin capsules and oven-dried at 60 °C for 24 h. The capsules were then closed for storage before analysis. Importantly, this taxonomic sorting corrected for the bias induced by the use of different plankton nets which would have sampled different size classes.

2.2.2. Elemental and isotopic analyses

Elemental and isotopic analyses of the medusae samples from 2018 were analyzed using an elemental analyzer (Thermo Scientific EA Flash 2000) coupled to a gas isotope ratio mass spectrometer (Thermo Scientific DELTA V Plus) at the stable Isotopes Laboratory of the “Pôle Spectrométrie Océan” (PSO-IUEM, Plouzané, France).

Elemental and isotopic analyses of the zooplankton samples of 2018 and of all samples from 2010 to 2016 were analyzed by a high sensitivity elemental analyzer ([Hansen and Sommer 2007](#)) connected to a gas isotope ratio mass spectrometer (Delta^{Plus} Advantage, Thermo Fisher Scientific) at GEOMAR, Helmholtz Centre for Ocean Research, Kiel, Germany.

Stable isotope values are expressed as permil (‰) using the δ notation (normalized to Vienna Pee Dee Belemnite and atmospheric N_2 for respectively carbon and nitrogen):

$$\delta X = \left(\frac{X_{\text{sample}}^H / X_{\text{sample}}^L}{X_{\text{std}}^H / X_{\text{std}}^L} - 1 \right) \times 1000$$

With X the element measured, X^H the amount of heavy isotope and X^L the amount of light isotope from the samples (X_{sample}) and standard (X_{std}). All C:N ratios are expressed by mass.

An internal standard—casein (Analysentechnik, Germany) in the “Pôle Spectrométrie Océan”, and acetanilide (Merck, Germany) in GEOMAR—was measured every sixth sample within each

sample run. The overall standard deviations measured in the “Pôle Spectrométrie Océan” were of ± 0.12 ‰ and ± 0.24 ‰ for respectively, nitrogen and carbon. At GEOMAR, the overall standard deviation for the low measurement range 2.5-8 $\mu\text{g N}$ and 5.0-80 $\mu\text{g C}$ was ± 0.25 ‰ and ± 0.2 ‰, respectively. The overall standard deviation for the higher measurement range 3-15 $\mu\text{g N}$ and 10.0- 140 $\mu\text{g C}$ was ± 0.2 ‰ and ± 0.15 ‰, respectively.

2.2.3. Data treatment

To make comparable the different sampling sites, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of copepods were subtracted from the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of medusae of the corresponding site (thus correcting for the isotopic baseline). It is important to mention here that, due to technical difficulties, copepod stable isotope signature is unavailable for Goby Lake (GLK) in 2018. We thus used data from previous years as a baseline.

2.3. Fatty Acids Analyses

Preparation and analysis of fatty acids samples were performed at the LIPIDOCEAN technical facility (Lemar, IUEM, Plouzané, France). The protocol used follows [Le Grand et al. \(2014\)](#) and [Mathieu-Resuge et al. \(2019\)](#) with slight modifications. All the glassware was heated 6 h at 450 °C prior to be used and all the solvents used were HPLC grade.

2.3.1. Extraction of lipids

A piece (0.8 g) of wet tissue was cut from the umbrella of the medusae. For the smallest medusae, tissues from several individuals were pooled to reach 0.8 g. Due to field constraints, these samples were first frozen at -20 °C for a few days before being placed in 6 ml of chloroform:methanol solution (2:1; v:v) to allow good preservation and extraction of the lipids. The preserved samples were then stored at -20 °C until preparation. The samples were agitated during 20-30 min to ensure a good lipid extraction prior to the next steps.

2.3.2. Separation of neutral and polar lipids

Samples were centrifuged at 1512 g for 15 min to separate the organic, lipid-containing phase from the aqueous phase containing medusae water. Half of the organic phase volume (3 ml) was transferred to 7 ml flasks. The content of the flasks was then evaporated to dryness in a centrifuge evaporator (Genevac EZ-2.3; SP Scientific), recovered with three washes of 0.5 ml of chloroform:methanol solution (98:2; v:v) and deposited at the top of a silica gel column (40 × 4 mm, silica gel 6 nm pore size, 63-200 µm particle size, deactivated with 6 % H₂O). Neutral lipids (NL) were eluted first, using 10 ml of a chloroform:methanol solution (98:2; v:v). Polar lipids (PL) were eluted second using 20 ml of methanol. Then, 2.3 µg of C23:0 (tricosanoic acid) internal standard was added to the NL and PL fractions.

2.3.3. Transesterification

The NL and PL fractions were then evaporated to dryness in a centrifuge evaporator (Genevac EZ-2.3; SP Scientific) and transesterified to obtain fatty acids methyl esters (FAME). The transesterification was performed by adding 0.8 ml of H₂SO₄:methanol (3.4 %; v:v), mixing, and heating at 100 °C for 10 min. After cooling, 0.8 ml of hexane and 1.5 ml of hexane-saturated distilled water were added. The vials were then homogenized by hand and centrifuged at 378 g for 1 min. The lower aqueous phase (without FAME) was discarded. The last step of adding hexane-saturated distilled water, homogenizing, and centrifuging, was repeated two more times to wash the FAME-containing phase. FAME were then analyzed by gas chromatography.

2.3.4. Fatty acids analysis by gas chromatography

FAME were analyzed in a Varian CP8400 gas chromatograph equipped with two splitless injectors (temperature = 220 °C), two flame-ionization detectors (temperature = 280 °C) and two parallel columns; one polar (DB-WAX; 30 m length, 0.25 mm diameter, 0.25 µm film thickness, Agilent), and one neutral (DB-5; 30 m length, 0.25 mm diameter, 0.25 µm film thickness, Agilent). This two columns set-up allows a better identification of FAME, and to solve co-elution problems. The gas chromatograph oven was programmed in temperature

(60-150 °C at 50 °C.min⁻¹, 150-170 °C at 3.5 °C.min⁻¹, 170-185 °C at 1.5 °C.min⁻¹, 185-225 °C at 2.4 °C.min⁻¹ and finally 225-250 °C at 5.5 °C.min⁻¹ and maintained for 15 min). The gaz vector used was dihydrogen (H₂). FAME were identified by their retention time compared to commercial standards (Supelco 37 Component FAME Mix, the PUFA n° 1 and 3 and the Bacterial Acid Methyl Ester Mix from Sigma) and laboratory-made intern standard mixtures obtained from marine animals, micro- and macroalgae.

2.3.5. Data treatment

FAME abundances were quantified, in each sample, by comparing the area of the corresponding peaks to the area of the peak corresponding to the 2.3 µg of the C23:0 internal standard.

Neutral (NLFA) and polar lipids' fatty acids (PLFA) were separated during the processing of the samples (see section 2.3.2.). NLFA and PLFA masses were added to obtain the composition of total lipids' fatty acids (TLFA). The proportion of the different fatty acids (FA) were then expressed as percentage of either NLFA, PLFA or TLFA. Only FAs with at least one sample contributing to more than 2 % of either NLFA or PLFA compositions were kept in the analyses.

Several indicators were then computed: The ratio of NLFA in TLFA (NLFA:TLFA). The ratio of n-3 polyunsaturated fatty acids (PUFA) on n-6 PUFA (n-3:n-6 ratio), and the ratios of eicosapentaenoic acid (20:5n-3, EPA) on docosahexaenoic acid (22:6n-3, EPA:DHA ratio). And finally, the average number of unsaturation per FA (U), calculated as:

$$U = \frac{\sum P_{FAn} \times n}{\sum P_{FAn}}$$

With P_{FAn} the proportion of a given fatty acid of unsaturation n .

Note that, the n-3:n-6 and EPA:DHA ratios, and the average unsaturation are available for NLFA and PLFA. These indicators, have the potential to yield valuable information on the nutrition (e.g. [Rocker et al. 2019](#)) or stress (e.g. [Tchernov et al. 2004](#)) of zooxanthellate jellyfishes. This is discussed further and detailed in section 4.1.2.

2.4. Chlorophyll a Measures and Zooxanthellae Presence or Absence

The *Mastigias papua saliii* collected from Clear Lake (CLM) in 2018 were unpigmented and transparent as opposed to medusae collected from the other sites. This suggested that these were non-zooxanthellate. In addition to visual examination, the presence of numerous zooxanthellae in their tissue was tested by comparing the chlorophyll a content of medusae sampled from the different sites in 2018.

For this purpose, *Mastigias papua* tissues not used for isotopic or fatty acids analyses were frozen at -20 °C. Samples were subsequently shipped to the laboratory where they were lyophilized and grinded to a powder. For each sampling site, five medusae of intermediate size (ca. five cm in bell diameter) were selected for chlorophyll a and pheophytin measurements. Chlorophyll a and pheophytin concentrations were then obtained by following the protocols of [Holm-Hansen et al. \(1965\)](#) and [Lorenzen \(1967\)](#). Pigments were extracted from 3 mg of powder in acetone for ca. 12 h at 6 °C in the dark. After extraction, the samples were centrifuged (3 min at 1814 g) and the fluorescence at 665 µm of the supernatant was measured before and after acidification in a fluorimeter. Chlorophyll a and pheophytin concentrations were subsequently calculated using the equations given in [Lorenzen \(1967\)](#). For each medusae, three replicates were made in order to deal with possible intra-individual variability.

2.5. 2010-2018 Data and Population Density

A potential link between the isotopic and elemental composition of medusae and their population density was investigated using data from *Mastigias papua etpisoni* oral arms (instead of umbrella, see Appendix) from OTM between 2010 and 2018. To make the different years comparable, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of copepods were subtracted to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of medusae of the corresponding year, thus correcting for the potential shifts in the isotopic baseline. The *Mastigias papua etpisoni* population density in OTM is evaluated monthly by the Coral Reef Research Foundation (CRRF) through net sampling (15 sampling points, replicated three times during the day, using a 50 cm diameter and 1 mm mesh size zooplankton net, Martin et al. 2006, Cimino et al. 2018). The population density used here are the evaluation the closest in time to the sampling date for isotopic and elemental composition.

2.6. Statistics

2.6.1. Isotopic, elemental and fatty acids indicators

Different indicators have been collected using the isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), elemental (C:N ratios) and fatty acid compositions of the medusae. These are: corrected $\delta^{13}\text{C}$, corrected $\delta^{15}\text{N}$, C:N, NLFA:TLFA, n-3:n-6, EPA:DHA, and average unsaturation the three latter being available for the two FA fractions (NLFA and PLFA). The effect of sampling site and medusae wet mass was tested on these indicators using ANCOVAs with permutations (10 000 permutations). Medusae wet mass was Log_{10} transformed to improve linearity and was used as the covariate whereas sampling site was used as the categorical factor. If Log_{10} transformed medusae wet mass was found to have a significant effect (either alone or through interaction with the sampling site), least-squares means (i.e. means corrected for the effect of medusae wet mass) were then compared using Tukey post-hoc tests. If Log_{10} transformed medusae wet mass was not found to have a significant effect, the statistical model was simplified to assess only the effect of sampling site. If normality and homoscedasticity were respected (assessed respectively through Shapiro-Wilk and a Bartlett tests), this was done by one-way ANOVA, followed by a Tukey post-hoc test. If normality and/or homoscedasticity were not respected a non-parametric Kruskal-Wallis test was used instead, followed by a Dunn post-hoc test.

Additionally, the FA compositions of the samples were investigated by principal component analysis (PCA). Two PCAs were performed for, respectively, NLFA and PLFA.

2.6.2. Correlations between the different indicators

Isotopic and elemental composition, and fatty acids data yield different potential indicators that can provide information on the nutrition of *Mastigias papua* (namely: corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, C:N ratios, NLFA:TLFA, the first and second principal components (PC1 and PC2) of PCAs, the n-3:n-6 and EPA:DHA ratios and the average unsaturation; the five later being available for NLFA and PLFA). To test whether these indicators correlated or not, pairwise Pearson's correlation coefficients were computed.

2.6.3. Chlorophyll a

Chlorophyll a content of the medusae from the different sites were compared by one-way ANOVA on log transformed data. The three replicates measured for each individual medusae were averaged to avoid pseudo-replication. Normality of residuals and homoscedasticity were checked using, respectively, a Shapiro-Wilk and a Bartlett tests. Subsequent Tukey post-hoc test were performed to compare the different sites.

2.6.4. 2010-2018 data

The relationship between *Mastigias papua etpisoni* population density and their corrected $\delta^{13}\text{C}$, corrected $\delta^{15}\text{N}$, and C:N ratios was tested by performing linear regressions using the mean isotopic or elemental value of each year as the variable and population density as the covariate. As sampling effort varied from year to year, each year was weighted by the number of medusae sampled for isotopic and elemental measures to improve the confidence on well sampled years.

3. Results

3.1. Presence of *Zooxanthellae*

A significant effect of sampling site on chlorophyll a concentration was detected (one-way ANOVA, $F = 114.2$, $p\text{-value} < 0.001$). Chlorophyll a concentration (given in μg of pigment per g of lyophilized medusa tissue) in *Mastigias papua saliii* from Clear Lake (CLM) was very low (below $0.04 \mu\text{g.g}^{-1}$) as compared to chlorophyll a content from medusae from other sampling sites ($30.8 \pm 27.3 \mu\text{g.g}^{-1}$; mean \pm s.d.). The differences between medusae from CLM and from other sites were always highly significant (Tukey post-hoc test $p\text{-value} < 0.001$).

This very low chlorophyll a content of medusae from CLM confirms that the absence of pigmentation was indeed related to an absence of zooxanthellae.

3.2. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N Ratios

Corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were significantly affected by both medusae wet mass, sampling sites and the interaction of the two factors (Table 2). The results are presented in Fig. 2.

The patterns related to medusae wet mass were different in corrected $\delta^{13}\text{C}$ and corrected $\delta^{15}\text{N}$. The corrected $\delta^{13}\text{C}$ of medusae from all sites increased with their size with the exception of the medusae from CLM (Fig. 2a). The corrected $\delta^{15}\text{N}$ was unaffected by medusae size in medusae from the lagoon, OTM and CLM (Fig. 2c). In medusae from NLK it increased with size whereas in medusae from GLK it decreased (Fig. 2c). Comparing the different sampling sites through their least-square means, corrected $\delta^{13}\text{C}$ and corrected $\delta^{15}\text{N}$ present a somewhat reversed pattern (Fig. 2b and d). Medusae from NLK have the highest corrected $\delta^{13}\text{C}$ (0.3 ‰) and the lowest corrected $\delta^{15}\text{N}$ (-2.5 ‰). By contrast, medusae from CLM have the lowest corrected $\delta^{13}\text{C}$ (-5.6 ‰) and the highest corrected $\delta^{15}\text{N}$ (5.9 ‰). Medusae from the other sites are positioned in between these two extremes (Fig. 2b and d).

C:N ratios, unlike corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, were only significantly affected by sampling site but not by either medusae wet mass or the interaction between medusae wet mass and sampling sites (Table 2). Hence, the effect of sampling sites on medusae's C:N ratios were analyzed using one-way ANOVA and was confirmed to be significant ($F = 72.5$, $p\text{-value} < 0.001$). C:N ratios were the highest in medusae from NLK and GLK (mostly comprised between 5 and 7, Fig. 3) and lowest in medusae from CLM (mostly comprised between 4.5 and 3.5, Fig. 3).

It is important to notice that, in both corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Fig. 2) and C:N ratios (Fig. 3), when comparing the different sampling sites, the same general order appears: NLK/GLK, OTM and CLM (with more variability on the position of GLK though).

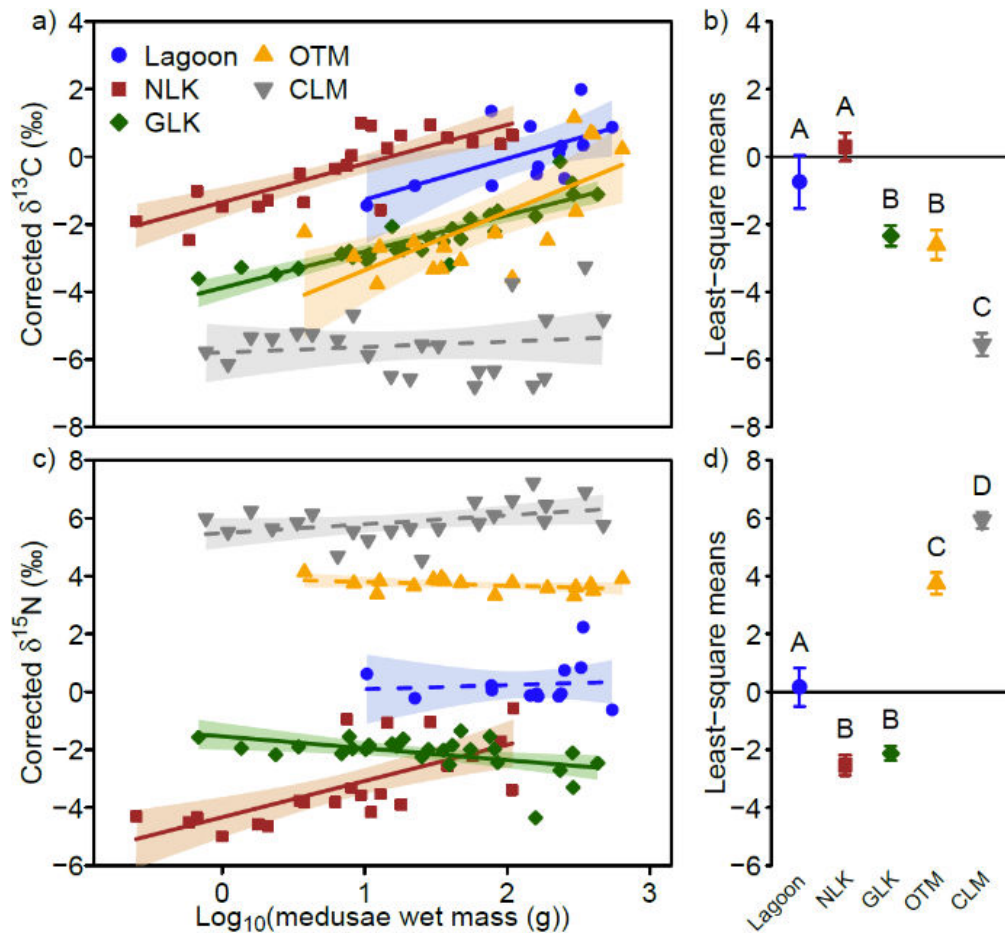


Fig. 2 Effect of *Mastigias papua* wet mass and sampling site on their corrected $\delta^{13}\text{C}$ (a and b), and corrected $\delta^{15}\text{N}$ (c and d). (a) and (c), present the raw data. Solid lines indicate a significant slope (p -value < 0.05). Shaded areas are 95 % C. I. around regression lines. (b) and (d) compare the least-square means (i.e. means corrected for the effect of medusae wet mass) in the different sampling sites. Error bars are 95 % C. I. around the least-square means. Statistically significant differences are indicated by different capital letters (A, B, C and D; Tukey post-hoc, p -value < 0.05)

Fig. 2 Effet de la masse fraîche et du site d'échantillonnage des *Mastigias papua* sur leur $\delta^{13}\text{C}$ corrigé (a et b), et $\delta^{15}\text{N}$ corrigé (c et d). (a) et (c) présentent les données brutes. Les lignes continues indiquent une pente significative (valeur $p < 0.05$). Les aires ombrées sont les I. C. 95 % autour des régressions. (b) et (d) comparent les moyennes aux moindres carrés (i.e. les moyennes corrigées de l'effet de la masse fraîche des méduses) dans les différents sites. Les barres d'erreur sont des I. C. 95 % autour des moyennes aux moindres carrés. Les différences statistiquement significatives sont indiquées par différentes lettres capitales (A, B, C et D ; Test post-hoc de Tukey, valeur $p < 0.05$)

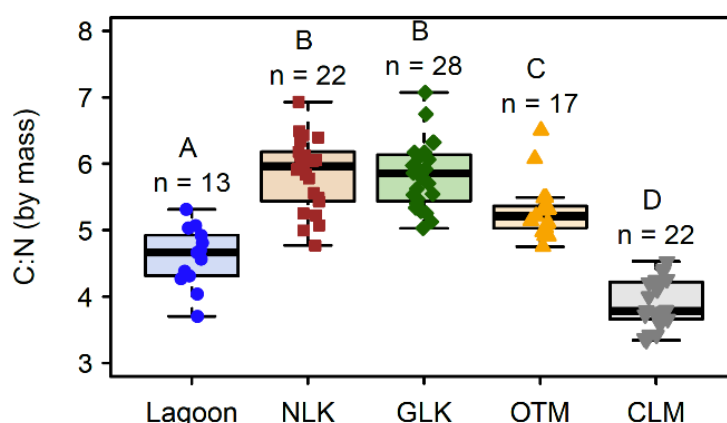


Fig. 3 Effect of *Mastigias papua* sampling site on their C:N ratios. Statistically significant differences are indicated by different capital letters (A, B, C and D; Tukey post-hoc test, p-value < 0.05). No significant effect of medusae wet mass has been found (see Table 2)

Fig. 3 Effet du site d'échantillonnage de *Mastigias papua* sur leurs ratios C:N. Les différences statistiquement significatives sont indiquées par différentes lettres capitales (A, B, C et D ; Test post-hoc de Tukey, valeur p < 0.05). Pas d'effet significatif de la taille n'a été trouvé (voir Table 2)

3.3. Fatty Acids

3.3.1. Proportions of neutral and polar lipids' fatty acids

The proportion of NLFA in TLFA (NLFA:TLFA) was not significantly affected by medusae mass alone but was significantly affected by sampling sites and by the interaction of medusae wet mass and sampling site (Table 2). NLFA:TLFA was unaffected by medusae wet mass in medusae from the lagoon, OTM and CLM but decreased with medusae wet mass in medusae from NLK and GLK (Fig. 4a). Comparing the different sampling sites through least square means (Fig. 4b), NLFA:TLFA were the highest in medusae from NLK and GLK (respectively 0.54 and 0.52), and the lowest in medusae from CLM (0.38). Medusae from OTM and from the lagoon had somewhat intermediate values (respectively 0.48 and 0.50) albeit not significantly different from the values found for the medusae from NLK and GLK (Fig. 4b). It is worth noting that, the general order NLK/GLK, OTM, CLM, already mentioned above (see section 3.2.) is found again in the NLFA:TLFA ratios of medusae from the different marine lakes.

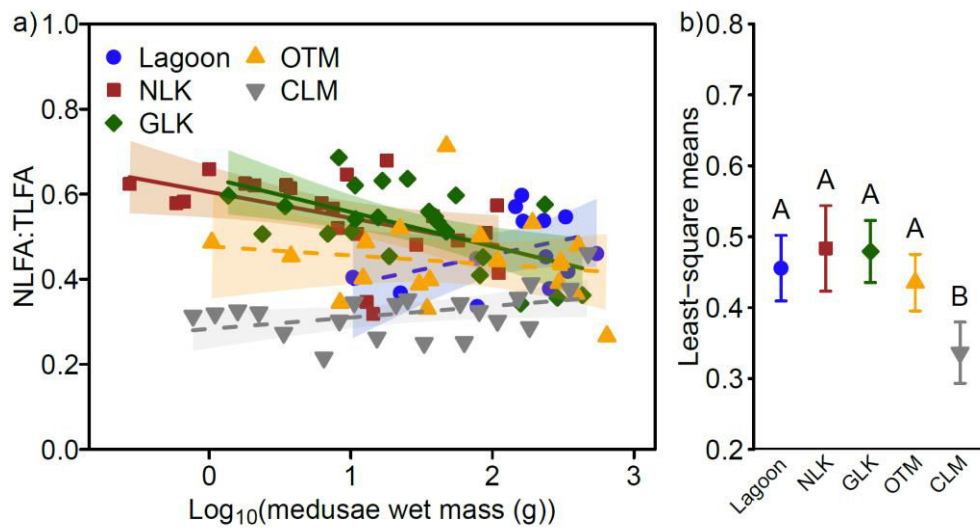


Fig. 4 Effect of *Mastigias papua* wet mass and sampling site on their proportion of neutral lipids' fatty acids (NLFA) in total lipids' fatty acids (TLFA). (a) presents the raw data. Solid lines indicate a significant slope (p -value < 0.05). Shaded areas are 95 % C. I. around regression lines. (b) compares the least-square means (i.e. means corrected for the effect of medusae wet mass) in the different sampling sites. Error bars are 95 % C. I. around the least-square means. Statistically significant differences are indicated by different capital letters (A and B; Tukey post-hoc, p -value < 0.05)

Fig. 4 Effet de la masse fraîche et du site d'échantillonnage des *Mastigias papua* sur leur proportion d'acides gras issus des lipides neutres (NLFA) dans les acides gras issus des lipides totaux (TLFA). (a) présente les données brutes. Les lignes continues indiquent une pente significative (valeur p < 0.05). Les aires ombrées sont les I. C. 95 % autour des régressions. (b) compare les moyennes aux moindres carrés (i.e. les moyennes corrigées de l'effet de la masse fraîche des méduses) dans les différents sites. Les barres d'erreur sont des I. C. 95 % autour des moyennes aux moindres carrés. Les différences statistiquement significatives sont indiquées par différentes lettres capitales (A, B, C et D ; Test post-hoc de Tukey, valeur p < 0.05)

Table 2 p-values obtained for the ANCOVAs with permutation (10 000 permutations) performed in this study. The covariate used was Log₁₀ transformed medusae wet mass (Mass) and the categorical factor was the sampling sites (Site). NS = non-significant (p-value > 0.05). NLFA = neutral lipids' fatty acids, PLFA = polar lipids' fatty acids, TLFA = total lipids' fatty acids, EPA = eicosapentaenoic acid (20:5n-3), DHA = docosahexaenoic acid (22:6n-3)

Table 2 valeurs p obtenues avec les ANCOVAs à permutation (10 000 permutations) réalisées au cours de cette étude. La covariable utilisée était la mass fraîche transformée par Log₁₀ (Mass) et le facteur catégorique était le site d'échantillonnage (Site). NS = non significatif (valeur p > 0.05). NLFA = acides gras des lipides neutres, PLFA = acides gras des lipides polaires, TLFA = acides gras des lipides totaux, EPA = acide eicosapentaénoïque (20:5n-3), DHA = acide docosahéxaénoïque (22:6n-3)

| Variable | Corrδ ¹³ C | Corrδ ¹⁵ N | C:N | NLFA:TLFA | n-3:n-6 | | EPA:DHA | | Unsaturation | |
|-----------|-----------------------|-----------------------|--------|-----------|---------|--------|---------|--------|--------------|--------|
| | | | | | NLFA | PLFA | NLFA | PLFA | NLFA | PLFA |
| Mass | <0.001 | <0.05 | NS | NS | <0.01 | <0.01 | <0.05 | NS | NS | NS |
| Site | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Mass:Site | <0.001 | <0.001 | NS | <0.01 | <0.01 | <0.001 | <0.05 | <0.05 | NS | NS |

3.3.2. General fatty acid composition

The overall fatty acid composition of *Mastigias papua* medusae sampled in this study is given in Table 3.

Saturated fatty acids (SFA) constituted ca. 37-46 % of TLFA, and were predominantly present in the neutral fraction (SFA constituted 42-56 % of NLFA as opposed to 30-45% of PLFA). The main FA constituting SFA were the 16:0 and the 18:0. The 16:0 was dominant in TLFA of medusae from all sampling sites except from CLM where it was the 18:0.

Polyunsaturated fatty acids (PUFA) accounted for 33-42 % of TLFA and were predominant in PLFA. The dominant PUFA found were the 20:4n-6 (arachidonic acid), the 20:5n-3 (eicosapentaenoic acid; EPA) and the 22:6n-3 (docosahexaenoic acid; DHA). Arachidonic acid tended to be more abundant in medusae from CLM as compared to other sites (ca. 15 % of TLFA in CLM as opposed to 4-7 % of TLFA in other sites). DHA displayed an opposed trend being less abundant in CLM as compared to other sites (3-4 % of TLFA in CLM as opposed to 7-10 % of TLFA in other sampling sites). EPA represented 7-9 % of TLFA in all sites except in GLK where it was lower (ca. 4 % of TLFA).

Monounsaturated fatty acids (MUFA) comprised 8-14 % of TLFA and were predominantly present in NLFA. The predominant MUFA was the 18:1n-9 which tended to be less abundant in medusae from CLM (ca. 2 % of TLFA) as opposed to medusae from other sites (6-10 % of TLFA).

Finally, some dimethyl acetals (DMA) were found. They comprised 4-6 % of TLFA in medusae from most sites (slightly higher in CLM, ca. 10 % of TLFA) and were predominant in PLFA. The predominant DMA was the 18:0DMA.

The FA relative composition was analyzed through principal component analyses (PCA, Fig. 5). For NLFA, the first principal component (PC1) represented more than 82.04 % of the variability (Fig. 5a). The second principal component (PC2), as a result, represented only a small fraction of the variability (6.92 % Fig. 5a). This suggest strong patterns in the NLFA composition. For PLFA, PC1 was less dominant, but together PC1 and PC2 still represented more than 85 % of the total variability (PC1: 49.31 %, PC2: 38.03 %, Fig. 5b).

In all cases PC1 separated well the medusae from CLM from the medusae from the other sites (Fig. 5). More finely, it is important to notice that, along PC1, for NLFA in particular, the general order GLK/NLK, OTM, CLM is again found. The PC1 correlated positively with the 16:0 and several PUFA (18:1n-9 and 22:6n-3 (DHA) in NLFA, 18:3n-6 and 18:4n-3 in PLFA, Fig. 5). It correlated negatively with the 18:0, the 18:0DMA in NLFA, and the 20:4n-6 (arachidonic acid) and 20,5n-3 (EPA) in PLFA (Fig. 5).

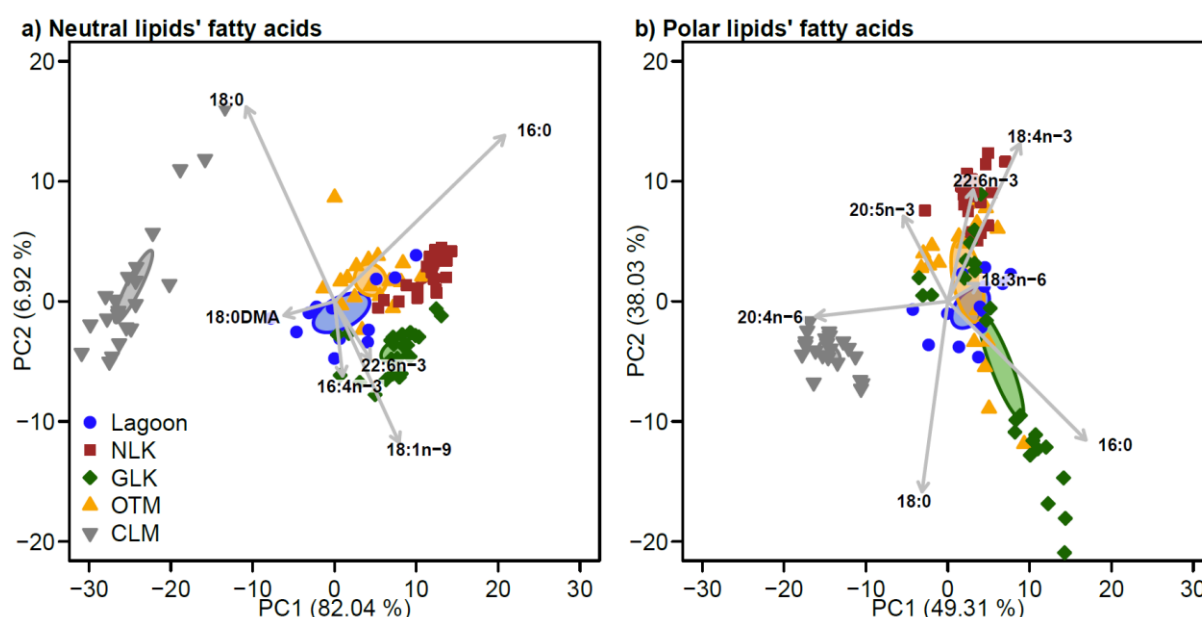


Fig. 5 Principal component analyses (PCA) of fatty acids compositions (%) of (a) neutral lipids, and (b) polar lipids of *Mastigias papua* medusae. Arrows and associated names represent the five fatty acids contributing the most to principal components 1 (PC1) and 2 (PC2). Colored areas correspond to 95 % confidence intervals around the means of the different sites sampled. Only fatty acids accounting for > 2 % in at least one sample were used

Fig. 5 Analyses en composantes principales (ACP) des compositions en acides gras (%) des lipides (a) neutres et (b) polaires des *Mastigias papua*. Les flèches et les noms associés représentent les cinq acides gras qui contribuent le plus aux composantes principales 1 (PC1) et 2 (PC2). Les aires colorées correspondent aux intervalles de confiance à 95 % autour des moyennes des différents sites échantillons. Seuls les acides gras représentant > 2 % dans au moins un échantillon ont été inclus

As noted above, the PC2 represented only a small fraction of the variability of NLFA (Fig. 5a). By opposition it represented an important part of the variability of PLFA (Fig. 5b). The way the PC2 of PLFA separates or clusters individuals varies according to the sampling site. In NLK, CLM

and, in a lesser extent the lagoon, medusae from the same site stayed clustered together, whereas in GLK and OTM the individual medusae were separated suggesting important individual variability in these two sites (Fig. 5b). PC2 does not separate well medusae from different sites (except, in some extent for the medusae from NLK, Fig. 5b). The PC2 of PLFA was generally positively correlated with PUFA (18:3n-6, 18:4n-3, 20:5n-3 (EPA) and 22:6n-3 (DHA)) and negatively correlated with SFA (16:0 and 18:0, Fig. 5b).

Table 3 → Relative composition (%; mean \pm s.d.) of fatty acids in *Mastigias papua* medusae from the different sampling sites. NLFA = neutral lipids' fatty acids, PLFA = polar lipids' fatty acids, TLFA = total lipids' fatty acids. SFA = saturated fatty acids, MUFA = mono-unsaturated fatty acids, PUFA = poly-unsaturated fatty acids, DMA = dimethyl acetals. The sums do not equal 100 % as unidentified fatty acids have not been included in this table and as only fatty acids accounting for > 2 % in at least one sample are represented

Table 3 → Composition relative (%; moyenne \pm écartype) des acides gras de *Mastigias papua* des différents sites échantillonnés. NLFA = acides gras des lipides neutres, PLFA = acides gras des lipides polaires, TLFA = acides gras des lipides totaux. SFA = acides gras saturés, MUFA = acides gras mono-insaturés, PUFA = acides gras polyinsaturés, DMA = diméthyl acetals. Les sommes ne sont pas égales à 100 % car les acides gras non identifiés ne sont pas inclus dans ce tableau et seuls les acides gras représentant > 2 % dans au moins un échantillon ont été inclus

Table 3 Lagoon / Lagon

| | Lagoon NLFA n = 13 | PLFA n = 13 | TLFA n = 13 |
|---------------|--------------------------|-----------------------|-----------------------|
| 14:0 | 2.19 (± 0.84) | 1.04 (± 0.17) | 1.64 (± 0.51) |
| 16:0 | 37.32 (± 5.12) | 23.86 (± 2.05) | 30.81 (± 3.57) |
| 17:0 | 0.87 (± 0.41) | 0.96 (± 0.39) | 0.91 (± 0.39) |
| 18:0 | 7.94 (± 1.73) | 13.74 (± 2.86) | 10.7 (± 2.08) |
| Σ SFA | 49.93 (± 3.9) | 40.38 (± 3.13) | 45.23 (± 2.2) |
| 16:1n-5 | — | — | — |
| 16:1n-7 | 3.59 (± 0.72) | 1.18 (± 0.24) | 2.43 (± 0.53) |
| 16:1n-9 | 0.39 (± 0.22) | — | 0.19 (± 0.1) |
| 18:1n-7 | 1.15 (± 0.47) | 1.33 (± 0.35) | 1.24 (± 0.38) |
| 18:1n-9 | 9.99 (± 2.65) | 4.24 (± 1.31) | 7.24 (± 2.13) |
| 20:1n-11 | 0.13 (± 0.06) | — | 0.07 (± 0.04) |
| 20:1n-9 | 0.31 (± 0.52) | 0.13 (± 0.18) | 0.22 (± 0.34) |
| 22:1n-9 | 0.25 (± 0.19) | 0.03 (± 0.02) | 0.14 (± 0.1) |
| Σ MUFA | 16.55 (± 2.01) | 7.21 (± 1.2) | 12.05 (± 1.79) |
| 16:2n-4 | 0.09 (± 0.04) | 0.02 (± 0.01) | 0.06 (± 0.02) |
| 16:4n-3 | 0.34 (± 0.24) | — | 0.17 (± 0.13) |
| 18:2n-6 | 1.21 (± 0.32) | 1.22 (± 0.29) | 1.21 (± 0.27) |
| 18:2n-9 | 1.96 (± 0.92) | 0.6 (± 0.3) | 1.32 (± 0.65) |
| 18:3n-3 | 0.62 (± 0.51) | 0.65 (± 0.23) | 0.63 (± 0.36) |
| 18:3n-6 | 1.82 (± 0.71) | 1.91 (± 0.88) | 1.84 (± 0.72) |
| 18:4n-3 | 2.35 (± 0.58) | 7.08 (± 1.67) | 4.59 (± 0.76) |
| 18:5n-3 | 0.32 (± 0.22) | 0.03 (± 0.02) | 0.18 (± 0.13) |
| 20:3n-3 | 0.08 (± 0.07) | 0.05 (± 0.03) | 0.06 (± 0.05) |
| 20:4n-6 | 3.47 (± 1.15) | 8.44 (± 1.94) | 5.94 (± 1.39) |
| 20:5n-3 (EPA) | 3.96 (± 0.74) | 10.71 (± 1.79) | 7.3 (± 1.37) |
| 21:5n-3 | 0.17 (± 0.14) | 0.13 (± 0.02) | 0.15 (± 0.07) |
| 22:4n-6 | 0.65 (± 0.19) | 0.94 (± 0.18) | 0.79 (± 0.16) |
| 22:5n-3 | 1.86 (± 0.83) | 1.92 (± 0.41) | 1.86 (± 0.56) |
| 22:5n-6 | 0.4 (± 0.17) | 0.59 (± 0.19) | 0.49 (± 0.14) |
| 22:6n-3 (DHA) | 7.32 (± 1.33) | 9.37 (± 1.46) | 8.35 (± 1.04) |
| Σ PUFA | 27.47 (± 3.26) | 44.38 (± 2.67) | 35.75 (± 2.49) |
| Σ n-3 | 17.22 (± 2.85) | 30.11 (± 2.62) | 23.49 (± 2.5) |
| Σ n-6 | 8.18 (± 1.39) | 13.64 (± 1.73) | 10.87 (± 1.34) |
| 16:0DMA | 0.63 (± 0.27) | 0.83 (± 0.28) | 0.73 (± 0.24) |
| 16:1n-7DMA | 1.26 (± 0.51) | 1.72 (± 0.39) | 1.47 (± 0.3) |
| 18:0DMA | 1.3 (± 0.38) | 1.65 (± 0.36) | 1.47 (± 0.26) |
| 20:1n-7DMA | — | 1.88 (± 0.58) | 0.89 (± 0.25) |
| Σ DMA | 3.19 (± 1.06) | 6.09 (± 0.68) | 4.55 (± 0.53) |

Table 3 Continued: NLK / Suite : NLK

| | NLK NLFA n = 22 | PLFA n = 22 | TLFA n = 22 |
|---------------|-----------------------|-----------------------|-----------------------|
| 14:0 | 1.65 (± 0.47) | 1.23 (± 0.17) | 1.45 (± 0.27) |
| 16:0 | 47.71 (± 2.87) | 19.41 (± 1.37) | 35.33 (± 3.71) |
| 17:0 | 0.27 (± 0.07) | 0.18 (± 0.04) | 0.23 (± 0.05) |
| 18:0 | 5.49 (± 0.66) | 7.78 (± 1.25) | 6.54 (± 0.92) |
| Σ SFA | 55.53 (± 2.62) | 29.4 (± 2.22) | 44.14 (± 3.24) |
| 16:1n-5 | — | — | — |
| 16:1n-7 | 2.02 (± 0.33) | 0.71 (± 0.05) | 1.42 (± 0.14) |
| 16:1n-9 | 0.19 (± 0.06) | 0.08 (± 0.02) | 0.14 (± 0.04) |
| 18:1n-7 | 0.35 (± 0.11) | 0.42 (± 0.1) | 0.38 (± 0.1) |
| 18:1n-9 | 12.07 (± 1.12) | 4.43 (± 0.5) | 8.65 (± 0.95) |
| 20:1n-11 | — | — | — |
| 20:1n-9 | 0.1 (± 0.02) | — | 0.06 (± 0.02) |
| 22:1n-9 | — | — | — |
| Σ MUFA | 15 (± 1.32) | 6.04 (± 0.45) | 10.97 (± 0.94) |
| 16:2n-4 | 0.18 (± 0.05) | 0.08 (± 0.01) | 0.14 (± 0.04) |
| 16:4n-3 | 0.03 (± 0.02) | — | 0.01 (± 0.01) |
| 18:2n-6 | 0.86 (± 0.1) | 0.81 (± 0.06) | 0.84 (± 0.07) |
| 18:2n-9 | 1.83 (± 0.28) | 0.83 (± 0.31) | 1.37 (± 0.2) |
| 18:3n-3 | 0.13 (± 0.04) | 0.15 (± 0.02) | 0.14 (± 0.03) |
| 18:3n-6 | 1.62 (± 0.24) | 2.56 (± 0.32) | 2.02 (± 0.24) |
| 18:4n-3 | 1.52 (± 0.29) | 13.17 (± 1.92) | 6.69 (± 1.65) |
| 18:5n-3 | 1.22 (± 0.32) | 0.06 (± 0.02) | 0.7 (± 0.2) |
| 20:3n-3 | 0.1 (± 0.03) | 0.06 (± 0.02) | 0.08 (± 0.02) |
| 20:4n-6 | 2.01 (± 0.68) | 8.26 (± 1.2) | 4.74 (± 1.02) |
| 20:5n-3 (EPA) | 3.8 (± 0.56) | 11.65 (± 1.2) | 7.23 (± 0.73) |
| 21:5n-3 | 0.05 (± 0.02) | 0.03 (± 0.01) | 0.04 (± 0.01) |
| 22:4n-6 | 0.38 (± 0.15) | 0.82 (± 0.18) | 0.57 (± 0.16) |
| 22:5n-3 | 1.36 (± 0.34) | 1.79 (± 0.23) | 1.55 (± 0.24) |
| 22:5n-6 | 0.11 (± 0.04) | 0.11 (± 0.04) | 0.11 (± 0.04) |
| 22:6n-3 (DHA) | 8.5 (± 0.69) | 10.85 (± 0.95) | 9.57 (± 0.53) |
| Σ PUFA | 24.1 (± 1.67) | 51.82 (± 2.1) | 36.3 (± 2.42) |
| Σ n-3 | 16.75 (± 1.04) | 37.79 (± 2.32) | 26.05 (± 1.89) |
| Σ n-6 | 5.29 (± 0.86) | 12.92 (± 1.26) | 8.62 (± 1.24) |
| 16:0DMA | 0.17 (± 0.04) | 1.19 (± 0.31) | 0.62 (± 0.17) |
| 16:1n-7DMA | 0.29 (± 0.11) | 2.57 (± 0.34) | 1.3 (± 0.33) |
| 18:0DMA | 0.42 (± 0.16) | 3.24 (± 1.05) | 1.66 (± 0.63) |
| 20:1n-7DMA | — | 1.76 (± 0.29) | 0.78 (± 0.22) |
| Σ DMA | 1 (± 0.3) | 8.82 (± 1.13) | 4.45 (± 1.05) |

Table 3 Continued: GLK / Suite : GLK

| | GLK NLFA n = 24 | PLFA n = 24 | TLFA n = 23 |
|---------------|-----------------------|------------------------|-----------------------|
| 14:0 | 1.78 (± 0.61) | 1.54 (± 0.58) | 1.62 (± 0.52) |
| 16:0 | 41.67 (± 3.27) | 27 (± 7.54) | 34.79 (± 4.43) |
| 17:0 | 0.2 (± 0.06) | 0.31 (± 0.08) | 0.24 (± 0.04) |
| 18:0 | 4.77 (± 1.14) | 15.36 (± 4.62) | 9.04 (± 2.01) |
| Σ SFA | 48.75 (± 2.81) | 45.17 (± 12.59) | 46.26 (± 5.53) |
| 16:1n-5 | 0.2 (± 0.16) | 0.45 (± 0.11) | 0.3 (± 0.09) |
| 16:1n-7 | 2.77 (± 1.25) | 0.84 (± 0.23) | 1.93 (± 0.73) |
| 16:1n-9 | 0.15 (± 0.09) | 0.02 (± 0.03) | 0.09 (± 0.04) |
| 18:1n-7 | 0.31 (± 0.1) | 0.43 (± 0.14) | 0.36 (± 0.1) |
| 18:1n-9 | 14.52 (± 1.68) | 3.67 (± 0.97) | 9.81 (± 1.44) |
| 20:1n-11 | 0.11 (± 0.05) | 1.71 (± 0.45) | 0.82 (± 0.27) |
| 20:1n-9 | 0.28 (± 0.09) | 0.01 (± 0.01) | 0.16 (± 0.06) |
| 22:1n-9 | 0.31 (± 0.3) | — | 0.18 (± 0.18) |
| Σ MUFA | 19.3 (± 2.42) | 7.56 (± 1.65) | 14.21 (± 1.83) |
| 16:2n-4 | 0.12 (± 0.05) | 0.2 (± 0.09) | 0.15 (± 0.04) |
| 16:4n-3 | 3.62 (± 1.46) | — | 2.1 (± 0.99) |
| 18:2n-6 | 1.08 (± 0.11) | 0.84 (± 0.23) | 0.98 (± 0.13) |
| 18:2n-9 | 1.62 (± 0.24) | — | 0.91 (± 0.19) |
| 18:3n-3 | 0.1 (± 0.01) | 0.17 (± 0.03) | 0.14 (± 0.02) |
| 18:3n-6 | 1.61 (± 0.17) | 4.64 (± 1.42) | 3.03 (± 0.85) |
| 18:4n-3 | 0.58 (± 0.29) | 8.19 (± 3.46) | 4.14 (± 2.16) |
| 18:5n-3 | 2 (± 0.75) | 0.05 (± 0.04) | 1.19 (± 0.51) |
| 20:3n-3 | 0.05 (± 0.03) | 0.05 (± 0.05) | 0.05 (± 0.04) |
| 20:4n-6 | 1.98 (± 1.06) | 7.85 (± 3.31) | 4.77 (± 2.48) |
| 20:5n-3 (EPA) | 2.42 (± 0.45) | 5.66 (± 2.45) | 3.99 (± 1.42) |
| 21:5n-3 | 0.3 (± 1.43) | 2.35 (± 0.9) | 1.17 (± 0.98) |
| 22:4n-6 | 1.07 (± 1.48) | 1.42 (± 0.59) | 1.27 (± 1) |
| 22:5n-3 | 1.56 (± 0.62) | 1.55 (± 0.6) | 1.56 (± 0.44) |
| 22:5n-6 | 0.39 (± 0.37) | 0.54 (± 0.79) | 0.44 (± 0.47) |
| 22:6n-3 (DHA) | 7.85 (± 1.66) | 5.7 (± 2.27) | 7.08 (± 1.21) |
| Σ PUFA | 26.81 (± 3.69) | 40.36 (± 11.97) | 33.71 (± 6.89) |
| Σ n-3 | 18.49 (± 2.96) | 24.05 (± 7.39) | 21.55 (± 3.88) |
| Σ n-6 | 6.55 (± 1.85) | 15.63 (± 4.97) | 10.87 (± 3.61) |
| 16:0DMA | 0.54 (± 0.39) | 1.29 (± 0.35) | 0.86 (± 0.2) |
| 16:1n-7DMA | — | 0.72 (± 0.18) | 0.31 (± 0.08) |
| 18:0DMA | 1.78 (± 1.2) | 4.4 (± 1.19) | 2.88 (± 0.6) |
| 20:1n-7DMA | — | — | — |
| Σ DMA | 2.32 (± 1.59) | 6.41 (± 1.55) | 4.05 (± 0.77) |

Table 3 Continued: OTM / Suite : OTM

| | OTM NLFA n = 18 | PLFA n = 18 | TLFA n = 18 |
|---------------|-----------------------|-----------------------|-----------------------|
| 14:0 | 2.26 (± 0.35) | 0.68 (± 0.15) | 1.3 (± 0.26) |
| 16:0 | 41.42 (± 3.15) | 22.99 (± 4.11) | 30.09 (± 3.92) |
| 17:0 | 0.41 (± 0.24) | 0.43 (± 0.25) | 0.42 (± 0.24) |
| 18:0 | 7.28 (± 2.69) | 11.25 (± 3.88) | 9.53 (± 2.81) |
| Σ SFA | 51.96 (± 3.46) | 36.08 (± 8.01) | 42.02 (± 6.29) |
| 16:1n-5 | — | — | — |
| 16:1n-7 | 3.28 (± 0.86) | 0.89 (± 0.29) | 1.82 (± 0.46) |
| 16:1n-9 | 0.28 (± 0.13) | 0.11 (± 0.09) | 0.18 (± 0.11) |
| 18:1n-7 | 1.09 (± 0.46) | 1.2 (± 0.42) | 1.14 (± 0.4) |
| 18:1n-9 | 9.22 (± 1.4) | 3.34 (± 0.43) | 5.67 (± 1.02) |
| 20:1n-11 | — | — | — |
| 20:1n-9 | 0.09 (± 0.05) | — | 0.03 (± 0.02) |
| 22:1n-9 | 0.41 (± 0.42) | 0.02 (± 0.02) | 0.18 (± 0.15) |
| Σ MUFA | 15.12 (± 1.61) | 5.97 (± 0.6) | 9.57 (± 1.19) |
| 16:2n-4 | 0.07 (± 0.05) | 0.02 (± 0.02) | 0.04 (± 0.02) |
| 16:4n-3 | 0.21 (± 0.07) | 0.05 (± 0.06) | 0.11 (± 0.05) |
| 18:2n-6 | 0.67 (± 0.3) | 0.79 (± 0.32) | 0.74 (± 0.3) |
| 18:2n-9 | 4.62 (± 0.85) | 1.47 (± 0.31) | 2.72 (± 0.59) |
| 18:3n-3 | 0.27 (± 0.18) | 0.54 (± 0.18) | 0.43 (± 0.17) |
| 18:3n-6 | 1.52 (± 0.37) | 2.24 (± 0.75) | 1.98 (± 0.62) |
| 18:4n-3 | 2.3 (± 0.57) | 7.95 (± 3.69) | 5.89 (± 2.83) |
| 18:5n-3 | 0.68 (± 0.3) | 0.1 (± 0.02) | 0.34 (± 0.18) |
| 20:3n-3 | — | 0.04 (± 0.04) | 0.03 (± 0.02) |
| 20:4n-6 | 2.72 (± 0.81) | 8.78 (± 2.12) | 6.47 (± 2) |
| 20:5n-3 (EPA) | 3.77 (± 1.3) | 11.74 (± 3.56) | 8.54 (± 2.56) |
| 21:5n-3 | 0.08 (± 0.01) | 0.11 (± 0.03) | 0.1 (± 0.02) |
| 22:4n-6 | 0.65 (± 0.23) | 0.99 (± 0.35) | 0.87 (± 0.33) |
| 22:5n-3 | 1.63 (± 0.51) | 2.13 (± 0.72) | 1.92 (± 0.57) |
| 22:5n-6 | 0.29 (± 0.1) | 0.58 (± 0.31) | 0.45 (± 0.17) |
| 22:6n-3 (DHA) | 6.28 (± 0.93) | 8.51 (± 1.77) | 7.66 (± 1.33) |
| Σ PUFA | 26.07 (± 2.55) | 46.99 (± 7.42) | 38.98 (± 6.2) |
| Σ n-3 | 15.32 (± 2.31) | 31.27 (± 5.72) | 25.12 (± 4.36) |
| Σ n-6 | 6.03 (± 1.07) | 14.22 (± 2.51) | 11.08 (± 2.54) |
| 16:0DMA | 0.24 (± 0.06) | 0.69 (± 0.12) | 0.52 (± 0.13) |
| 16:1n-7DMA | 0.72 (± 0.26) | 1.8 (± 0.58) | 1.35 (± 0.39) |
| 18:0DMA | 1.15 (± 0.28) | 2.9 (± 0.5) | 2.21 (± 0.44) |
| 20:1n-7DMA | — | 1.89 (± 0.96) | 1.18 (± 0.76) |
| Σ DMA | 2.12 (± 0.54) | 7.57 (± 0.9) | 5.43 (± 1.06) |

Table 3 Continued and end: CLM / Suite et fin : CLM

| | CLM NLFA n = 21 | PLFA n = 21 | TLFA n = 21 |
|---------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| 14:0 | 1.39 (\pm 0.43) | 0.42 (\pm 0.13) | 0.75 (\pm 0.22) |
| 16:0 | 19.59 (\pm 6.08) | 14.15 (\pm 1.86) | 16.01 (\pm 2.99) |
| 17:0 | – | 1.63 (\pm 0.3) | 1.08 (\pm 0.21) |
| 18:0 | 19.77 (\pm 3.43) | 17.03 (\pm 1.47) | 17.92 (\pm 1.72) |
| Σ SFA | 42.29 (\pm 9.22) | 34.98 (\pm 2.79) | 37.45 (\pm 4.34) |
| 16:1n-5 | 2.41 (\pm 0.79) | – | 0.8 (\pm 0.28) |
| 16:1n-7 | 1.76 (\pm 0.55) | 1.25 (\pm 0.25) | 1.42 (\pm 0.25) |
| 16:1n-9 | 1.36 (\pm 0.82) | 0.39 (\pm 0.38) | 0.72 (\pm 0.37) |
| 18:1n-7 | 1.64 (\pm 0.45) | 2.39 (\pm 0.36) | 2.14 (\pm 0.34) |
| 18:1n-9 | 2.39 (\pm 0.63) | 2.06 (\pm 0.23) | 2.17 (\pm 0.26) |
| 20:1n-11 | 0.1 (\pm 0.11) | – | 0.04 (\pm 0.04) |
| 20:1n-9 | 0.15 (\pm 0.17) | 0.1 (\pm 0.06) | 0.12 (\pm 0.06) |
| 22:1n-9 | 1.85 (\pm 1.35) | – | 0.62 (\pm 0.41) |
| Σ MUFA | 12.48 (\pm 1.99) | 6.49 (\pm 0.71) | 8.51 (\pm 0.84) |
| 16:2n-4 | – | 2.22 (\pm 1.1) | 1.44 (\pm 0.61) |
| 16:4n-3 | – | 0.15 (\pm 0.05) | 0.1 (\pm 0.03) |
| 18:2n-6 | 1.58 (\pm 0.52) | 3.77 (\pm 0.39) | 3.04 (\pm 0.37) |
| 18:2n-9 | – | – | – |
| 18:3n-3 | 0.69 (\pm 0.28) | 1.17 (\pm 0.18) | 1.02 (\pm 0.18) |
| 18:3n-6 | – | – | – |
| 18:4n-3 | 3.95 (\pm 1.2) | 1 (\pm 0.4) | 1.99 (\pm 0.7) |
| 18:5n-3 | 0.08 (\pm 0.15) | – | 0.03 (\pm 0.06) |
| 20:3n-3 | 3.42 (\pm 3.28) | – | 1.15 (\pm 1.14) |
| 20:4n-6 | 5.42 (\pm 2.45) | 19.74 (\pm 2.42) | 14.91 (\pm 2.22) |
| 20:5n-3 (EPA) | 2.29 (\pm 1.05) | 11.33 (\pm 1.26) | 8.3 (\pm 1.28) |
| 21:5n-3 | – | 0.05 (\pm 0.04) | 0.04 (\pm 0.02) |
| 22:4n-6 | 2.06 (\pm 0.88) | 2.13 (\pm 0.32) | 2.11 (\pm 0.4) |
| 22:5n-3 | 2.14 (\pm 1) | 2.14 (\pm 0.59) | 2.16 (\pm 0.5) |
| 22:5n-6 | 0.47 (\pm 0.45) | 0.67 (\pm 0.13) | 0.61 (\pm 0.19) |
| 22:6n-3 (DHA) | 2.08 (\pm 0.97) | 4.3 (\pm 0.7) | 3.57 (\pm 0.73) |
| Σ PUFA | 25.38 (\pm 6.06) | 50.04 (\pm 2.85) | 41.76 (\pm 3.6) |
| Σ n-3 | 14.66 (\pm 3.16) | 20.39 (\pm 1.89) | 18.52 (\pm 1.59) |
| Σ n-6 | 10.72 (\pm 3.84) | 27.42 (\pm 2.69) | 21.8 (\pm 2.73) |
| 16:0DMA | 2.82 (\pm 1) | 1.47 (\pm 0.49) | 1.91 (\pm 0.46) |
| 16:1n-7DMA | 4.23 (\pm 1.33) | 1.97 (\pm 0.41) | 2.71 (\pm 0.33) |
| 18:0DMA | 8.65 (\pm 2.49) | 3.61 (\pm 0.65) | 5.26 (\pm 0.71) |
| 20:1n-7DMA | – | – | – |
| Σ DMA | 15.7 (\pm 4.51) | 7.05 (\pm 1.42) | 9.88 (\pm 1.22) |

3.3.3. *n*-3:*n*-6 and EPA:DHA ratios

With the exception of the effect of medusae wet mass on EPA:DHA ratios in PLFA, the two ratios, in both FA fractions, were significantly affected by the medusae wet mass, the sampling sites and the interaction of the two factors (Table 2). Results are presented in Fig. 6.

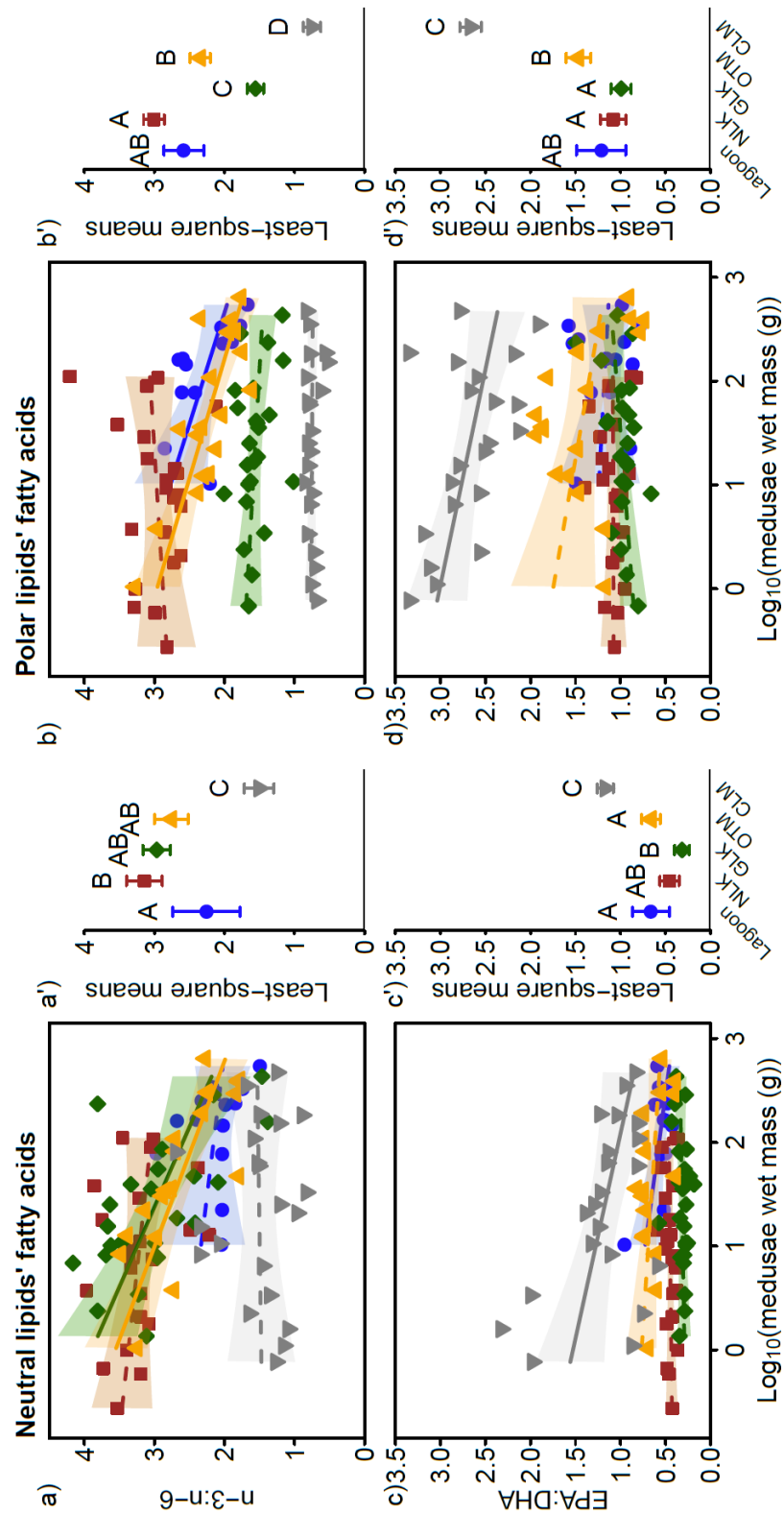


Fig. 6 ← Effect of *Mastigias papua* wet mass and sampling site on two ratios derived from fatty acid composition. Top row: ratios of n-3 polyunsaturated fatty acids on n-6 polyunsaturated fatty acids. Bottom row: ratios of eicosapentaenoic acid (20:5n-3, EPA) on docosahexaenoic acid (22:6n-3, DHA). Columns correspond to the different fatty acid fractions (neutral and polar). Larger graphics (noted “x”) are linear regressions between indicators and medusae size: solid lines indicate a significant slope (p-value < 0.05). Shaded areas are 95 % C. I. around regression lines. Smaller graphics (noted “x'”) compare the least-square means (i.e. means corrected for the effect of medusae wet mass) in the different sampling sites. Error bars are 95 % C. I. around the least-square means. Statistically significant differences are indicated by different capital letters (A, B, C and D; Tukey post-hoc, p-value < 0.05)

Fig. 6 ← Effet de la masse fraîche et du site d'échantillonnage des *Mastigias papua* sur deux ratios issus de la composition en acide gras. Ligne du haut : ratios des acides gras polyinsaturés n-3 sur les acides gras polyinsaturés n-6. Ligne du bas : ratios de l'acide eicosapentaénoïque (20:5n-3, EPA) sur l'acide docosahexaénoïque (22:6n-3, DHA). Les colonnes correspondent aux différentes fraction lipidiques (neutre et polaire). Les graphiques les plus grands (notés « x ») sont les regressions linéaires entre la masse fraîche des méduses et l'indicateur : les lignes continues indiquent une pente significative (valeur p < 0.05). Les aires ombrées sont les I. C. 95 % autour des régressions. Les graphiques plus petits (notés « x' ») comparent les moyennes aux moindres carrés (i.e. les moyennes corrigées de l'effet de la masse fraîche des méduses) dans les différents sites. Les barres d'erreur sont des I. C. 95 % autour des moyennes aux moindres carrés. Les différences statistiquement significatives sont indiquées par différentes lettres capitales (A, B, C et D ; Test post-hoc de Tukey, valeur p < 0.05)

Patterns related to medusae wet mass are generally conserved between the different FA fractions (NLFA and PLFA), within the same indicator, with few exceptions (Fig. 6). The n-3:n-6 ratios of the medusae, decreased with medusae wet mass in both PLFA and NLFA of medusae from OTM, in the PLFA of medusae from the lagoon, and in the NLFA of medusae from GLK (Fig. 6a and b). For the other combinations of sampling-sites and FA fraction, no effect of medusae wet mass was found (Fig. 6a and b). The EPA:DHA ratios size related patterns are different from the ones observed in the n-3:n-6 ratios indicators and not as pronounced. Regardless of the FA fraction, the EPA:DHA ratios decreased with medusae mass in medusae from CLM, but not in medusae from the other sampling site (Fig. 6c and d).

Comparing the sampling sites to each other's through their least square means, the general order GLK/NLK, OTM, CLM already found in other indicators is found again in EPA:DHA ratios of both FA fraction (although reversed, Fig. 6c' and d') and for the n-3:n-6 ratios in NLFA (Fig.

6a'). The n-3:n-6 ratios of PLFA present a different pattern with the medusae from GLK having a lower position relative to other sites as compared to other indicators (Fig. 6b').

3.3.4. Average unsaturation

The average unsaturation was unaffected by medusae wet mass in both NLFA and PLFA (Table 2). The model was thus simplified to take in account sampling site only. Sampling site was found to have an important effect in both NLFA (Kruskal-Wallis test, $\chi^2 = 15.5$, p-value < 0.001) and PLFA (Kruskal-Wallis test, $\chi^2 = 62.8$, p-value < 0.001). In NLFA, the average unsaturation was relatively similar from one site to another (generally between 1 and 1.5, Fig. 7a). By comparison, in PLFA, larger differences were observed (Fig. 7b). The average unsaturation of PLFA was rather constant and high in medusae from the lagoon, NLK and CLM (between 2 and 3 as a function of the site with a range size within a site never exceeding 0.5, Fig. 7b). By opposition, medusae from GLK and OTM had much more variable average unsaturation of their PLFA (range respectively ca. 0.75-2.75 and 1.25-2.75, Fig. 7b).

3.4. Correlations Between the Different Indicators

The correlations between the different indicators of the nutrition of *Mastigias papua* are presented in Fig. 8. With the exception of most of the correlations involving the PC2, and the unsaturation, all correlations tested were statistically significant ($\alpha = 0.05$). The Pearson's correlation coefficients (r_p) generally indicated strong positive or negative correlations between most indicators tested (excluding not significant correlations, 65.2 % of correlations tested have $r_p > 0.5$ or $r_p < -0.5$, and 18.8 % have $r_p > 0.75$ or $r_p < -0.75$). Whereas most indicators were positively correlated to each other's, the corrected $\delta^{15}\text{N}$ and the EPA:DHA ratios (from both FA fractions) were negatively correlated with most others indicators (Fig. 8). Some indicators had markedly weaker correlations than most other indicators (most notably PC2 and unsaturation from either NLFA or PLFA, Fig. 8). Interestingly however, the correlation between the PC2 and unsaturation of corresponding lipid fraction was strong ($r_p = -0.74$ in NLFA and $r_p = 0.93$ in PLFA, Fig. 8).

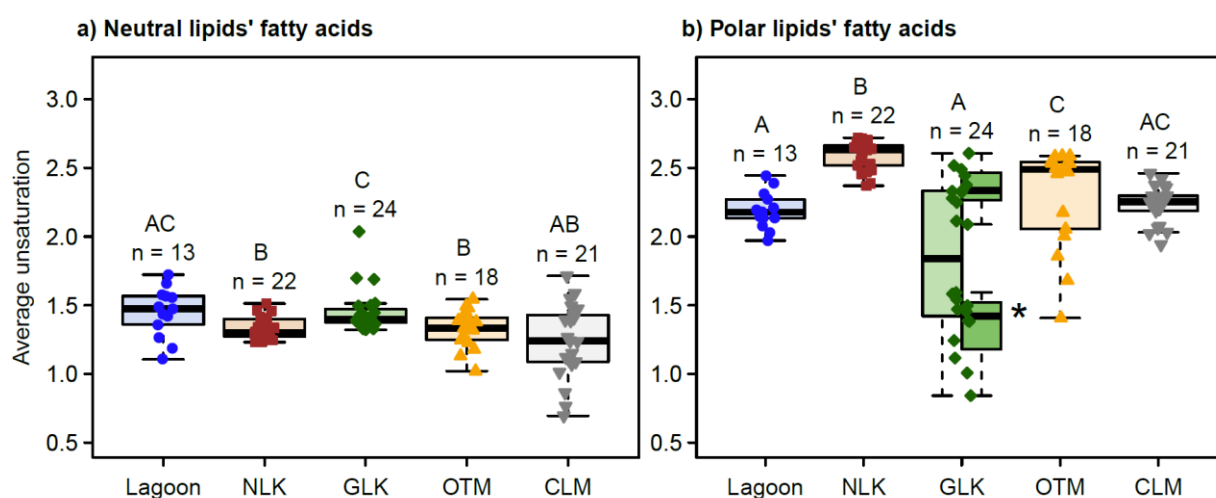


Fig. 7 Effect of sampling sites of *Mastigias papua* on their average unsaturation in (a) neutral lipids' fatty acids (NLFA) and in (b) polar lipids' fatty acids (PLFA). Statistically significant differences are indicated by different capital letters (A, B and C; Dunn post-hoc tests, p-value < 0.05). No significant effect of medusae wet mass has been found (see table 2). *In PLFA, the medusae from GLK were clearly split in two groups (highlighted by the darker green boxplots). The group with the lowest unsaturation was significantly different from all other groups (Dunn post-hoc test, p-value < 0.05)

Fig. 7 Effet du site d'échantillonnage de *Mastigias papua* sur l'insaturation moyenne dans (a) les acides gras des lipides neutres (NLFA) et (b) les acides gras des lipides polaires (PLFA). Les différences statistiquement significatives sont indiquées par différentes lettres capitales (A, B et C ; test post-hoc de Dunn, valeur p < 0.05). Pas d'effet significatif de la taille n'a été trouvé (voir Table 2). * dans les PLFA, les méduses de GLK étaient clairement séparée en deux groupes (montrés par les boxplots plus sombres). Le groupe avec l'insaturation la plus basse était significativement différent de tout les autres groupes (test post-hoc de Dunn, valeur p < 0.05)

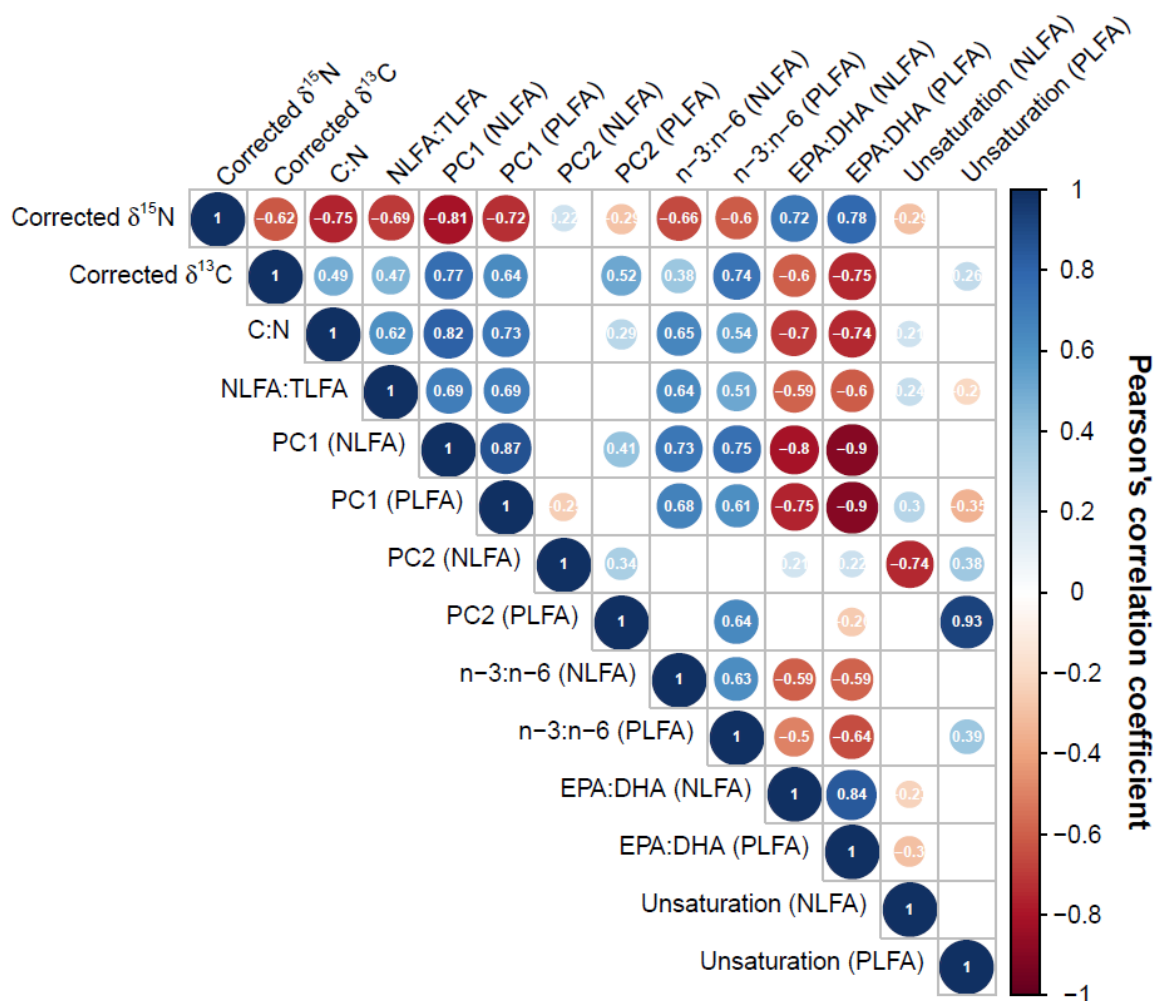


Fig. 8 Correlations (Pearson's correlation coefficient) between the different indicators of nutrition of *Mastigias papua*. Empty cases correspond to non-significant correlations (p-value > 0.05). NLFA = neutral lipids' fatty acids, PLFA = polar lipids' fatty acids, TLFA = total lipids' fatty acids, PC1 = first principal component of the ACP, PC2 = second principal component of the ACP (see Fig. 5), EPA = eicosapentaenoic acid (20:5n-3), DHA = docosahexaenoic acid (22:6n-3)

Fig. 8 Corrélations (coefficient de corrélation de Pearson) entre les différents indicateurs de la nutrition de *Mastigias papua*. Les cases vides correspondent à des corrélations non significatives (valeur p > 0.05). NLFA = acides gras des lipides neutres, PLFA = acides gras des lipides polaires, TLFA = acides gras des lipides totaux, PC1 = première composante principale de l'ACP, PC2 = seconde composante principale de l'ACP (voir Fig. 5), EPA = acide eicosapentaénoïque (20:5n-3), DHA = acide docosahéaénoïque (22:6n-3)

3.5. Inter-Annual Variability in the Nutrition of Medusae of Ongeim'l Tketau: Effect of Population Density

Data from 2010 to 2018 revealed significant relationships between corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and Log_{10} transformed *Mastigias papua etpisoni* population density in OTM (respectively $R^2 = 0.86$, $p\text{-value} < 0.01$ and $R^2 = 0.7$, $p\text{-value} < 0.05$, Fig. 9a and b). For C:N ratios however, the relationship was not significant at $\alpha = 0.05$ (i.e. $0.05 < p\text{-value} < 0.1$; Fig. 9c).

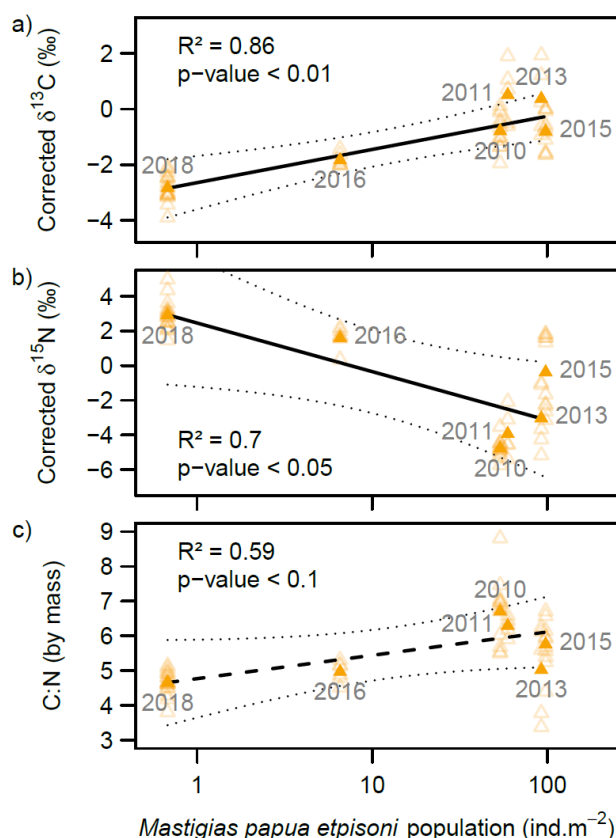


Fig. 9 Relationship between corrected $\delta^{13}\text{C}$ (a), corrected $\delta^{15}\text{N}$ (b) and mass C:N ratios (c) (oral arms data) and medusae population density of *Mastigias papua etpisoni* in Ongeim'l Tketau (OTM) across different sampling years. Regressions have been performed on the weighted means of each year (weighted by the number of medusae sampled; $n = 12, 5, 5, 10, 6$ and 18 for respectively 2010, 2011, 2013, 2015, 2016 and 2018). Empty symbols are the raw data; full symbols are the means of each sampling. Dotted lines are 95 % C. I. around regressions

Fig. 9 Relations entre les $\delta^{13}\text{C}$ corrigés (a), $\delta^{15}\text{N}$ corrigés (b) et ratios C:N massiques (c) (données des bras péri-buccaux) et la densité de population de *Mastigias papua etpisoni* dans Ongeim'l Tketau (OTM) au cours des différentes années d'échantillonnage. Les régressions ont été réalisées sur les moyennes pondérées de chaque année (pondérées par le nombre de méduses échantillonnées, $n = 12, 5, 5, 10, 6$ et 18 pour respectivement 2010, 2011, 2013, 2015, 2016 et 2018). Les symboles vident correspondent aux données brutes ; les symboles pleins sont les moyennes. Les lignes pointillées correspondent aux I. C. 95 % autour des régressions

4. Discussion

4.1. Interpretation of the Indicators Used in this Study

Different indicators were used in this study. In this section (4.1.), we summarize how each of these indicators are interpreted in light of the published literature (sections 4.1.1. and 4.1.2.). We then discuss how these indicators would be expected to be correlated with each other (section 4.1.3.). Then, we use this as a framework for the interpretation of these indicators in the context of our results and our problematic of the plasticity of the nutrition of *Mastigias papua* (sections 4.2. to 4.4.).

4.1.1. Isotopic and elemental composition

Stable isotopes (mainly bulk carbon and nitrogen) are a widely used tool in trophic ecology. They have been emphasized as valuable trophic markers for jellyfishes (Pitt et al. 2009), and for photosymbiotic organisms (Ferrier-Pagès and Leal 2018). In the case of mixotrophic photosymbiotic holobionts, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures can be used to assess the relative importance of autotrophy and heterotrophy (e.g. Muscatine et al. 1989a, Reynaud et al. 2002, Alamaru et al. 2009, Ferrier-Pagès et al. 2011, reviewed in Ferrier-Pagès and Leal 2018):

$\delta^{13}\text{C}$: $\delta^{13}\text{C}$ tend to increase with autotrophy and decrease with heterotrophy (e.g. Muscatine et al. 1989a, Swart et al. 2005, Einbinder et al. 2009, Ferrier-Pagès et al. 2011). Two mechanisms are at play: (1) Carbon derived through autotrophic sources is enriched relative to carbon derived from heterotrophic sources (Ferrier-Pagès et al. 2011, Ferrier-Pagès and Leal 2018); (2) Increasing photosynthesis results in decreased fractionation during the uptake of dissolved inorganic carbon, thereby inducing an increase of $\delta^{13}\text{C}$ (“Depletion-Diffusion hypothesis”, see Muscatine et al. 1989a, Swart et al. 2005, Ferrier-Pagès and Leal 2018). Importantly, due to this effect, the observed increase of $\delta^{13}\text{C}$ in umbrella seen along medusae size-gradients (Fig. 2) cannot be interpreted as an increase in autotrophy relative to heterotrophy, but may instead be an artefact of the organ shape that may have reduced the supply of dissolved inorganic carbon from the surrounding water (see Appendix).

$\delta^{15}\text{N}$: $\delta^{15}\text{N}$ tend to decrease with autotrophy and increase with heterotrophy (Ferrier-Pagès et al. 2011, Ferrier-Pagès and Leal 2018) although this can be cofounded by other

factors such as recycling (Alamaru et al. 2009, Reynaud et al. 2009) or decreased fractionation at high photosynthesis rates (Muscatine and Kaplan 1994, Baker et al. 2011).

C:N ratios: Non-zooxanthellate jellyfishes have generally low C:N ratios (ca. 3.8, Ikeda 2014, Molina-Ramírez et al. 2015). Zooxanthellae, in contrast, can reach higher C:N ratios (e.g. Alamaru et al. 2009). Moreover, C:N ratios increase in both the host and its symbionts when nitrogen becomes more available (either as dissolved nutrients, Muscatine et al. 1989b, Belda et al. 1993; or as prey, Cook et al. 1988). Thus, high C:N ratios would indicate dominant autotrophy while low C:N ratios would indicate dominant heterotrophy.

4.1.2. Fatty acid composition

As with stable isotopes, fatty acids (FA) are now widely used as trophic markers (e.g. Dalsgaard et al. 2003, Pitt et al. 2009). In photosymbiotic organisms, FA can be used as indicators of the relative importance of autotrophy and heterotrophy (e.g. Seeman et al. 2013, Mies et al. 2018, Radice et al. 2019) or of their general health status (e.g. Rocker et al. 2019). Moreover, the separation of neutral lipids' fatty acids (NLFA) and polar lipids' fatty acids (PLFA) allows us to resolve different processes. As NLFA are predominantly reserve lipids, they reflect mostly trophic interactions whereas PLFA, being predominantly membrane lipids are more subjected to physiologic processes (Dalsgaard et al. 2003).

NLFA:TLFA ratios: NLFA:TLFA (i.e. the proportion of neutral lipids' fatty acids of total lipids' fatty acids) is indicative of the amount of reserve lipids (Dalsgaard et al. 2003). Non-zooxanthellate jellyfishes contain only little lipid reserves (Donnelly et al. 1994, Doyle et al. 2007), but zooxanthellae can store massive amounts of lipids in the form of droplets particularly when under nutrient limitation (Muller-Parker et al. 1996, Jiang et al. 2014, Rosset et al. 2015). In corals, high NLFA:TLFA ratios have been correlated with the presence of zooxanthellae (Imbs et al. 2010). As a result, NLFA:TLFA ratios would be higher in dominantly autotrophic medusae and lower in dominantly heterotrophic medusae.

PC1: In both NLFA (Fig. 5a) and PLFA (Fig. 5b), PC1 (principal component 1) positively correlated with fatty acids (FA) typically associated with zooxanthellae (e.g. 16:0, 18:4n-3, 18:1n-9, 22:6n-3, see e.g. Al-Moghrabi et al. 1995, Dalsgaard et al. 2003, Papina et al. 2003, Treignier et al. 2008, Mortillaro et al. 2009, Imbs et al. 2010, Leone et al. 2015, Revel et al.

2016, Radice et al. 2019) and negatively correlated with FA typically found in coral hosts (e.g. 20:4n-6, Imbs et al. 2007, Treignier et al. 2008) but also in non-zooxanthellate jellyfishes (e.g. 18:0, 20:4n-6, 20:5n-3, see Fukuda and Nakamura 2001, Leone et al. 2015, Prieto et al. 2018, Tilves et al. 2018). This suggests that the PC1 obtained in this study would be indicative of the importance of zooxanthellae. PC1, in this study, would be high in mostly autotrophic medusae and lower in mostly heterotrophic medusae.

PC2: PC2 (principal component 2) is also available for both NLFA and PLFA but explains only a little fraction of the variability of NLFA (Fig. 5a) and will thus only be discussed in the context of PLFA (Fig. 5b). In PLFA, PC2 is positively correlated with many n-3 PUFA and negatively correlated with saturated fatty acid (SFA, Fig. 5b). Moreover, PC2 appears to separate better individuals with zooxanthellae than individuals without (Fig. 5b). Increases in 16:0 or decreases in n-3 PUFA have been linked to nutrient limitations, or more generally to stress of zooxanthellae (Al-Moghrabi et al. 1995, Tolosa et al. 2011, Tagliafico et al. 2017, Rocker et al. 2019). Thus, in this study, the PC2 of PLFA would decrease with medusae stress.

n-3:n-6 ratios: In scleractinian corals, n-6 PUFA are markers of the host (Imbs et al. 2007, 2014) or can be obtained through predation (Figueiredo et al. 2012), whereas most n-3 PUFA are furnished by the zooxanthellae (e.g. Treignier et al. 2008). Hence, low n-3:n-6 ratios would be indicative of an holobiont dominated by the host (and hence mostly heterotrophic) whereas high n-3:n-6 ratios would be indicative of an holobiont dominated by the symbionts (and hence mostly autotrophic). However, these ratios may also be influenced by stress. Indeed, decreases in n-3 PUFA have been correlated with thermal stress in corals (Tolosa et al. 2011 see also Tagliafico et al. 2017). This would be particularly true in PLFA which more closely reflect physiological processes (Dalsgaard et al. 2003).

EPA:DHA ratios: EPA is a common FA whereas DHA can be marker of dinoflagellates (Dalsgaard et al. 2003). Therefore, in the context of a cnidarian-zooxanthellae symbiosis EPA:DHA ratios would be negatively correlated to the density of zooxanthellae (Rocker et al. 2019). They would thus be indicative of the balance between autotrophy and heterotrophy.

Unsaturation: Unsaturation of membrane FA control membrane fluidity (e.g. Cossins and Prosser 1978). In cnidarian-zooxanthellae symbioses, good membrane fluidity is central to the symbiosis health (Tchernov et al. 2004). However, stress (e.g. heat, nutrient limitation)

can increase the proportion of SFA and reduce the proportion of PUFA (primarily n-3 PUFA, Al-Moghrabi et al. 1995, Tolosa et al. 2011, Tagliafico et al. 2017) and therefore, reduce unsaturation. Thus, a reduced unsaturation, particularly in PLFA—which comprise membrane FA—could be interpreted as a sign of stress. This could be confused by temperature (Cossins and Prosser 1978), but it is unlikely in the context of this study where the temperatures of the different sampling sites did not differ much (measured at 5 m deep; hottest: CLM 33 °C, coldest: Lagoon 30 °C).

4.1.3. Validation of the isotopic, elemental and fatty acid indicators for *Mastigias papua*

In summary, most indicators used in this study allow us to assess the relative contribution of autotrophy and heterotrophy to the holobiont nutrition. $\delta^{13}\text{C}$, C:N ratios, NLFA:TLFA, PC1, and n-3:n-6 ratios (of both NLFA and PLFA), through varied mechanisms, are expected to increase with autotrophic nutrition. Conversely, $\delta^{15}\text{N}$, and EPA:DHA ratios (of both NLFA and PLFA) are expected to increase with heterotrophic nutrition. It should thus be expected that $\delta^{13}\text{C}$, C:N ratios, NLFA:TLFA, PC1, and n-3:n-6 ratios on one hand, and $\delta^{15}\text{N}$, and EPA:DHA ratios on the other hand would be positively correlated to each other. Conversely, $\delta^{13}\text{C}$, C:N ratios, NLFA:TLFA, PC1, and n-3:n-6 ratios should be negatively correlated to $\delta^{15}\text{N}$, and EPA:DHA ratios. This is indeed what is observed in the dataset (Fig. 8).

PC2 and unsaturation, particularly in PLFA, would be expected to be related to stress. It is thus expected that their correlation coefficients with other indicators would be lower but that they would be well correlated to each other. This corresponds to what is observed (between PC2 and unsaturation of PLFA, $r_P = 0.93$, Fig. 8).

Overall, the good agreement between the different indicators used to assess the relative contribution of autotrophy and heterotrophy to the holobiont nutrition (Fig. 8) allows us to be confident in the interpretation of the results from this study that are explored below.

4.2. Site-by-Site Interpretations of the Nutrition of *Mastigias papua*

4.2.1. Clear Lake (CLM)

Mastigias papua saliii from CLM at the time of the sampling were all non-zooxanthellate and therefore, provide a baseline for what a purely heterotroph *Mastigias papua* would be. They were characterized by the lowest $\delta^{13}\text{C}$, C:N ratios, NLFA:TLFA, PC1, and n-3:n-6 values and the highest $\delta^{15}\text{N}$, and EPA:DHA ratio of all the populations sampled here (Figs. 3-6), which is in line with a pure heterotrophic nutrition.

Mastigias papua saliii from CLM were also characterized by a decrease in the EPA:DHA ratio with size (Fig. 6d and c). It has been noted above (section 4.1.2.) that in photosymbiotic organisms, the EPA:DHA ratio can be negatively correlated with the density of zooxanthellae (Rocker et al. 2019). However, in the context of the non-zooxanthellate medusae from CLM, this interpretation cannot be made. Instead, the decrease in EPA:DHA ratios might be due to an increase in trophic level (Dalsgaard et al. 2003). Similar increases in trophic levels with size have been documented in non-zooxanthellate jellyfishes and have been related to the possibility of capture of larger prey (Graham and Kroutil 2001, Fleming et al. 2015). However, it would be expected that $\delta^{15}\text{N}$ would increase with size (see Fleming et al. 2015). If a slight increase is indeed seen, it is not significant (Fig. 2 but see Fig. A1), raising caution on the interpretation of increasing trophic level with size in medusae from CLM.

4.2.2. Uet era Ngermeuangel (NLK)

Whereas *Mastigias papua saliii* from CLM represent the pure heterotrophy, *Mastigias papua remiliiki* from NLK, along with *Mastigias papua nakamurai* from GLK (see section 4.2.3.), represent the other end of the spectrum as they were the most autotrophic population sampled in this study. Medusae from NLK had the highest $\delta^{13}\text{C}$, C:N ratios, NLFA:TLFA, PC1, and n-3:n-6 values and the lowest $\delta^{15}\text{N}$, and EPA:DHA ratio of all the populations sampled (along with medusae from GLK, Figs. 3-6).

The $\delta^{15}\text{N}$ values of the medusae from NLK increased with their size (Fig. 2c) whereas their NLFA:TLFA ratio decreased (Fig. 4a). This suggests that the larger medusae tended to be more heterotrophic than smaller ones.

4.2.3. Goby Lake (GLK)

Mastigias papua nakamurai from GLK was, in many ways, very similar to *Mastigias papua remiliiki* from NLK. Medusae from GLK, along medusae from NLK are interpreted as the most autotrophic population sampled here (see Figs. 3-6). However, medusae from GLK differed from medusae from NLK in displaying more signs of stress:

The medusae from GLK had the greater spread along the PC2 and on the average unsaturation of their PLFA of all sampled populations, being essentially separated in two groups (Figs. 5b, 7b). One group was characterized by some n-3 PUFA (mainly 18:4n-3, 20:5n-3 and 22:6n-3) whereas the other group was more characterized by SFA (16:0 and 18:0). The group with high SFA, low n-3 PUFA and hence, low unsaturation can be interpreted as being subjected to stress (Tolosa et al. 2011, Tagliafico et al. 2017). The clear split in two groups of the medusae from GLK in term of stress (Figs. 5b and 7b) suggest that the stress is individual-specific. Why some individuals experience more stress than others is unclear, but this does not appear to be linked to their size as no significant relationship between medusae wet mass and either the PC2 or unsaturation of their PLFA have been found (result not shown). This stress, through a decrease in n-3 PUFA (Tolosa et al. 2011, Tagliafico et al. 2017) might also explain why the n-3:n-6 ratios of the PLFA from medusae from GLK is relatively low (Fig. 6b').

The slight, but significant decrease in the $\delta^{15}\text{N}$ values of the medusae from GLK (Fig. 2c) could suggest that they tend towards more heterotrophy when growing. However, this is not supported by FA data. The decrease in the NLFA:TLFA (Fig. 4a), and the n-3:n-6 ratios from NLFA (Fig. 6a) with medusae size would, at the opposite, suggest that larger medusae are more autotrophic. It is therefore impossible to be conclusive on size-related pattern in the medusae from GLK.

4.2.4. Ongeim'l Tketau (OTM)

Mastigias papua etpisoni from OTM, in terms of nutrition, was in between medusae from CLM on one hand, and medusae from NLK and GLK on the other hand (generally of intermediate $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C:N ratios, NLFA:TLFA, PC1, n:3-n:6 and EPA:DHA ratios Figs. 3-6).

Similar to medusae from GLK, albeit not in the same extent, medusae from OTM displayed sign of stress (spread and rather low values of PC2 and unsaturation in their PLFA, Figs. 5b and 7b).

The decrease in the n-3:n-6 ratios with medusae size in both lipid fractions (Fig. 6a and b) could suggest that larger medusae are more heterotrophic. However, this is not reflected in isotopic data which makes this pattern uncertain.

4.2.5. Lagoon

Finally, *Mastigias papua* lagoon population appeared to have an intermediate to dominantly autotrophic nutrition. As a function of the indicator, medusae from the lagoon can be more akin to medusae from GLK and NLK (e.g. $\delta^{13}\text{C}$, Fig. 2b) or to medusae from OTM (e.g. C:N, Fig. 3). Due to the smaller sample size (13 individuals of a restricted size range), finer patterns are difficult to discuss.

4.3. General Pattern, and the Plasticity of the Nutrition of *Mastigias papua*

4.3.1. A gradient from pure heterotrophy to predominant autotrophy

A strong pattern, supported by all relevant indicators (Fig.7), emerges from the data: the medusae populations sampled in this study were generally ordered: NLK/GLK, OTM and CLM from the most autotrophic to the most heterotrophic (medusae from the lagoon being apparently akin to medusae from OTM in terms of autotrophy-heterotrophy balance, Fig. 10). This order, however, is only qualitative. It would thus be valuable to estimate quantitatively what each of the ends of this spectrum represent in terms of e.g. carbon or nitrogen sources. The heterotrophic end of the spectrum is easy to quantify: as the medusae from CLM had no symbionts, this corresponds to 100 % heterotrophy. The more autotrophic end of the spectrum however, is harder to characterize. Zooxanthellate jellyfishes are generally mixotrophic, using both autotrophic and heterotrophic nutrition pathways concomitantly (Kremer 2005, Welsh et al. 2009). Generally, all the carbon necessary for respiration, can be fulfilled, and often exceeded by zooxanthellae's photosynthesis (e.g. Kremer et al. 1990, Kikinger 1992, Verde and McCloskey 1998, Jantzen et al. 2010) while predation is still needed

to meet nitrogen and phosphorus requirements (Kremer 2005, Welsh et al. 2009). To our knowledge, nitrogen budgets have not been realized for *Mastigias papua*, but carbon budgets are available (McCloskey et al. 1994). McCloskey et al. (1994) found that 97 % of host daily carbon demand for respiration and growth could be provided by zooxanthellae in medusae from OTM. This score was higher in medusae from the lagoon (143 %). The estimations of McCloskey et al. (1994) provide a characterization of what could be the autotrophic end of the spectrum characterized here: the medusae would derive enough carbon from their symbionts photosynthesis to meet their respiration and growth requirements.

4.3.2. The influence of medusae size

Differences in nutrition can arise as a function of medusae size in non-zooxanthellate medusae (e.g. Graham and Kroutil 2001, Fleming et al. 2015) and in zooxanthellate medusae (McCloskey et al. 1994). Such variations according to size have been observed in this study. However, they are often difficult to interpret (see sections 4.2.3. and 4.2.4.). One aspect can however be retained. No shared size-related patterns have been found when comparing the different sampling sites. This suggests that the existence, and the nature, of size-related patterns in the nutrition of zooxanthellate medusae, might not be determined by the medusae size itself, but rather by how medusae size interact with variable environmental conditions.

4.3.2. Variability in time

The results discussed above are from 2018 only. As such, they represent a snapshot of the nutrition of *Mastigias papua* in Palau. Over longer time scales, however, the nutrition of *Mastigias papua* would most likely change. The marine lakes of Palau are dynamic environments at various time scales (e.g. Orem et al. 1991, Dawson and Hamner 2005, Martin et al. 2006), and their dynamics can affect dramatically the dynamics of *Mastigias papua* populations (Dawson et al. 2001, Martin et al. 2006). Evidence for strong variations in nutrition of *Mastigias papua* can be seen in the medusae from CLM. In the past decades, this population has been observed to be successively zooxanthellate and non-zooxanthellate at least four times (Dawson et al. 2001, Gerda Ucharm unpublished data). Subtler variations in nutrition are also suggested by variations in isotopic and elemental compositions of the medusae from

OTM from year to year (Fig. 9). Such variability in nutrition is not surprising and has been already documented in other photosymbiotic organisms, often associated with seasonality (e.g. Verde and McColskey 1998, Swart et al. 2005, Ferrier-Pagès et al. 2011).

Importantly, this variability in time implies that the patterns documented in this study are not dependent on the sub-species of *Mastigias papua* considered (in spite of genetic differences, Swift et al. 2016). Rather, our results suggest that *Mastigias papua* is able to display an important plasticity in its nutrition (sensu Levis and Pfennig 2016) from pure heterotrophy to dominant autotrophy (Fig. 10). Important plasticity of the nutrition has already been reported in other photosymbiotic cnidarians such as scleractinian corals (Teece et al. 2011, Fox et al. 2019) but this study is, to our knowledge, the first documenting this plasticity in zooxanthellate jellyfishes.

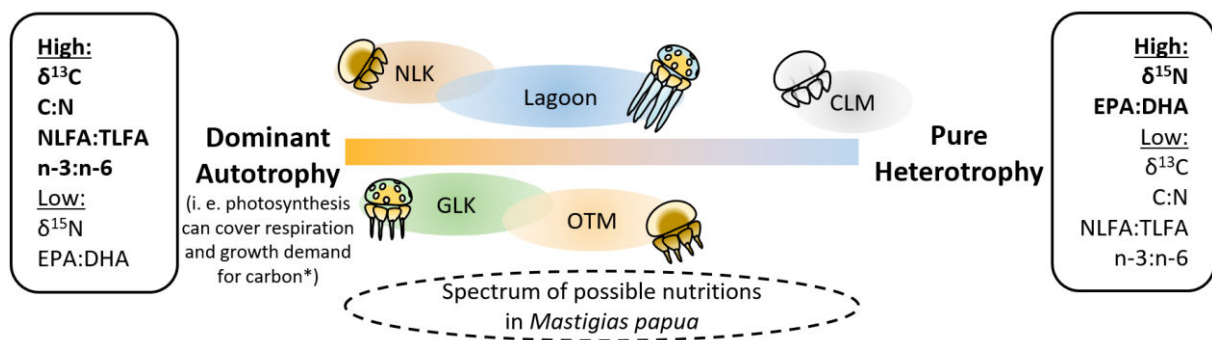


Fig. 10 Conceptual diagram: The spectrum of possible nutrition modes in *Mastigias papua* medusae. From dominant autotrophy (left) to complete heterotrophy (right) with the trophic indicators used in this study. The colored ovals correspond to the relative position of the nutrition of the medusae populations sampled in 2018 during this study. Note that these are not fixed in time. NLK = Uet era Ngermeuangel, GLK= Goby Lake, OTM = Ongeim'l Tketau, CLM = Clear Lake. * see McCloskey et al. (1994)

Fig. 10 Diagramme conceptuel : Le spectre des modes de nutrition possibles chez les méduses de l'espèce *Mastigias papua*. De l'autotrophie dominante (à gauche) à l'hétérotrophie complète (à droite) avec les indicateurs trophiques utilisés dans cette étude. Les ovals colorés correspondent à la position relative de la nutrition des populations de méduses échantillonnées en 2018 pour cette étude. Noter que ces positions ne sont pas fixes dans le temps. NLK = Uet era Ngermeuangel, GLK= Goby Lake, OTM = Ongeim'l Tketau, CLM = Clear Lake. * voir McCloskey et al. (1994)

4.4. Environmental Determinisms of the Nutrition of *Mastigias papua*

If the variations documented in the nutrition of *Mastigias papua* populations are not fully linked to their genotype, then the sources of these variations might be found in their environments. The extreme case of the non-zooxanthellate medusae from CLM can provide a first indication. Zooxanthellate jellyfishes, as scleractinian corals, can experience bleaching (loss of zooxanthellae) linked with heat stress (Dawson et al. 2001, McGill and Pomoroy 2008, Newkirk et al. 2018, Klein et al. 2019). For Palau's *Mastigias papua* specifically, the threshold at which bleaching occurs is estimated to be around 31.5 °C (Dawson et al. 2001). And indeed, we found that CLM was the hottest lake at the time of our sampling (the temperature at 5 m was of 33 °C in CLM as opposed to 29 °C to 31.5 °C in other sites). This gives a first, admittedly extreme way, in which the environment can influence the nutrition of *Mastigias papua*. More generally, the relative importance of heterotrophy and autotrophy in *Mastigias papua* nutrition are likely to be impacted by the availability of different resources (prey, light and dissolved inorganic nutrients, see e.g. Verde and McCloskey 1998, Kremer 2005).

The availability of these different resources might, in turn, be influenced by changes in the medusae populations densities. Medusae populations can deplete prey stocks and therefore affect their own food supply (Schneider and Behrends 1994, Lucas 2001, Goldstein and Riisgård 2016). The *Mastigias papua* populations of marine lakes of Palau could be very prone to such mechanisms. Palau marine lakes are characterized by a simple planktonic food chain (e.g. only two species of copepods in most lakes, Hamner et al. 1982, Saitoh et al. 2011), and often important populations of *Mastigias papua* (Hamner et al. 1982, Cimino et al. 2018). Medusae from the family Mastigiidae, to which belongs *Mastigias papua*, have been documented to have important predation impacts on zooplankton communities (Garcia and Durbin 1993, West et al. 2009 see also Bezio et al. 2018). From this, it could be hypothesized that the higher the population of *Mastigias papua*, the lower the population of zooplankton. This top-down control on the zooplanktonic prey could then result in a feedback on the *Mastigias papua* medusae population, possibly reducing the contribution of heterotrophy to its nutrition. If this is true, it would be expected that the indicators of the nutrition of *Mastigias papua* would follow density-dependent patterns. This is indeed what have been found here, using isotopic and elemental data from 2010 to 2018 in OTM (Fig. 9). *Mastigias papua* medusae tend to be more heterotrophic (low $\delta^{13}\text{C}$ and C:N ratios and high $\delta^{15}\text{N}$) when

population densities are low, and more autotrophic (high $\delta^{13}\text{C}$ and C:N ratios and low $\delta^{15}\text{N}$) when population densities are high. At the time of the sampling in 2018, medusae population in OTM was low (Fig. 9). Thus their nutrition, more heterotrophic than in medusae from NLK and GLK, might be explained by a greater availability of prey.

4.5. Conclusions and Implications for the ecology of Zooxanthellate Jellyfishes

The plasticity of the nutrition of zooxanthellate jellyfishes was previously characterized mostly through extreme cases (e.g. *Phyllorhiza punctata* zooxanthellate and non-zooxanthellate populations, Bolton and Graham 2004, *Cephea cephea* loss of zooxanthellae at the medusae stage, Sugiura 1969). Here, it is shown that subtler changes occur in the nutrition of zooxanthellate jellyfishes. This has important implications for their ecologies. A great plasticity in the nutrition of zooxanthellate jellyfishes implies that they could occupy more diversified ecological niches, possibly reducing their vulnerability to environmental changes. This could partly explain why they may be favored by eutrophication (e.g. Stoner et al. 2011), unlike other photosymbiotic organisms such as scleractinian corals (e.g. Lapointe et al. 2019). This plasticity also explains the ability to survive bleaching (Dawson et al. 2001, this study) provided that prey is present in sufficient amounts. Another important repercussion of this plasticity of the nutrition might be on zooxanthellate jellyfishes' ability to bloom. Dawson and Hamner (2009) have found that zooxanthellate jellyfishes are generally less likely to bloom as compared to non-zooxanthellate jellyfishes. One of their hypothesis to explain this characteristic is that zooxanthellate jellyfishes could rely on one energy source when the other is scarce. This would effectively result in a smoothed energy income and, therefore, less variability in populations (hence, less blooms). The plasticity of the nutrition of *Mastigias papua* characterized here gives support to this hypothesis.

The present conclusions were, however, derived from one species of zooxanthellate jellyfish in one specific location. Zooxanthellate jellyfishes are diverse (see e.g. Sugiura 1969, Bouillon et al. 1988, Straehler-Pohl and Toshino 2015 reviewed in Djeghri et al. 2019) but, with the exception of the model organism *Cassiopea* spp. (Ohdera et al. 2018), their ecology remains little known. It is thus important to test to which extent the conclusions of the present study are transferable to other species.

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Appendix: *Organ-specific patterns and their importance for the interpretation of $\delta^{13}\text{C}$ signatures*

Introduction

Isotopic and elemental composition of oral arms (as opposed to umbrella) were also obtained for the medusae sampled in 2018. This also allows for the comparison with previous year (see Fig. 9 in main text) which sampled oral arms.

Here, we discuss organ-specific patterns and the important consequence they have for the interpretation of the $\delta^{13}\text{C}$ signatures.

Method

The isotopic and elemental data was obtained following the same protocol as detailed in the main text (see section 2.2. in main text). They were however not corrected by the isotopic baseline as we do not compare between the different sites here.

ANCOVAs with permutations (10 000 permutations) were performed to assess the effect of medusae wet mass and organ. These were performed separately on each sampling site and used the organ (umbrella versus oral arms) as the categorical factor and Log_{10} transformed medusae wet mass as the covariate. It was checked; 1) if the slopes for the different organs were significantly different (i.e. p-value of the interaction organ-medusae mass < 0.05); 2) if not, it was checked if the slopes were significant (i.e. p-value of the effect of the medusae mass < 0.05); and 3) if the intercept for the different organs were different (i.e. p-value of the effect of the organ < 0.05).

Results

The $\delta^{13}\text{C}$ signature of *Mastigias papua* varied according to organ and the medusae wet mass. In medusae from Uet era Ngermeuangel (NLK), Goby Lake (GLK) and Ogeim l'Tketau (OTM) the patterns are similar: With medusae size, the $\delta^{13}\text{C}$ in oral arms tend to stay constant whereas it increases in the umbrella (Fig. A1). In the medusae from the lagoon, the $\delta^{13}\text{C}$ tend to increase with size. Oral arms have a lower $\delta^{13}\text{C}$ than umbrella but have no different slopes

in relation to medusae size (Fig. A1). In medusae from Clear Lake (CLM) size have no significant effect, and oral arms have a lower $\delta^{13}\text{C}$ signature than umbrella (Fig. A1).

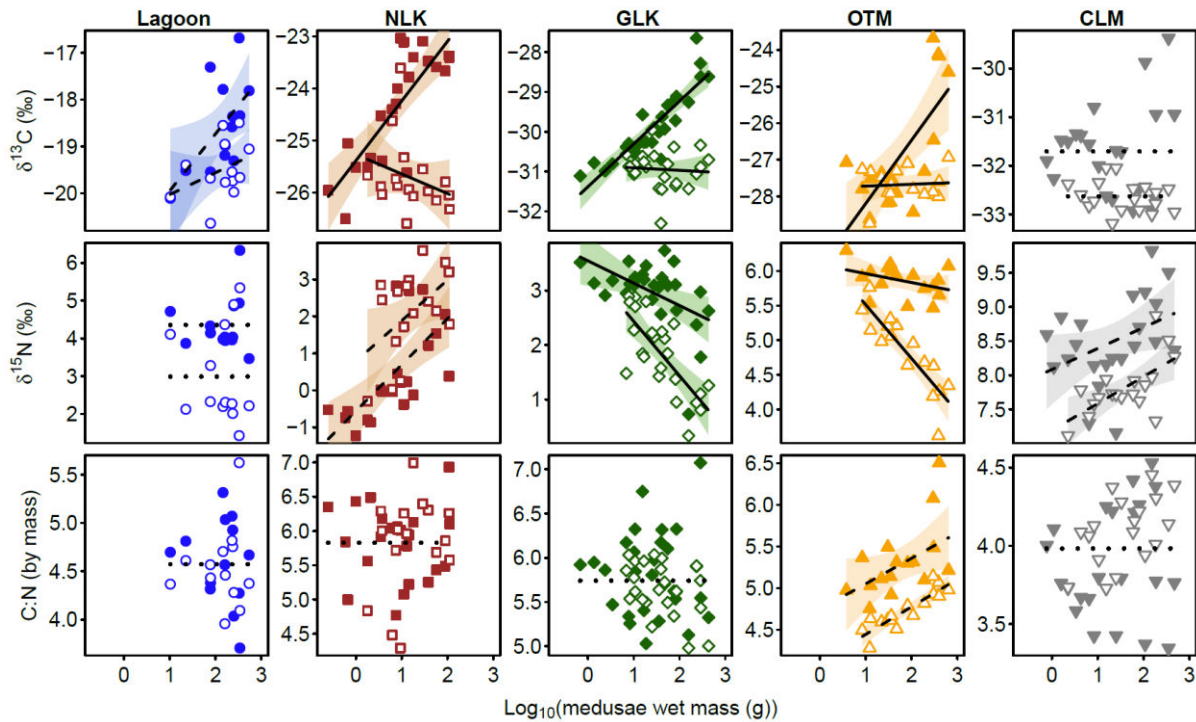


Fig. A1 Effect of medusae size (wet mass) and organ (umbrella (full symbols) versus oral arms (empty symbols)) on $\delta^{13}\text{C}$ (top panels), $\delta^{15}\text{N}$ (middle panels) and mass C:N ratios (bottom panels) in *Mastigias papua* in the different sites sampled in 2018. Horizontal dotted lines without shaded areas indicate no significant effect of medusae wet mass. If there is no effect of the organ only one line is drawn. Dashed lines indicate significant effect of size with no significant difference of slopes between umbrella and oral arms. Solid lines indicate significant effect of the interaction wet mass-organ (i.e. significantly different slopes between umbrella and oral arms). Shaded areas are 95 % C. I. around regression lines. Statistics are based on ANCOVAs with permutations. The threshold for statistical significance was set at $\alpha = 0.05$. Note the different scales on the y-axes

Fig. A1 Effet de la taille des méduses (masse fraîche) et de l'organe (ombrelle (symboles pleins) contre bras péri-buccaux (symboles vides)) sur les $\delta^{13}\text{C}$ (haut), $\delta^{15}\text{N}$ (milieu) et ratios C:N massiques (bas) chez *Mastigias papua* dans les différents sites échantillonnés en 2018. Les lignes pointillées horizontales sans aires ombrées indiquent l'absence d'effet significatif de la masse fraîche. S'il n'y a pas d'effet de l'organe échantillonné, seule une ligne est marquée. Les lignes en traits-tillés indiquent un effet significatif de la masse fraîche mais sans différence de pentes entre l'ombrelle et les bras péri-buccaux. Les lignes pleines indiquent un effet significatif de l'interaction masse fraîche-organe (i.e. pentes significativement différentes entre l'ombrelle et les bras péri-buccaux). Les aires ombrées correspondent aux I. C. 95 % autour des régressions. Ces statistiques sont basées sur des ANCOVAs à permutations. Le seuil de significativité statistique est à $\alpha = 0.05$. Noter les différentes échelles sur l'axe des y

The $\delta^{15}\text{N}$ signature of *Mastigias papua* also varied according to organ and the medusae wet mass. Medusae from GLM and OTM presented similar patterns; $\delta^{15}\text{N}$ signatures tended to decrease with medusae size. This decrease was faster in oral arms than in umbrella (Fig. A1). Medusae from NLK and CLM presented an opposed pattern with their $\delta^{15}\text{N}$ increasing with medusae size (albeit without significant differences in slopes between oral arms and umbrella, Fig. A1). The $\delta^{15}\text{N}$ of medusae from the lagoon was not significantly influenced by medusae size (Fig. A1). Lastly, in medusae from most sites (Lagoon, GLK, OTM, and CLM) the $\delta^{15}\text{N}$ of oral arms was lower than the $\delta^{15}\text{N}$ of umbrella. This pattern was however reversed in medusae from NLK (Fig. A1).

Finally, the C:N ratios of medusae were neither influenced by their size nor the organ sampled in most sites (Lagoon, NLK, GLK, and CLM, Fig. A1). The only exception was medusae from OTM which had increasing C:N ratios with size, and oral arms of lower C:N ratios than umbrella (Fig. A1).

Discussion

A strong pattern of $\delta^{13}\text{C}$ data in this study is an increase of the values with medusae size in umbrella of medusae of all sites except in medusae from Clear Lake (CLM, Fig. A1). This increase is thus seen only in medusae with zooxanthellae but is generally not mirrored in their oral arms (Fig. A1). This is odd, as an increase in $\delta^{13}\text{C}$ due to increased autotrophy (see section 4.1.1. in main text) would be expected to be stronger in oral arms which contain more zooxanthellae (Muscattine et al. 1986). Hence we hypothesize that the increases seen in the $\delta^{13}\text{C}$ signatures of the umbrella, but not in oral arms, are explained by another mechanism linked to the shapes of these organs:

In this regard it is important to consider further the “depletion-diffusion hypothesis” (see Muscattine et al. 1989, reviewed in Ferrier-Pagès and Leal 2018). *In-hospite* pools of dissolved inorganic carbon have two sources: (1) respiration (of both symbionts and host) and (2) diffusion from the surrounding sea-water; and one sink: symbionts’ photosynthesis. When photosynthesis is high, the carbon pool gets depleted resulting in less fractionation during inorganic carbon uptake by the algae and thus an increase in $\delta^{13}\text{C}$ values (Muscattine et al. 1989, Swart et al. 2005, Ferrier-Pagès and Leal 2018 see also Fry 1996). In this context the

different shapes of the oral arms and of the umbrella may be of importance: The umbrella, with medusa growth, gets thicker whether oral arms become more and more complex (Uchida 1926). The complex shape of oral arms would favor diffusion of dissolved inorganic carbon from surrounding water whether this would get more and more limited in the umbrella as it gets thicker (Fig. A2). This limitation of the diffusion in the umbrella would then increase fractionation even without increase of photosynthesis. Therefore, the different shapes of the umbrella and of the oral arms would thus explain their differences in the evolution of their $\delta^{13}\text{C}$ signatures along medusae sizes. Importantly, this implies that the strong size-specific patterns seen in $\delta^{13}\text{C}$ data cannot be interpreted as variation in nutrition.

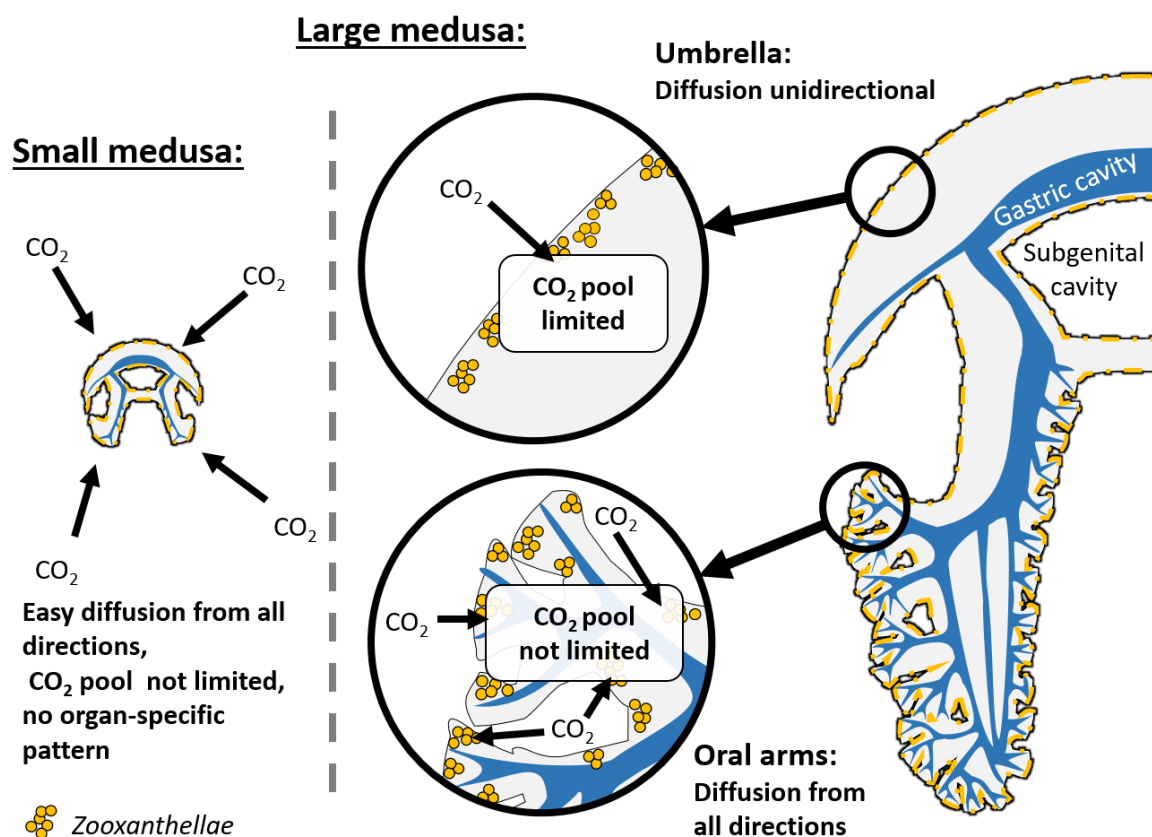


Fig. A2 Hypothesized effects of medusa size and organ shapes on the diffusion of dissolved CO₂ from the sea-water too zooxanthellae through the host tissues. Due to their complex shape, the oral arms would favor diffusion unlike the umbrella. Blue = digestive system, orange = zooxanthellae. See Uchida (1926) for details on *Mastigias* anatomy and development

Fig. A2 Effets hypothétiques de la taille des méduses et de la forme des organes sur la diffusion du CO₂ dissous depuis l'eau de mer vers les zooxanthelles à travers les tissus de l'hôte. Du fait de leur forme complexe, les bras péri-buccaux favoriseraient la diffusion, contrairement à l'ombrelle. Bleu = système digestif, orange = zooxanthelles. Voir Uchida (1926) pour les détails de l'anatomie et du développement de *Mastigias*

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General discussion

Discussion générale



1. Introduction

Jellyfishes (i.e. pelagic cnidarians, [Lucas and Dawson 2014](#)) play an integrative role in marine ecosystems. They are important components of pelagic food webs ([Purcell 1997](#), [Hays et al. 2018](#)), and thereby affect nutrient cycling ([Pitt et al. 2009a](#)) and carbon export ([Lebrato et al. 2012](#)). Jellyfishes, however, differ notably from most other pelagic metazoans through the ability of some of them to bloom ([Boero et al. 2008](#), [Dawson and Hamner 2009](#), [Lucas and Dawson 2014](#), [Pitt et al. 2014](#)). Population dynamics of jellyfishes in marine ecosystems are controlled by their recruitment, growth and survival which are themselves controlled (among other factors) by the nutrition of the jellyfishes. Hence, understanding jellyfishes' nutrition is key to understand the role of jellyfishes in ecosystem functioning. Whereas most jellyfishes are purely heterotrophic, acquiring their nutrition from predation (see e.g. [Purcell 1997](#)) many other supplement this heterotrophic nutrition through a photosymbiosis with zooxanthellae (see e.g. [Kremer et al. 1990](#), [Kikinger 1992](#), [Verde and McCloskey 1998](#)).

These zooxanthellate jellyfishes and their nutrition have been the focus of this thesis. Research on zooxanthellate jellyfishes have mostly focused at the model organism *Cassiopea* spp. ([Ohdera et al. 2018](#)). Other species have received relatively less attention and the information concerning them is often scattered across the literature with little synthesis (e.g. on photosynthesis: [Verde and McCloskey 1998](#) – on strobilation: [Astorga et al. 2012](#), [Helm 2018](#) – on polyp formation: [Lucas et al. 2012](#) – on impacts on ecosystem functioning: [Pitt et al. 2009a](#) – on blooming ability: [Dawson and Hamner 2009](#)). The first step of this thesis was therefore to synthesize this information. Then experimental and field works were performed to investigate the nutrition of zooxanthellate jellyfishes with particular emphasis on variations between different life-stages or size classes or between populations living in different environments.

2. Scientific Contributions of this Thesis

2.1. Diversity and Traits of Zooxanthellate Jellyfishes

The Chapter I of this thesis provides a global assessment of zooxanthellate jellyfishes species. The conclusions are that symbioses between zooxanthellae and medusozoans originated a minimum of seven times, five of which concern jellyfish-containing taxa. From this census of

zooxanthellate jellyfishes, it was estimated that 20 to 25 % of scyphozoans species are zooxanthellate (including facultative symbionts). Thus, being zooxanthellate is by no means an exception in Scyphozoa but is rather common.

The Chapter I also identifies three important, emergent, characteristics of zooxanthellate jellyfishes: (1) at the medusa stage, their nutrition is generally mixotrophic. In this regard, zooxanthellate medusae are, to our knowledge, the largest pelagic mixotrophs. This sets them apart from both smaller pelagic photosymbiotic species (e.g. radiolarians), or large benthic photosymbiotic species (e.g. scleractinian corals). (2) zooxanthellate polyps, although being able to host zooxanthellae do not rely much on them. This makes zooxanthellate jellyfishes' polyps more akin to other, non-zooxanthellate, jellyfish polyps than to small scleractinian corals in terms of ecology. (3) Zooxanthellae play a key role in strobilation. This gives them the potential to exert a control on the recruitment of new individuals into medusa populations.

2.2. Significance of Zooxanthellae and Mixotrophic Nutrition in the Life-Cycle of Jellyfishes

2.2.1. Polyp phase

The Chapter I highlighted previous works (e.g. [Sugiura 1963](#), [Rahat and Adar 1980](#), [Hofmann and Kremer 1981](#), [Prieto et al. 2010](#)) that suggest that zooxanthellae are of small importance for the nutrition of zooxanthellate jellyfishes' polyps. This has led to the hypothesis that zooxanthellate and non-zooxanthellate polyps should not differ much in their ecologies. This research question was tested in Chapter II. The results were that the non-zooxanthellate *Aurelia* sp. polyps and the zooxanthellate *Cassiopea* sp. polyps reacted in the same way to presence or absence of light, prey and added nutrients. This would indeed support the above stated hypothesis that zooxanthellate and non-zooxanthellate polyps react in the same way to resource availability.

2.2.2. Strobilation

Another pattern highlighted by Chapter I is that zooxanthellae play a role in strobilation (see e.g. [Sugiura 1964, 1969](#), [Rahat and Adar 1980](#), [Newkirk et al. 2018](#)). Although the experiment described in Chapter II was not design to specifically study strobilation, it provided one new

observation (albeit admittedly anecdotal). Zooxanthellae are known to multiply in the forming ephyra during strobilation (e.g. [Ludwig 1969](#)). This was also observed in the experiment described in Chapter II but additionally, this was observed in one polyp held in the dark (Chapter II). This multiplication of zooxanthellae could only have been supported through the heterotrophy of the host. This observation should be confirmed through more experimental work, but may help explain the role of zooxanthellae during strobilation in scyphozoans.

2.2.3. Medusa phase

For many species of zooxanthellate jellyfishes, both heterotrophic and autotrophic inputs are generally needed at the medusa phase ([Kremer 2005](#), [Welsh et al. 2009](#)). In Chapter III *Cassiopea* sp. medusae only grew when given light and prey thus tending to confirm the pattern of a reliance on both autotrophy and heterotrophy. Aside from this general mixotrophy, previous works have suggested that the size (or age) of a medusa can affect its balance of autotrophy and heterotrophy (see [Sugiura 1969](#), [McCloskey et al. 1994](#)). This has been confirmed by the results presented in Chapter IV where variations of nutrition are indeed found along size gradients. However, the previous works have not compared medusae from different environments (but see [McCloskey et al. 1994](#)). Hence, it was not possible to decipher whether the variations associated with medusae size were strictly linked to ontogeny or if they could be affected by the environmental conditions. By documenting different size-related patterns as a function of the sampling site, the results of Chapter IV demonstrate that environment can indeed impact the size-related pattern in the nutrition of zooxanthellate jellyfishes. This, along with other lines of evidences suggests that the nutrition of zooxanthellate medusae is highly plastic.

2.3. Plasticity in the Nutrition of Zooxanthellate Jellyfishes

The fact that populations or individuals can be either zooxanthellate or non-zooxanthellate in some species ([Dawson et al. 2001](#), [Bolton and Graham 2004](#)) suggests that the nutrition of zooxanthellate jellyfishes can be highly variable for a given species. The aforementioned examples are rather extreme cases, and it would be expected that finer gradients in the balance between autotrophy and heterotrophy of zooxanthellate jellyfishes can be found.

However, these gradients are little documented in the existing literature (but see [McCloskey et al. 1994](#)). The results presented in Chapter IV demonstrate that such gradients can indeed be found. Moreover, the fact that the nutrition within a given population can change in time shows that variation of nutrition is not (only) linked to the genotype but is also a response to changes in environmental conditions. Hence, this documented variability is in fact a form of phenotypic plasticity (sensu [Levis and Pfennig 2016](#)). In the specific case of *Mastigias papua* from Palau, their nutrition can occupy a whole spectrum from complete heterotrophy to dominant autotrophy, where photosynthesis can provide all respiration and growth carbon requirements (Chapter IV). This plasticity of the nutrition of zooxanthellate jellyfishes would then have important consequences for their ecology.

3. Implications for the Ecology of Zooxanthellate Jellyfishes

Some aspects of the ecology of zooxanthellate jellyfishes, in the light of the published literature, have been discussed in Chapter I. The results garnered during this thesis, and more particularly, the wide nutritional plasticity of *Mastigias papua* medusae documented in Chapter IV, shed new light on some of the aspects of the ecology of zooxanthellate jellyfishes.

3.1. Population Dynamics

3.1.1. Blooming ability

One remarkable aspect of zooxanthellate jellyfishes' population dynamics is their generally lower ability to bloom as compared to non-zooxanthellate jellyfishes ([Dawson and Hamner 2009](#)). Several hypotheses have been proposed to explain it. First, the symbiosis with zooxanthellae might imply trade-offs ([Dawson and Hamner 2009](#), see also Chapter I and references therein). This first hypothesis has not been much explored but would be a valuable direction for future research (see below, section 4.2.). The other hypotheses relate to the availability of resources and are detailed in Chapter I. Briefly, [Dawson and Hamner \(2009\)](#) proposed two possible hypotheses: (1) zooxanthellae allow zooxanthellate jellyfishes to access a more stable resource (autotrophy). (2) zooxanthellate jellyfishes are able to switch resources if one becomes limiting. In both cases, this results in a smoother energy income for

the medusae population. As a result, this would make populations less variable and thus, less prone to form blooms as compared to non-zooxanthellate jellyfishes. Another hypothesis was proposed in Chapter I: zooxanthellate jellyfishes, to bloom, might need both autotrophic and heterotrophic resources. By relying on two different resources they might be more likely to get a mismatch (in the vocabulary of the match-mismatch hypothesis, see e.g. [Cushing 1990](#)), which would result in an absence of bloom. The new results of this thesis allow us to discriminate which of these hypotheses is the most plausible. Indeed, the wide plasticity in the nutrition of *Mastigias papua* medusae documented in Chapter IV supports the hypothesis of [Dawson and Hamner \(2009\)](#) according to which it is the ability of zooxanthellate jellyfishes to switch resources that reduce their ability to bloom.

3.1.2. Density-dependent processes

Another important insight gained through the results of Chapter IV is the existence of density-dependent processes affecting the nutrition of zooxanthellate jellyfishes' populations. Such processes have already been documented in non-zooxanthellate jellyfishes (e.g. [Lucas 2001](#), [Goldstein and Riisgård 2016](#)). Moreover, due to their simple food web, high medusae biomass and enclosed topography (e.g. [Hamner et al. 1982](#), see also Fig. 1), the marine lakes from Palau would be environments very prone to such processes. The fact that density-dependent processes are observed in these ecosystems may thus not be very surprising. Interestingly however, some differences may be hypothesized between density-dependent limitation of prey stocks by zooxanthellate and non-zooxanthellate jellyfishes. In non-zooxanthellate jellyfishes, the depletion of prey-stocks represents a strict limitation that can lead to decreased medusae sizes ([Lucas 2001](#), [Goldstein and Riisgård 2016](#)). In zooxanthellate jellyfishes by contrast, this decrease in size might be mitigated by the alternative energy source that is autotrophy.



Fig. 1 Important population density of *Mastigias papua remeliiki* medusae in Uet era Ngermeuangel, a marine lake of Palau (Micronesia). Larger medusae in this picture are ca. 15 cm in bell diameter. Photograph taken by Philippe Pondaven

Fig. 1 Fortes densités de population de méduses *Mastigias papua remeliiki* dans Uet era Ngermeuangel, un lac marin de Palaos (Micronésie). Les ombrelles des plus grandes méduses dans cette image mesurent ca. 15 cm de diamètre. Photographie prise par Philippe Pondaven

3.2. Changes in Environment

3.2.1. Temperature-induced bleaching

The results of Chapters II and IV have implications for the reaction of zooxanthellate jellyfishes to bleaching events. The results from Chapter II confirm that zooxanthellate and non-zooxanthellate polyps react in similar ways to resource availability. This supports the inference made in Chapter I that bleached zooxanthellate polyp populations would be little influenced by the loss of their zooxanthellae (Fig. 2). The wide spectrum of the nutrition of *Mastigias papua* found in Chapter IV gives more support to the inference that, if enough prey is available,

some zooxanthellate jellyfish species might be able to cope with bleaching (Chapter I). From this, several scenarios of the reaction of zooxanthellate jellyfishes to bleaching may be hypothesized (Fig. 2): (1) mass mortality, (2) reacquisition of zooxanthellae, (3) switching to 100 % heterotrophic nutrition. Then, as noted in Chapter I, the role of zooxanthellae in strobilation may exert a control in the recruitment of young medusae possibly leading to senescence of the medusa population (Fig. 2).

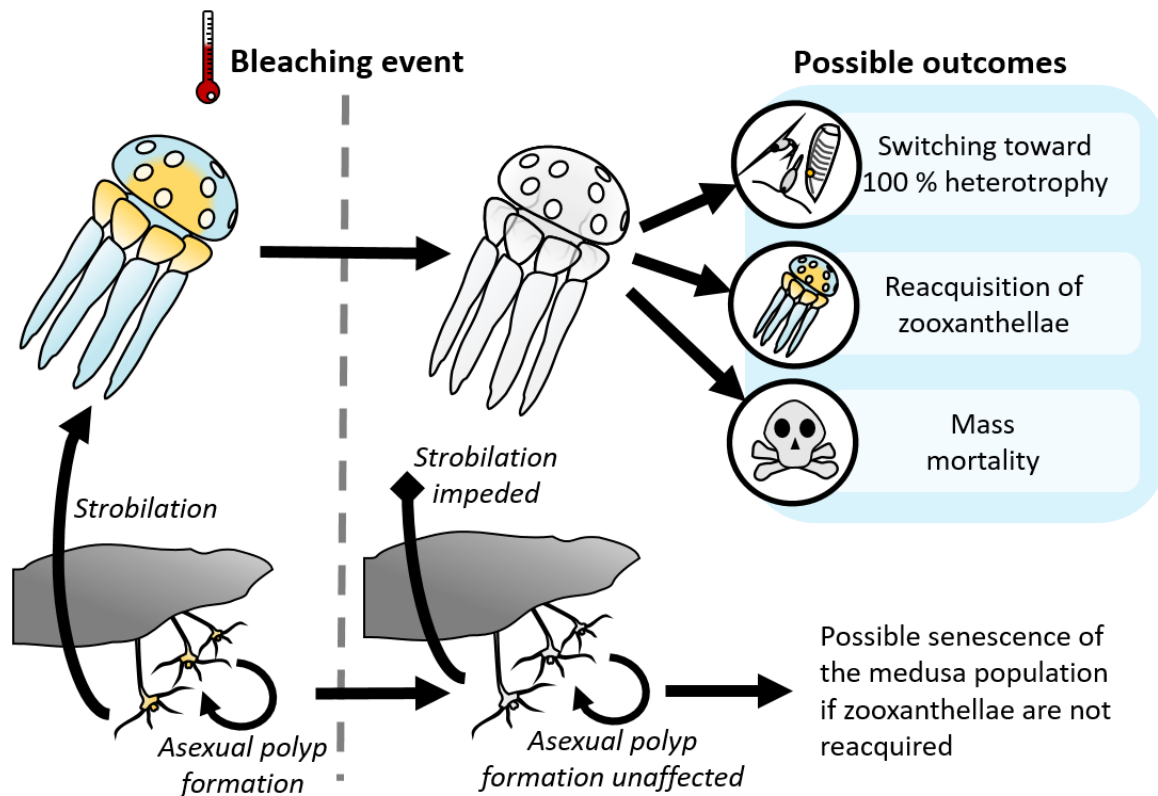


Fig. 2 Possible effects of bleaching on zooxanthellate jellyfishes' populations

Fig. 2 Possibles effets du blanchissement sur les populations des méduses à zooxanthelles

3.2.2. Eutrophication

In Chapter I, it was noted that zooxanthellate jellyfishes appear to be favored by eutrophication (e.g. [Stoner et al. 2011](#)) contrary to other photosymbiotic cnidarians such as scleractinian corals (e.g. [Lapointe et al. 2019](#)). The argument advanced to explain this discrepancy was that zooxanthellate medusae, by being pelagic, were generally unlikely to experience the negative effects of eutrophication (e.g. reduced light, enhanced competition with macroalgae, see Chapter I). The benthic polyps, likewise, would be little influenced by

eutrophication as they rely little on their zooxanthellae (Chapter I, confirmed by the results of Chapter II). The wide plasticity of the nutrition of *Mastigias papua* (Chapter IV) may be an additional mechanism to explain why zooxanthellate jellyfishes appear to be favored by eutrophication: They would be able to adapt to changes in resources.

4. Knowledge Gaps and Future Directions

Naturally, this thesis is far from having answered all the questions that surround the ecology and the nutrition of zooxanthellate jellyfishes. Many knowledge gaps remain to be explored. Therefore, here are given some possible future directions for the research on zooxanthellate jellyfishes.

4.1. Technical Difficulties in the Study of the Nutrition of Zooxanthellate Jellyfishes

One important tool that has been used during this thesis was trophic markers (stable isotopes and fatty acids). These are undoubtedly valuable tools for the study of the nutrition of jellyfishes (Pitt et al. 2009b) and photosymbiotic organisms (Ferrier-Pagès and Leal 2018, Mies et al. 2018). However, many difficulties remain regarding their use.

More particularly, in the case of stable isotopes, difficulties arise from both specificities of jellyfishes, and specificities of photosymbioses. The isotopic signatures of photosymbiotic organisms are affected by complex processes of mixing and fractionation (Ferrier-Pagès and Leal 2018). The results of Chapter III provide some first insights of how these processes are at play in zooxanthellate jellyfishes. However, the effects of varied levels of photosynthesis or predation remain to be determined precisely. This has been attempted during this thesis but, due to technical difficulties, it did not yield exploitable results. Other difficulties of the use of stable isotopes to assess the nutrition of zooxanthellate jellyfishes are linked to uncertainties inherent to jellyfish isotopic studies. Methods for the preparation and conservation of jellyfish samples for isotopic analyses are non-trivial (Fleming et al. 2011, Kogovšek et al. 2014, MacKenzie et al. 2017). The trophic fractionation factors of jellyfishes are apparently unusual (i.e. high fractionation for carbon, low fractionation for nitrogen, Malej et al. 1993, D'Ambra et al. 2014), and have proven difficult to apply to infer trophic levels (Fleming et al. 2015). An

additional difficulty is linked to the type of organ sampled which can differ in isotopic signature in non-zooxanthellate jellyfishes (D'Ambra et al. 2014). In zooxanthellate jellyfishes, this can be further complicated by organ-specific fractionation due to photosynthesis (Chapter IV). Hence, to be able to better understand how stable isotopes behave in zooxanthellate jellyfishes, more experimental work is needed. These works should particularly aim to assess trophic fractionation factors (between prey and the host, between dissolved inorganic nutrients and the symbionts, and between the symbionts and their host), and the effect of varied levels of predation, photosynthesis or recycling of elements within the holobiont.

4.2. Zooxanthellate Jellyfishes as Models of Photosymbioses

Cassiopea is one genus of zooxanthellate jellyfish that has long been used as a model for the study of cnidarian-zooxanthellae symbioses in general (Freudenthal 1962, Ohdera et al. 2018). It has deepened the understanding of the cell biology of the cnidarian-zooxanthellae symbioses (see e.g. Colley and Trench 1985) or of the infection by diverse symbionts strains (e.g. Mellas et al. 2014). However, zooxanthellate jellyfishes other than *Cassiopea* might have potential to study of cnidarian-zooxanthellae symbioses.

From a theoretical standpoint, establishments of symbioses are of particular interest to biologists (e.g. López-García et al. 2017). One important aspect to study is the trade-offs associated with the symbioses, notably how symbioses can shift from mutualism to parasitism (see e.g. Bronstein 2001). In the specific case of cnidarian-zooxanthellae symbioses, such shifts have been documented (e.g. Sachs and Wilcox 2005, Lesser et al. 2013, Hartmann et al. 2019 see also Wooldridge 2010). Importantly, a shift from mutualism to parasitism is likely to imply trade-offs (but see Bronstein 2001). Some zooxanthellate jellyfishes might present unique occasion to explore some of these trade-offs. For instance, it has been proposed to compare zooxanthellate and non-zooxanthellate populations of *Phyllorhiza punctata* to understand why the latter are less likely to bloom (Dawson and Hamner 2009). Another group that could be of great interest is Cepheidae, as most of them apparently lose their symbionts ontogenetically (Sugiura 1969, Chapter I). To date, almost nothing is known of the reasons of this loss of symbionts and this would be an interesting and valuable research axis to pursue. However, this could be limited by some technical issues. *Cassiopea*, as a biologic model, has

the advantage of being relatively easy to maintain in laboratory ([Ohdera et al. 2018](#)). The Cepheidae, by opposition are generally harder to maintain but progress in jellyfish husbandry are constantly made (e.g. [Ramondenc et al. 2019](#)) and, in the specific case of Cepheidae, bubble wrap may be part of the solution ([Monterey Bay Aquarium 2019](#)).

4.3. Can we Generalize?

The most notable gap in our knowledge of zooxanthellate jellyfishes might be related to their diversity. Zooxanthellate jellyfishes are indeed diverse both from a phylogenetic and ecologic standpoint (Chapter I). Results from Chapter IV illustrate how, within a given species, the nutrition mode can vary widely. Inter-specific differences have the potential to be even more marked. In Chapter I, we give three emergent traits of zooxanthellate jellyfishes and emphasize their importance for the ecology of these species. However, for most zooxanthellate jellyfish species it remains unclear to which extent these traits are applicable.

4.3.1. Ecology of the polyps and the lack of an outgroup

Two key traits of zooxanthellate polyps given in Chapter I are: (1) zooxanthellate jellyfish's polyps do not rely much on their zooxanthellae for survival and asexual formation of polyps. And (2) zooxanthellae play a critical (but little understood) role in strobilation.

These conclusions are, however, entirely based on Kolpophoran polyps (see references in Chapter I). Hence, they may reflect traits unique to Kolpophorae rather than convergent traits of the diverse zooxanthellate jellyfishes' lineages. In which context, the polyps of *Linuche unguiculata* could be used as an outgroup. *Linuche unguiculata* is a Coronatae, the sister group to Discomedusae ([Kayal et al. 2018](#)). Moreover, the anatomy of its polyps is unlike those of Kolpophorae (see e.g. [Ortiz-Corp's et al. 1987](#), [Jarms et al. 2002](#)) suggesting that they might have a contrasted ecology. They may also have different chemical cues for strobilation as compared to Discomedusae polyps ([Helm and Dunn 2017](#)). Finally, cultivation methods for *Linuche unguiculata* polyps are readily available ([Jarms et al. 2002](#)). It would therefore be valuable to experimentally assess the role of zooxanthellae in the survival, growth and asexual

reproduction of *Linuche unguiculata* polyps and test whether they display, or not, the traits found in other zooxanthellate scyphozoans polyps.

4.3.2. Diversity of the nutrition of the medusae

The way zooxanthellate jellyfishes acquire their nutrition is most likely critical to their ecology (Chapter I). The results of Chapter IV show that this nutrition can vary a lot within a given species. This highlights that studies in varied environments are needed to fully assess the range of nutrition a zooxanthellate jellyfish might display. However, very few species of zooxanthellate jellyfishes are sufficiently known in this regard (e.g. *Cassiopea*, *Mastigias* and *Cotylorhiza* see Table 1). This lack of knowledge is even more apparent in hydrozoans and cubozoans zooxanthellate jellyfishes' nutrition on which very little is known (with the exception of *Velella velella* e.g. [Zeman et al. 2018](#)). Moreover, it is unlikely that the findings from one species are readily transferable to another. For instance, comparing three Kolpophorae medusae, one can find very contrasted nutrition modes: *Cassiopea* medusae is apparently very dependent on its symbionts (e.g. [Mortillaro et al. 2009](#)), *Cephea* medusae is not ([Sugiura 1969](#)), and *Mastigias* medusae may display a complete spectrum from dominant autotrophy to pure heterotrophy (Chapter IV). Therefore, to understand the roles of zooxanthellate jellyfishes in their respective ecosystems, more species need to be investigated in varied environments.

5. Conclusions

In summary, this thesis gives a synthetic assessment of the diversity, traits, and ecology of zooxanthellate jellyfishes. The significance of zooxanthellae in their nutrition has been assessed through both laboratory experiments and field observations. Most notably, the high plasticity of the nutrition of zooxanthellate jellyfishes has been characterized using the *Mastigias papua* medusae from the Palau archipelago as a model. This work highlights how diverse zooxanthellate jellyfishes are, both in terms of phylogeny and in terms of nutrition and subsequent ecology. There are, however, still many gaps and uncertainties in our knowledge of zooxanthellate jellyfishes that remain to be explored.

Table 1 Overview of the studies on the nutrition of Kolpophorae medusae. The column “Autotrophy” inventories studies assessing photosynthesis and/or inorganic nutrient uptake. The column “Heterotrophy” inventories assessing predation. The column “Mixotrophy” inventories studies assessing the relative contribution of autotrophy and heterotrophy. Light grey boxes indicates cases in which limited information is available (e.g. only one population sampled). Dark grey boxes represent cases for which no information has been found

Table 1 Vue synthétique des études portant sur la nutrition de la phase méduses des Kolpophorae. La colonne « Autotrophy » présente des études portant sur la photosynthèse et/ou la prise de nutriments inorganiques. La colonne « Heterotrophy » présente des études portant sur la prédation. La colonne « Mixotrophy » présente des études portant sur la contribution relative de l'autotrophie et de l'hétérotrophie. Les cases gris clair indiquent des cas où peu d'information est disponible (e.g. une seule population échantillonnée). Les cases gris foncé indiquent des cas pour lesquels aucune information n'a été trouvée

| Family, Genus | Presence/absence of zooxanthellae | Autotrophy | Heterotrophy | Mixotrophy |
|--------------------------|--|---|---|---|
| Cassiopeidae | | | | |
| <i>Cassiopea</i> | e.g. Freudenthal 1962, Verde and McCloskey 1998, Klein et al. 2019 | e.g. Drew 1972, Verde and McCloskey 1998, Freeman et al. 2016 | Larson 1997 | Mortillaro et al. 2009, Welsh et al. 2009 |
| Mastigiidae | | | | |
| <i>Mastigias</i> | e.g. Sugiura 1963, Muscatine et al. 1986, Dawson et al. 2001 | Muscatine and Marian 1982, McCloskey et al. 1994 | Hamner et al. 1982 | Chapter IV |
| <i>Phyllorhiza</i> | Bolton and Graham 2004 | Pitt et al. 2005, see also the Appendix | García and Durbin 1993, Peach and Pitt 2005 | |
| Cepheidae | | | | |
| <i>Cephea</i> | Sugiura 1969, Straehler-Pohl and Jarms 2010 | | | |
| <i>Cotylorhiza</i> | Kikinger 1992, Prieto et al. 2010 | Kikinger 1992 | Kikinger 1992, Pérez-Ruzafa et al. 2002 | Kikinger 1992 |
| <i>Marivagia</i> | see Chapter I | | | |
| <i>Netrostoma</i> | Straehler-Pohl and Jarms 2010 | | | |
| Thysanostomatidae | | | | |
| <i>Thysanostoma</i> | see Chapter I | | | |
| Versurigidae | | | | |
| <i>Versuriga</i> | see Chapter I | | | |

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**Appendix: Preliminary assessment of the
photosynthesis and respiration rates of
Phyllorhiza punctata medusae**

**Appendice : Evaluation préliminaire des taux de
photosynthèse et de respiration des méduses
de l'espèce *Phyllorhiza punctata***



Introduction

Phyllorhiza punctata Lendenfeld, 1884, is a zooxanthellate jellyfish species that is seen with some concern due to its invasive abilities (Graham et al. 2003, Bayha and Graham 2014). Depending on the population, *Phyllorhiza punctata* medusae can be zooxanthellate or not (Bolton and Graham 2004) making them an interesting model to compare zooxanthellate and non-zooxanthellate jellyfishes (Dawson and Hamner 2009). This may begin by a fuller understanding of its nutrition ecology. Whereas the predatory, heterotrophic nutrition of *Phyllorhiza punctata* medusae has been well characterized (García and Durbin 1993, D’Ambra et al. 2001, Peach and Pitt 2005, West et al. 2009, Bezio et al. 2018), there is no, to my knowledge, published assessment of its photosynthesis and respiration rates. Therefore, I want to present a preliminary assessment of the photosynthesis and respiration rates of *Phyllorhiza punctata* medusae performed through oxymetric measurements.

Material and Methods

Oximetry measures were performed on five *Phyllorhiza punctata* medusae of different umbrella diameter (range: 3.2-6.6 cm) obtained from Océanopolis (Brest Aquarium, France). Their photosynthesis and respiration levels were measured as follows: For each measure, two acrylic incubation chambers (volume = 0.975 L), equipped with a pump to allow effective mixing of the water volume, were set in a 24 °C water bath. One medusa was introduced in the first chamber whereas the other served as blank, filled with incubation water only. The system was left to stabilize for ca. 15 min. Then, changes in oxygen concentration in the two chambers were monitored using oxygen probes (FDO 925-3 dissolved oxygen probe, WTW, USA) mounted in the chambers. The incubations lasted for 1 hour and measures were taken every 30 s. For each medusa incubations were done in the dark and in a gradient of five or six levels of irradiance (range 10-220 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$, measured using a spherical light sensor: QSL – 2100, Biospherical Instruments Inc., USA). The dark incubation was considered representative of the respiration (assumed unaffected by irradiance). The incubations in light allowed for the measures of the net photosynthesis of the medusae at varied irradiance.

Measures of oxygen consumption and/or production were checked for quality graphically (linearity of the relationship between incubation time and oxygen level). The consumptions or

productions of oxygen due to the medusae, δO_{2med} , were then calculated using the following formula, allowing for the correction of the effect of the incubation water:

$$\delta O_{2med} = (\delta O_{2exp} \times V) - (\delta O_{2blank} \times (V - V_{med}))$$

With δO_{2exp} and δO_{2blank} the consumption or production of O_2 in the experimental and in the blank chamber respectively. V the volume of the incubation chamber and V_{med} the volume of the medusa (obtained via medusa mass and assuming a density equal to sea water: 1.025).

The δO_{2med} obtained during the incubation in the dark was considered as respiration rates R . Photosynthesis rates P were then obtained by subtracting R to the δO_{2med} obtained under light conditions.

Photosynthesis-irradiance curves (P/I curves) were then obtained for each medusa using the following model:

$$P = P_{max} (1 - e^{-I/k})$$

With P_{max} the maximum photosynthesis, I the irradiance and k the half-saturation intensity.

Results and Discussion

The estimations of the parameters of the P/I curves were significant for all the medusae except for the smallest individual (Fig. 1) probably due to a too small signal to be reliably detected. The photosynthesis rates per individuals increased with medusae size (Fig. 1) but were similar when normalized to the wet mass (data not shown). The irradiances to which the medusae were exposed during the measures were not of high intensity ($< 250 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ as opposed to $> 1500 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ in full sunlight). This calls caution when interpreting the estimation of P_{max} , and of the saturating irradiance. However, extrapolating from the models, the saturating light can be estimated to be ca. $400 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ (value obtained for $P = 0.95 P_{max}$ and $k = 130 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$, Fig. 1). This value is within the range of those reported in other zooxanthellate jellyfishes (Kremer et al. 1990, McCloskey et al. 1994, Verde and McCloskey 1998).

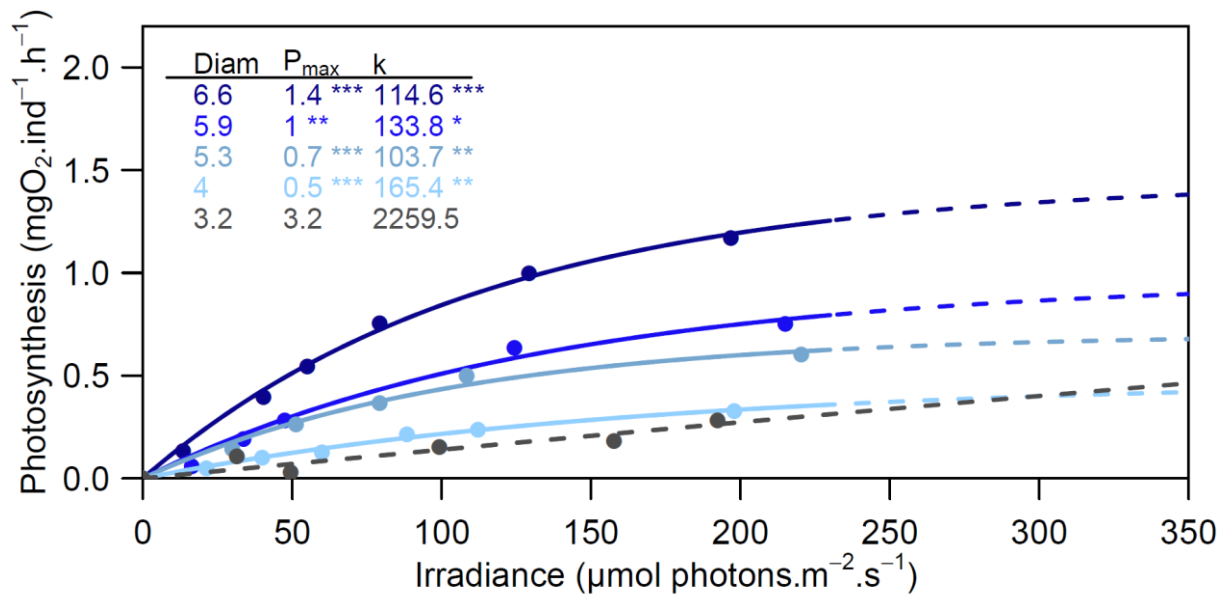


Fig. 1 Photosynthesis-Irradiance curves of five *Phyllorhiza punctata* medusae of different umbrella diameter (Diam). P_{max} and k are the estimated parameters of the curves. * = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001

Fig. 1 Courbes photosynthèse-Irradiance de cinq méduses de l'espèce *Phyllorhiza punctata* de différents tailles (diamètre de l'ombrelle ; Diam). P_{max} et k sont les paramètres estimés pour les courbes. * = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001

Comparing maximum photosynthesis rates, P_{max} , to respiration rates, they appear to be in the same range (Fig. 2). This suggests that carbon respiration requirements can be more or less fulfilled by photosynthesis in *Phyllorhiza punctata* medusae when well exposed to light. However, particularly when accounting for the day and night cycles, the photosynthesis would only provide a fraction of the respiration requirements. This is in the low range of what photosynthesis furnishes in other zooxanthellate jellyfishes (e.g. Drew 1972, Kremer et al. 1990, Kikinger 1992, McCloskey et al. 1994, Verde and McCloskey 1998). This low contribution of photosynthesis to the total carbon demand, however, need to be confirmed, as medusae stress in the small enclosures might have artificially increased respiration rates.

Photosynthesis rates also appears to increase faster than respiration rates with medusae size (Fig. 2), which is similar to what has been reported for *Mastigias papua* (McCloskey et al. 1994). However, due to the small size range represented here, to the small sample size, and the large overlap of the confidence intervals, these results remain inconclusive.

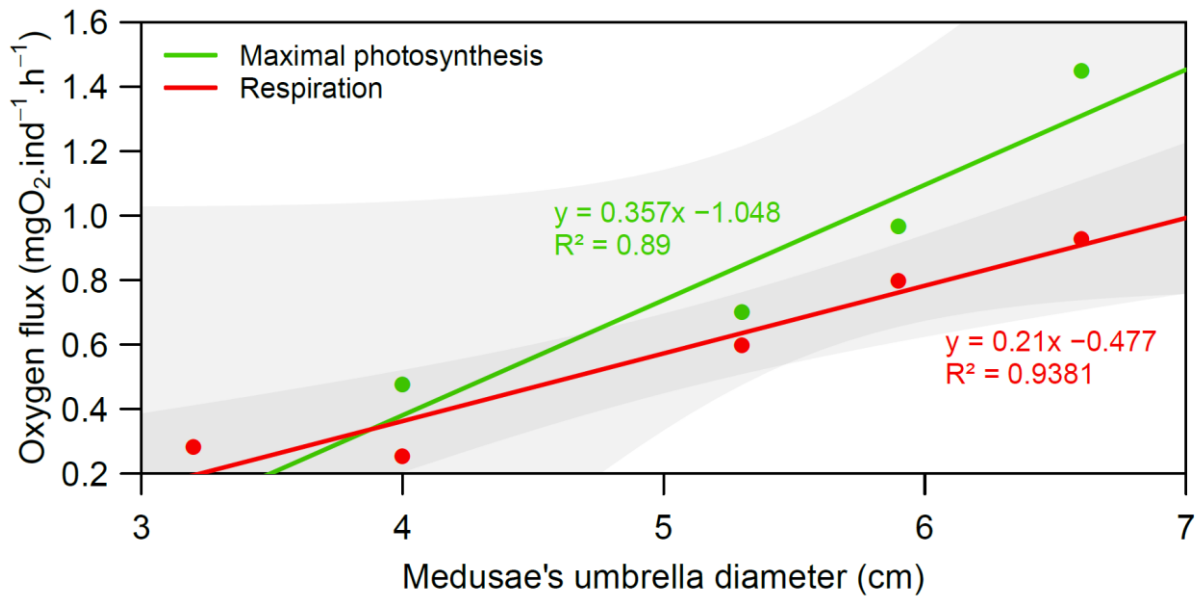


Fig. 2 Maximal photosynthesis and respiration of *Phyllorhiza punctata* medusae as a function of their umbrella diameter. Shaded areas correspond to 95 % confidence intervals around the regressions. Linear regressions were preferred to proper allometries due to the small sample size, they are therefore only indicative of general trends. Note that the photosynthesis of the smallest medusae as not been used as no reliable estimate was obtained (see text)

Fig. 2 Photosynthèse maximale et respiration de méduses de l'espèce *Phyllorhiza punctata* en fonction du diamètre de leur ombrelle. Les aires grisées correspondent aux intervalles de confiance à 95 % autour des régressions. Des régressions linéaires ont été préférées à des allométries du fait de la petite taille d'échantillon, elles ne peuvent donc représenter que des tendances générales. Noter que la photosynthèse maximale de la plus petite méduse n'a pas été utilisée du fait de l'absence d'obtention d'une estimation valide (voir texte)

When replacing these results in the larger context of this thesis, it is important to emphasize that these should not be seen as characteristic of all *Phyllorhiza punctata* medusae. This species is known for its zooxanthellate and non-zooxanthellate populations (Bolton and Graham 2004) which suggest it has a plasticity in its nutrition comparable to the one observed in *Mastigias papua* (Chapter IV). Hence, it would be expected that the contribution of heterotrophy and autotrophy to the nutrition might vary a lot from a population to another or between individuals (e.g. linked to size, see Fig. 2).

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Titre : Variabilité et Plasticité de la Nutrition des Méduses à Zooxanthelles : Apports expérimentaux et de terrain

Mots clés : Photosymbiose, Mixotrophie, Scyphozoa, Symbiodiniaceae, Marqueurs trophiques

Résumé :

Alors que la majorité des méduses sont des hétérotrophes strictes, certaines vivent en photosymbiose avec des Dinophyceae autotrophes (« zooxanthelles »). Ces méduses à zooxanthelles, en tant qu'holobiontes, sont mixotrophes, dérivant leur nutrition à la fois de la prédation et de la photosynthèse. Toutefois, l'importance relative de l'autotrophie et de l'hétérotrophie dans la nutrition peuvent varier en fonction de l'ontogénie, de la phylogénie, ou de l'écologie. De telles variations ont d'importantes conséquences pour la dynamique des populations de ces organismes. Il est donc important de pouvoir caractériser la variabilité et la plasticité de la nutrition des méduses à zooxanthelles pour comprendre leur écologie. Au cours de cette thèse, la nutrition des méduses à zooxanthelles a été étudiée par le biais d'expériences de laboratoire et d'observations de terrain. Une première expérience a permis de confirmer des résultats précédents suggérant que la nutrition autotrophe est de faible importance pour les polypes des méduses à zooxanthelles. Une seconde expérience a mis en évidence comment les compositions isotopiques et élémentaires des méduses à zooxanthelles peuvent être utilisées pour étudier leur nutrition. Ces résultats sont ensuite confrontés aux observations de terrain : La nutrition de la méduse à zooxanthelles *Mastigias papua* a été étudiée dans son environnement naturel (Palaos) via l'étude de leur compositions isotopiques, élémentaires, mais aussi en acides gras. Ces résultats de terrain démontrent l'importante plasticité de la nutrition de *Mastigias papua*, pouvant aller de la pure hétérotrophie, une autotrophie dominante. L'existence d'une telle plasticité dans la nutrition des méduses à zooxanthelles aide à comprendre certains aspects centraux de leur écologie, tels que leur tendance à former moins de blooms que les méduses sans zooxanthelles, ou leurs réactions aux évènements de blanchissement induit par la température.

Title: Variability and Plasticity of the Nutrition of Zooxanthellate Jellyfishes: Insights from experimental and field studies

Key words: Photosymbiosis, Mixotrophy, Scyphozoa, Symbiodiniaceae, Trophic markers

Abstract:

Whereas most jellyfishes are strictly heterotrophic organisms, some of them undergo a photosymbiosis with autotrophic Dinophyceae ("zooxanthellae"). These zooxanthellate jellyfishes, as holobionts, are mixotrophic deriving nutrition from both predation and photosynthesis. However, the relative importance of autotrophic and heterotrophic nutrition can vary as a function of ontogeny, phylogeny and ecology. Such variations of nutrition have important consequences for the population dynamics of these organisms. It is therefore central to characterize the variability and the plasticity of the nutrition of zooxanthellate jellyfishes to understand their ecology. In this thesis, the nutrition of zooxanthellate jellyfishes was investigated using laboratory experimental systems and field studies. A first experiment allowed to confirm previous findings that autotrophic nutrition is of small importance for the polyp of zooxanthellate jellyfishes. A second experiment assessed how elemental and isotopic compositions of zooxanthellate jellyfishes could be used to study their nutrition. The findings of this experiment are then confronted with results from the field: The nutrition of zooxanthellate *Mastigias papua* medusae was studied in their natural environments (Palau) through the use of isotopic, elemental but also fatty acids compositions. These field results demonstrate the wide plasticity of the nutrition of *Mastigias papua* ranging from pure heterotrophy to dominant autotrophy. The existence of such a wide plasticity in the nutrition of zooxanthellate jellyfishes helps to understand some crucial aspect of their ecology such as their generally low ability to bloom relative to non-zooxanthellate jellyfishes, or their reactions to temperature-induced bleaching.