LAS "MEDUSAS" COMO DEPREDADORES Y PRESAS. ESTUDIO DE SU METABOLISMO RESPIRATORIO Y COMPOSICIÓN BIOQUÍMICA.

"JELLYFISH" AS PREDATORS AND PREY: STUDYING THEIR RESPIRATORY METABOLISM AND BIOCHEMICAL COMPOSITION.

Doctorado en Oceanografía y Cambio Global

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Memoria presentada para optar al título de Doctor en Oceanografía y Cambio Global

Doctoral dissertation for the Ph. D. degree in oceanography and Global Change

Dirigida por los Doctores:

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Las Palmas de Gran Canaria - Diciembre 2020

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Escuela de
Doctorado

D. SANTIAGO HERNÁNDEZ LEÓN COORDINADOR DEL PROGRAMA DE DOCTORADO EN OCEANOGRAFÍA Y CAMBIO GLOBAL DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA,

INFORMA,

Que la Comisión Académica del Programa de Doctorado, en su sesión de fecha ..... tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada " Las "medusas" como depredadores y presas. Estudio de su metabolismo respiratorio y composición bioquímica" presentada por el doctorando D. Daniel Rickue Bondyale Juez y dirigida por la Doctora May Gómez Cabrera y codirigida por el doctor Theodore T. Packard.

Y para que así conste, y a efectos de lo previsto en el Artº 11 del Reglamento de Estudios de Doctorado (BOULPGC 7/10/2016) de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a...de.....de dos mil.....

Santiago
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UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

ESCUELA DE DOCTORADO

Programa de doctorado en Oceanografía y Cambio Global

Título de la Tesis

LAS "MEDUSAS" COMO DEPREDADORES Y PRESAS. ESTUDIO DE SU METABOLISMO RESPIRATORIO Y COMPOSICIÓN BIOQUÍMICA.

Tesis Doctoral presentada por D. Daniel Rickue Bondyale Juez

Dirigida por el Dra. May Gómez Cabrera

Codirigida por el Dr. Theodore T. Packard

El/la Director/a,	**El/la Codirector/a**	**El/la Doctorando/a,**
(firma)	(firma)	(firma)
GOMEZ CABRERA MARIA MILAGROSA -	PACKARD THEODORE TRAIN -	BONDYALE JUEZ DANIEL RICKUE -

Las Palmas de Gran Canaria, a 15 de Diciembre de 2020

A mis "mitocondrias",

AGRADECIMIENTOS/ACKNOWLEDGEMENTS

Quiero agradecer la oportunidad al programa de ayudas para la formación de profesorado universitario del Ministerio de Educación y Formación Profesional, a la Universidad de Las Palmas de Gran Canaria, a la Facultad de Ciencias del Mar y al programa de doctorado de oceanografía y cambio global. Contar con programas e instituciones públicas destinados a que las personas tengamos acceso a la investigación y a la educación es un privilegio que valoro y querría que fuese perenne.

La criptica dedicatoria, "A mis *mitocondrias*" viene motivada por la relación que tienen estos orgánulos con esta tesis y con la generación de energía. Pero, evidentemente, la dedicación es sobre todo figurada, no literal, y se refiere a aquellas personas que han sido mi fuente personal de energía. No de una energía que pueda ser medida o cuantificada, pero de una energía emocional sin la cual la autosuperación, en mi opinión, es imposible. En lugar de *Adenosine triphosphate*, el ATP del que me proveen estas mitocondrias emocionales sería Apoyo-Talento-Pasión, Atención-Tiempo-Paciencia, Amistad-Trabajo-Preguntas, Autoestima-Tenacidad-Perseverancia, Alegrías-Triunfo-Positividad... He intentado usar este ATP especial para convertirlo en este trabajo por lo que una parte de todas esas personas está aquí presente. Hay muchas "mitocondrias" incansables a las que agradecer. El trabajo aquí presentado pertenece sobre todo a la familia del grupo EOMAR. Quiero dar las gracias a May, Ted, Ico, Vane y Mayte, personas con las que he pasado muchas horas y cuya influencia y dedicación está presente en esta tesis. No quiero dejar fuera del agradecimiento también a todas las demás personas que forman o han formado parte de la familia EOMAR en algún momento ya que su compañía siempre ha sido agradable y estimulante. Agradezco también la labor de numerosos profesores a lo largo de mi vida cuyas lecciones individuales no recuerdo como no recuerdo todas las comidas que he comido pero de igual manera han contribuido seguro a lo que soy hoy. De igual, o tal vez, mayor medida me siento enormemente afortunado por el apoyo de amigos y familia cuyos nombres llenarían muchas páginas.

I will also like to thank all the people which have provided me of opportunities to experience collaboration and integration in the scientific community. A special thanks goes to Jennifer Purcell whose contributions to the jellyfish research community have been extensive and it has been a privilege to collaborate with her in some publications. Also I acknowledge the participation of Javier Almunia and Ester Alonso of the Loro Parque Foundation who in several occasions have provided the organisms to do this research. Anna Belcher and Kathryn Cook have also been instrumental to experience a collaboration with other institutions I thank them and their colleagues for their positive and very inclusive attitudes, especially Nathan Hubbot a fellow "medusologist" who opened the doors of his house without hesitation before even knowing me. I will also like to mention all the people that received us in their institutions during our time in the United States and Mexico it was wonderful observing the work of other research centres and being able to share.

Finally, I would like to thank Alexandra Elbakyan whose effort to make research more accessible has been vital.

“In the end respiration and burning are equivalent; the slight delay in the middle is what we know as life.”

Nick Lane, The vital Question

“The real world is in a much darker and deeper place than this, and most of it is occupied by jellyfish and things. We just happen to forget all that. Don't you agree? Two-thirds of earth's surface is ocean, and all we can see with the naked eye is the surface: the skin.”

Haruki Murakami, The Wind-Up Bird Chronicle

RESUMEN

El estudio del zooplancton ha sido siempre bastante crustaceocéntrico lo cual no es de extrañar ya que son componentes mayoritarios del zooplancton y sus exosqueletos les permiten no ser dañados tan gravemente durante los muestreos. Sin embargo, esto ha contribuido a que otros grupos también abundantes pasen más desapercibidos como es el caso del plancton gelatinoso. Los organismos de este grupo con su alto contenido en agua tienen una gran fragilidad. Además, el plancton gelatinoso puede alcanzar tamaños grandes que limitan su captura por métodos tradicionales de muestreo de plancton y aquellos muestreados son fácilmente dañados o destruidos en el proceso. Junto a esto a la hora de estudiar el contenido digestivo de un hipotético depredador, un organismo gelatinoso se descompondrá más rápidamente que, por ejemplo, el exoesqueleto de otras presas del grupo de los crustáceos. Todo ello ha contribuido al menosprecio del plancton gelatinoso tanto en su rol como depredador planctónico como de presa. En este trabajo se busca contribuir al, a veces escaso, conocimiento sobre algunos miembros del plancton gelatinoso y su rol como depredador y presa.

En concreto, para estimar cuantitativamente el consumo de presas necesario para estos organismos se estudió su metabolismo respiratorio. Durante el trabajo se revisaron métodos convencionales de medida de respiración a través de la tasa de consumo de oxígeno empleando incubaciones y monitorizando la concentración de O_2 mediante el método Winkler, electrodos u optodos. Luego estos resultados se compararon con medidas de la respiración empleando técnicas enzimáticas que no requieren de incubación ni de muestreo del organismo vivo como es el análisis cinético del sistema de transporte de electrones que controlan la actividad respiratoria a nivel celular. Con ello se buscó validar e incorporar una metodología alternativa que permitiese trabajar con estos frágiles organismos aun estando dañados durante el muestreo. A través del estudio de la respiración pudimos cuantificar la cantidad de carbono necesaria para satisfacer esta actividad metabólica. Además, se emplearon estas medidas para no solo investigar el flujo y la transformación de carbono sino también para estudiar las conversiones a nivel energético que tienen lugar en el medio marino.

Por otro lado, para entender su importancia como presa se analizó su contenido en sustancias orgánicas como proteínas, lípidos y carbohidratos. El contenido en dichas sustancias se empleó también para estimar su contenido energético y arrojar luz sobre la depredación cada vez más estudiada que sufren estos organismos con un mayor contenido en agua que otras presas.

Específicamente se trabajó sobre todo con dos especies de escifozoos (*Aurelia aurita* y *Pelagia noctiluca*) y dos especies de hidrozoos (*Physalia physalis* y *Velella velella*). Estas últimas han sido rara vez estudiadas a nivel de fisiología respiratoria o contenido orgánico. Por tanto, en este trabajo seguramente están presentes las primeras medidas documentadas de respiración y composición en dichos integrantes del pleuston.

Por otro lado, el estudio de la respiración y composición de estos organismos nos ha permitido documentar los cambios sufridos a nivel bioquímico y metabólico además de morfológico durante la transición de *Aurelia aurita* de larva a juvenil (éfira a medusa) y durante prolongados periodos de inanición. Estos estudios ecofisiológicos podrían explicar en parte algunos de los mecanismos que permiten la supervivencia en condiciones adversas, como por ejemplo la inanición. Seguramente estos procesos han contribuido a que este tipo de organismos haya logrado un importante éxito evolutivo al sobrevivir con un diseño muy parecido desde los comienzos de la vida eucariota pluricelular compleja siendo uno de los primeros metazoos.

ABSTRACT

The study of zooplankton has always been quite crustacean focused which is not surprising as they are a major component of zooplankton and their exoskeletons allows them not to be so severely damaged during sampling. However, this has contributed to paucity of research on other groups that are also abundant. Gelatinous plankton is one-such understudied group. The organisms of this group, with their high water content are very fragile. In addition, gelatinous plankton can reach large sizes that limit their capture by traditional plankton sampling techniques and those sampled are easily damaged or destroyed in the process. Together with this, when studying the digestive content of a hypothetical predator, a gelatinous organism will decompose faster than, for example, the exoskeleton of other prey of the crustacean group. All this has contributed to the disdain of gelatinous plankton both in its role as a planktonic predator and as a prey. Here, we seek to contribute to the, sometimes scarce, knowledge about some members of the gelatinous plankton and their roles as predator and prey.

In particular, their respiratory metabolism was studied to quantitatively estimate their consumption of prey. During the work, conventional methods for measuring respiration through the rate of oxygen consumption were reviewed using incubations and monitoring the concentration of O_2 by means of the Winkler method, electrodes or optodes. These results were then compared with respiration measurements using enzymatic techniques that do not require incubation or sampling of the living organism such as the kinetic analysis of the Electron Transport System (ETS) that controls respiratory activity at the cellular level. The aim was to validate and incorporate an alternative methodology that would allow working with these fragile organisms even if they were damaged during sampling. Using respiration results we were able to quantify the amount of carbon needed to satisfy this metabolic activity. In addition, these measures were used to not only investigate the flow and transformation of carbon but also to study the energy conversions that take place in the marine environment.

On the other hand, in order to understand its importance as a prey, its content in organic substances such as proteins, lipids and carbohydrates was analysed. The content of these substances was also used to estimate their energy content and to shed light on the increasingly studied predation suffered by these organisms with a higher water content than other prey.

Specifically, we worked mainly with two species of scyphozoans (*Aurelia aurita* and *Pelagia noctiluca*) and two species of hydrozoans (*Physalia physalis* and *Velella velella*). The latter have rarely been studied at the level of respiratory physiology or organic content. Therefore, this work probably contains come of the first documented measurements of respiration and composition in these pleuston members are surely present.

On the other hand, the study of the respiration and composition of these organisms has allowed us to document the changes suffered at a biochemical and metabolic level as well as morphologically during *Aurelia aurita*'s transition from larva to juvenile (ephyra to jellyfish) and during prolonged periods of starvation. These ecophysiological studies could partially explain some of the mechanisms that allow survival in adverse conditions, such as starvation. These processes have probably contributed to the significant evolutionary success of this type of organism, which has survived with a very similar design since the beginning of complex multicellular eukaryotic life, being one of the first metazoans.

PRESENTACIÓN DE LA TESIS

La tesis que aquí se presenta bajo el título "LAS "MEDUSAS" COMO DEPREDADORES Y PRESAS. ESTUDIO DE SU METABOLISMO RESPIRATORIO Y COMPOSICIÓN BIOQUÍMICA" se ha desarrollado dentro del Programa de Doctorado en Oceanografía y Cambio Global de la Universidad de Las Palmas de Gran Canaria y ha estado dirigida por los doctores May Gómez y Ted Packard. En ella se incluyen 5 contribuciones originales (una de ellas ya publicada) enmarcadas principalmente dentro del proyecto de investigación PERSEO (ProID201710051) del Gobierno de Canarias y por el Fondo Europeo de Desarrollo Regional - fondos FEDER y MERMAC (CEI 2017-10) de CEI Canarias: Campus Excelencia Atlántico Tricontinental; otorgados a la Dra. May Gomez. Durante la tesis se colaboró también con el proyecto de la beca FULLBRIGHT de la Dra. Jennifer Purcell. En este periodo de tiempo se realizó también una estancia en el British Antarctic Survey (Cambridge, Inglaterra) y en el National Oceanography Centre (Southampton, Inglaterra) coincidiendo con una colaboración para el análisis de muestras de micronecton y zooplancton dentro del proyecto COMICS (NE/M020762/1 & NE/M020835/1).

Esta tesis está estructurada en dos partes. Una primera que engloba la Introducción con un resumen del estado de la materia y los principales objetivos del trabajo, las contribuciones originales en formato de artículo científico con sus respectivas discusiones y conclusiones en cada contribución. Estos capítulos están escritos íntegramente en inglés, cumpliendo con ello los requisitos para la obtención de la Mención Internacional del Título de Doctor (Real Decreto 99/2011 del 28 de enero por el que se regulan las enseñanzas oficiales de doctorado, Art.15). La segunda parte de la tesis incluye un resumen en castellano del estado de la materia y resúmenes de las investigaciones realizadas incluyendo los objetivos de cada investigación y una recopilación de las conclusiones generales más relevantes en castellano. Con ello se cumple el formato de la tesis escrita en una lengua distinta a la española según lo expuesto en el Reglamento de Estudios de Doctorado de la Universidad de Las Palmas de Gran Canaria (BOULPGC 9 de enero de 2013, modificado en el BOULPGC de 4 marzo de 2019 Capitulo III, Artículo 10).

El desarrollo de esta tesis no habría sido posible sin el apoyo económico recibido del Ministerio de Educación y Formación Profesional en el marco del contrato predoctoral del programa de ayudas para formación del profesorado universitario (Becas FPU - 2015-036973).

CONTENT

CHAPTER 1

GENERAL INTRODUCTORY BACKGROUND

1.1. Oxygen, respiration and life

Oxygen (O_2) is normally found as a diatomic molecule in gaseous form at room temperature. This simple molecule has had great implications for the appearance and development of life as we know it on this planet.

Certainly the most direct connection between O_2 and the existence of life on this planet is its participation in the respiratory processes, however, this was not always the case. According to Canfield (2005)'s descriptions, more than 2.5 billion years ago, life on Earth was prokaryotic and employed primarily anoxic processes. Free oxygen was a rare gas in Earth's atmosphere. It is speculated that some microorganisms of this time already had metabolic pathways capable of taking advantage of low concentrations of O_2 resulting from minor chemical processes. These microbes would be the great beneficiaries of the change that would occur. There is great uncertainty about how exactly this process took place, but what has been confirmed is that approximately 2400 million years ago, at the beginning of the Proterozoic, the concentration of atmospheric O_2 had been increasing rapidly in the last thousands of years in what is known as the Great Oxidation (Holland, 2006) (Fig. 1.1). It is suspected that one of the main processes responsible, or even primarily responsible, was oxygenic photosynthesis. It is considered that those responsible for this novel process were primitive benthic microcyanobacteria. As Sanchez-Baracaldo (2015) explains, these evolved and diversified throughout the Proterozoic and then benthic macrocyanobacteria appeared, which continued with the increase of O_2. Around the end of the Proterozoic, planktonic macro- and microcyanobacteria appeared, giving way to the Phanerozoic with its characteristic of high oceanic primary production. This enhanced primary productivity, based on chlorophyll and enhanced by nitrogen fixation, would have, and continues to have, great consequences for life on earth. Knoll (2003) describes in an excellent way that the novelty of this process does not lie in the use of light energy for the generation of organic matter and ATP, which other photosynthetic bacteria already did, but in the generation of oxygen, as a secondary product, from ripping electrons from H_2O (photolysis of water).

The increase in O_2 caused by cyanobacterial photosynthesis was initially limited to the photic zone because the process required light. In these sun-lit waters, oxidation reactions caused by increasing oxygen concentrations, led to the decrease of other, more reduced, electron donors that were used by older photoautotrophs. This furthered the dominance of the cyanobacteria (Knoll et al., 2016). However, after thousands of years, the oxygen-rich near-surface waters mixed into the deep-dark ocean and the associated changes in seawater chemistry shifted the metabolism of the microbial world to aerobic respiration. This biological revolution based on the new process of cyanobacterial photosynthesis then reached far beyond the ocean. Henry's Law, governing gas exchange at the sea surface, ensured that the oxygen in the atmosphere and in the planet's soils increased in parallel with its increase in the ocean. Then, as in the ocean, the accompanying chemical changes in the atmosphere and soils shifted all the terrestrial

metabolism to aerobic respiration. Only in rare habitats or habitats isolated from the atmosphere, such as the waters in geothermal zones (geysers and hot springs), deep-earth zones, and oceanic oxygen minimum zones, would original anaerobes survive. [Later, after the evolution to eukaryotes, some of these microbes would colonize the enteric digestive systems of metazoans.]

In view of this increase in O_2 concentration, authors such as Margulis & Sagan (1995) propose that the so-called "oxygen holocaust" took place in which those organisms capable of implementing strategies to take advantage of this O_2 survived. Those prokaryotes capable of using O_2 for energy generation benefited. However, Knoll et al. (2016) nuanced these statements by adding that along with oxygen, other electron acceptors such as sulphates or nitrates also increased, hence, the diversity of anaerobic and chemosynthetic processes also increased (Lane, 2017). In any case, the increase in oxygen also allowed organisms to develop what we know as aerobic respiration. This process was able to generate energy by means of electron transports that ended with the reduction of O_2 to H_2O. This reaction took place in electron-accepting molecular structures known as O2Red. Brochier-Armanet et al. (2009) documents various groups of bacteria and archaea capable of performing aerobic respiration. Of particular interest for the next life transition are the bacteria containing O2red type A as they share a high genetic similarity with the genetic material in the mitochondria.

The change in O_2 concentration altered the composition of bacterial communities, but also created the conditions for a leap in the diversity of life on the planet. So far, only prokaryotic organisms of simple cellular complexity and lacking organelles and cell nuclei have been mentioned. But after the great oxidation, eukaryotic organisms with nucleus and cellular organelles also appeared, chloroplasts and mitochondria were among these organelles. Genetic evidence suggests that during the Proterozoic, when oxygen had only slightly increased in surface waters, a prokaryotic cell ended up inside another prokaryotic cell and neither the first was digested nor the second perished. They had managed to live together in this new union (Lane, 2017; Roger et al., 2017). This union proved to be stable and advantageous and over time this new cell formation specialized its novel arrangement forming what we know today as eukaryotic cells (Margulis & Sagan, 1995). This process is known as endosymbiosis (Sagan, 1967; Gray, 2017). Much evidence suggests that the first endosymbiosis that gave rise to eukaryotic cells was between a proteobacterium and an archaeon (Williams et al., 2013) where the proteobacterium would become what we now know as a mitochondrion and the archaeon host would become the cell that houses the rest of the organelles and cell nucleus (Roger et al., 2017). There are still open questions on how eukaryogenesis took place and what type of proteobacteria was responsible but this union constitutes the eukaryotic cells that are found in all the organisms of the Eukarya Domain (Gabaldón, 2018; Martijn et al., 2018). Other organelles originated by later endosymbiosis are plastids such as chloroplasts (Archibald, 2015). In this new cell type, eukaryotes, two fundamental aspects of life are evident: cooperation and oxygen are key to life. This is supported by the fact that between the two main domains (Bacteria and Archaea) they managed to create a new domain with extraordinary and emerging properties capable of competing with the rest of the forms of life, surviving and evolving up to the present day. On the other hand, whether we are talking about mitochondria or chloroplasts, these two organelles of endosymbiotic origin are related, although in different ways, to oxygen and its role in energy transformation.

However, at the time when endosymbiosis took place, which would culminate in the formation of the mitochondria, oxygen was not very abundant (Knoll et al., 2016). Therefore, oxygen processing was not necessarily the only form of energy production of

these first endosymbionts (Roger et al., 2017). The real advantage would be that with time and with the increase of O_2 in the environment, the endosymbiont proteobacteria specialized in the energetic production of molecules such as adenosine triphosphate (ATP). In addition, they gave up part of their genetic material that was no longer necessary to the nucleus or directly lost it (Lane, 2017). This occurred because living inside another cell obviates the need for some of this material. This specialisation achieved an advantageous efficiency in generating and managing ATP production, i.e. harnessing energy (Lane & Martin, 2015).

In addition to the formation of eukaryotic cells, another major step in life was the development of a complex multicellularity. Already at the beginning of the Proterozoic (2.5-1.9 billion years ago), some filamentous formations of cyanobacteria and other types of bacteria have been identified (Rokas, 2008) (Fig. 1.1). Even some protists started to show filamentous formations soon after their appearance. But during the last quarter of the Proterozoic (a billion years ago), atmospheric and hydrospheric oxygen, the resource for energy transformation, increased and the means to process it efficiently were developed. This allowed a step towards greater diversification in eukaryotic life (Hedges et al., 2004). New cellular complexities appeared, not only in the form of new cellular types derived from evolutionary and endosymbiotic processes, but also more complex cellular conformations. This was a multicellularity with specialised cells, which we will refer to as pluricellularity. Genetic evidence suggests that the ancestors of many of today's pluricellular life forms were single-celled protists that formed multicellular colonies that developed pluricellularity between 1000 and 400 million years (Ruiz-Trillo et al., 2007; Rokas, 2008). The fossil record shows unambiguous fossils of plant, animal and fungal lineages from 600 million years ago (Rokas, 2008).

However, it should be noted that oxygen was not the only key condition for the emergence of animal life. Examples of animals with important types of anaerobic metabolism and animals capable of surviving in low oxygen concentrations are given as evidence that oxygen was not the only requirement for the emergence of pluricellular eukaryotic life (Mills & Canfield, 2014). When the first animals appeared, oxygen concentrations were significantly lower than they are today. Therefore, it should be noted that, although necessary, there are other conditions to be investigated (Mills & Canfield, 2014). However, although the increase in O_2 concentration has not been the only factor that conditioned the appearance of animal life, it has had key implications for the diversification and evolution of such life dependent on respiratory processes (Knoll, 1991; Sperling et al., 2013).

The exact timing and protagonists of each step in the animal evolutionary process is something that continues to be constantly researched. But, it is recognized that the phylum Cnidaria is one of the first groups to develop with differentiated tissue and a basic nervous system. Then it branched from the line that would later correspond to all bilateral groups (Cunningham et al., 2017). This group, the cnidarians, has been diversifying into corals, anemones, jellyfish and siphonophores, among many others (Park et al., 2012), some of which are the subject of this paper. The cnidarians have a highly successful evolutionary design as they have managed to endure with their strategy until the present day. For example, there are fossil records of jellyfish from the end of the Proterozoic and beginning of the Cambrian, 600-500 million years ago. These jellyfish are very similar to their current forms (Cartwright et al., 2007; Young & Hagadorn, 2010) (Fig. 1.1). These simple designs could have emerged when the concentration of O_2 was lower. Yet, they have managed to reproduce and evolve while surviving numerous environmental changes in the planet. Given their history, these types of organisms

should be able to teach us much about the functioning of animal life throughout the evolution of planet Earth.

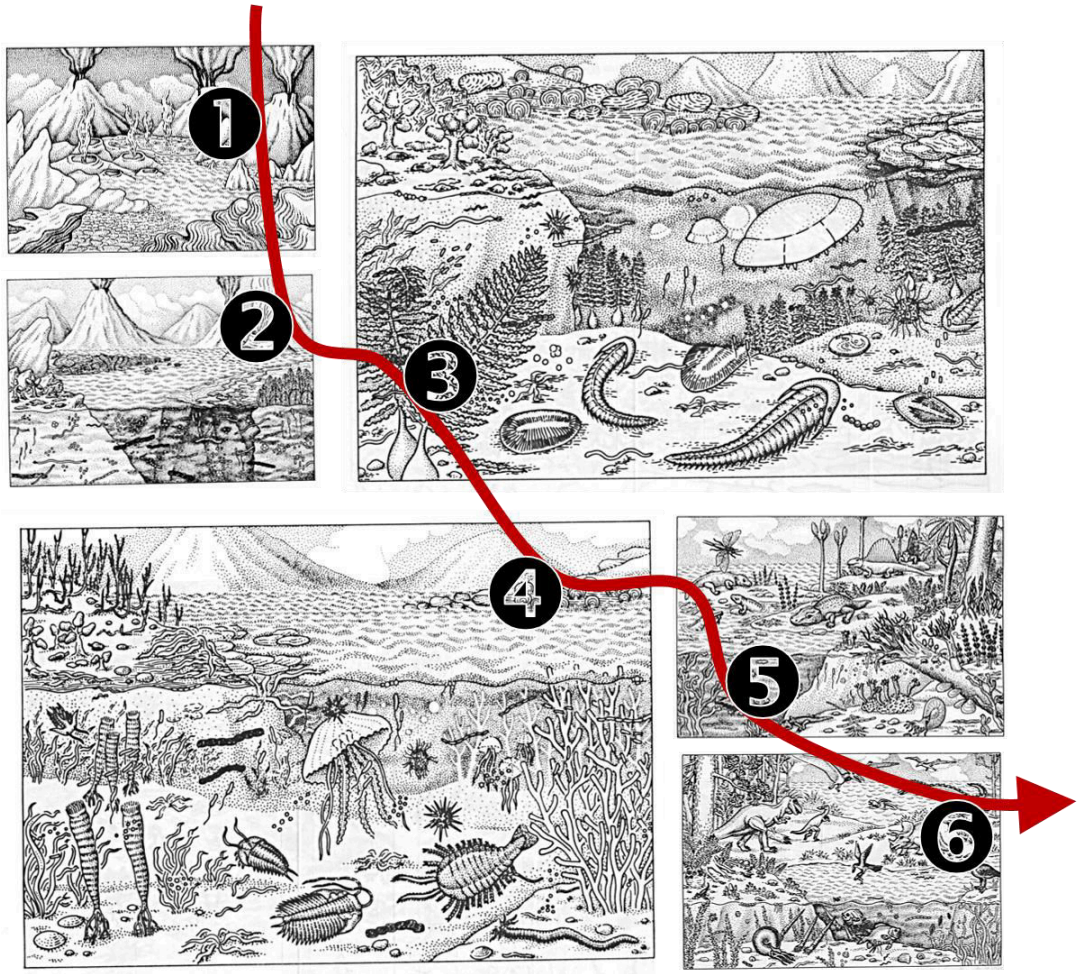

Figure 1.1. Margulis & Sagan (1995)'s summary vignettes of the evolution of life on planet earth from around 3 billion years ago to 70 million years ago with emphasis on the appearance of the Cnidarians (3 and 4). This figure emphasises the importance of oxygen (2) and associated processes for the appearance of eukaryotic life on the planet, and among these early inhabitants were jellyfish reigning in the marine environment with a morphology and strategy similar to that which we observe today. (1) Representation of the Archean Eon, approximately 4000 to 2500 years ago, with hydrothermal activity and bacterial biofilms that would remain in the fossil record as stromatolites (Nutman et al., 2016). (2) Representation of the beginning of the Proterozoic Eon, about 2500 million years ago, with the appearance of cyanobacteria and their photosynthetic production of O_2. (3) Representation of the end of the Proterozoic Eon, about 600 million years ago, with the appearance of the first eukaryotic and pluricellular soft life forms. (4) Representation of the beginning of the Phanerozoic Eon, Paleozoic Era, Cambrian Period, about 500 million years ago, and the continuous development of pluricellular life with the appearance of hard structures and the emergence of simple autotrophic and heterotrophic life forms. (5) Representation of the end of the Paleozoic Era, around 300 million years ago, with the development of vertebrates and marine and terrestrial plants. (6) Representation of the Mesozoic Era, between 200 and 70 million years ago, with large aerial, terrestrial and marine reptiles coexisting with small mammals and abundant plant life.

1.2. The role of jellyfish

As described in the previous section, in the Cambrian, during its famous explosion of metazoan life (Conway Morris, 2000), the Cnidarians diversified in their different classes (Park et al., 2012). These include the strictly sessile life forms such as the Anthozoa class (includes anemones and corals) and the lesser known Staurozoa; the obligate parasites of the Myxozoa class; and the “jellyfish” of the Cubozoa (boxjellyfish), Scyphozoa ("true jellyfish") and Hydrozoa (includes hydroids and siphonophores among others) classes (Collins, 2002). The latter in some species have more complex life cycles that combine sessile and planktonic life phases. Among all these, this work will focus mainly on organisms of the classes Scyphozoa and Hydrozoa that will be introduced later on. The planktonic forms of organisms of the phylum Cnidaria are classified within a polyphyletic group of non-taxonomic use called gelatinous plankton that includes planktonic life forms of various phyla (tunicates, ctenophores, cnidarians…) that share a series of characteristics among which it stands out having a high fragility due to its high water content (Purcell, 2009).

During these first periods of enormous marine diversification, gelatinous plankton, especially jellyfish, must have been among the main marine super predators (Richardson et al., 2009) (Fig. 1.2). Their predation and reproduction strategies must have been successful enough to ensure that they did not change much from the forms of the fossil record and survived in the presence of more modern predators that appeared millions of years later, such as fish or marine mammals. Some authors have gone so far as to suggest that the anthropogenic effects on ecosystems and climate change that negatively impact fish could be beneficial to jellyfish that have survived major changes in the planet and will not hesitate to regain their role as once-monopolistic pelagic predators (Parsons & Lalli, 2002; Richardson et al., 2009) (Fig. 1.2). The reality is that, for many researchers, the predatory role of jellyfish in the food chain has gone unnoticed due to the attention paid to non-gelatinous predators. However, jellyfish and other members of the gelatinous plankton may be the major competitors of zooplanktivorous fish species as well as predators of fish larvae and eggs. This double role makes it difficult for jellyfish-dominated ecosystems to recover (Lynam et al., 2007; Pauly et al., 2009). Purcell et al. (1999) lists a number of characteristics that make jellyfish great competitors. Jellyfish feed continuously without satiating at natural concentrations of food. They capture small and large prey, prey on larval stages of their competitors, and when food is scarce, they can shrink rather than die. They tolerate difficult conditions of low dissolved oxygen concentration, feed passively reducing energy costs of hunting, and take advantage of favourable conditions for the rapid formation of large blooms. In addition, they often have a rapid succession of short generations alternating between stages of sexual and asexual reproduction. Some of these characteristics will be addressed later.

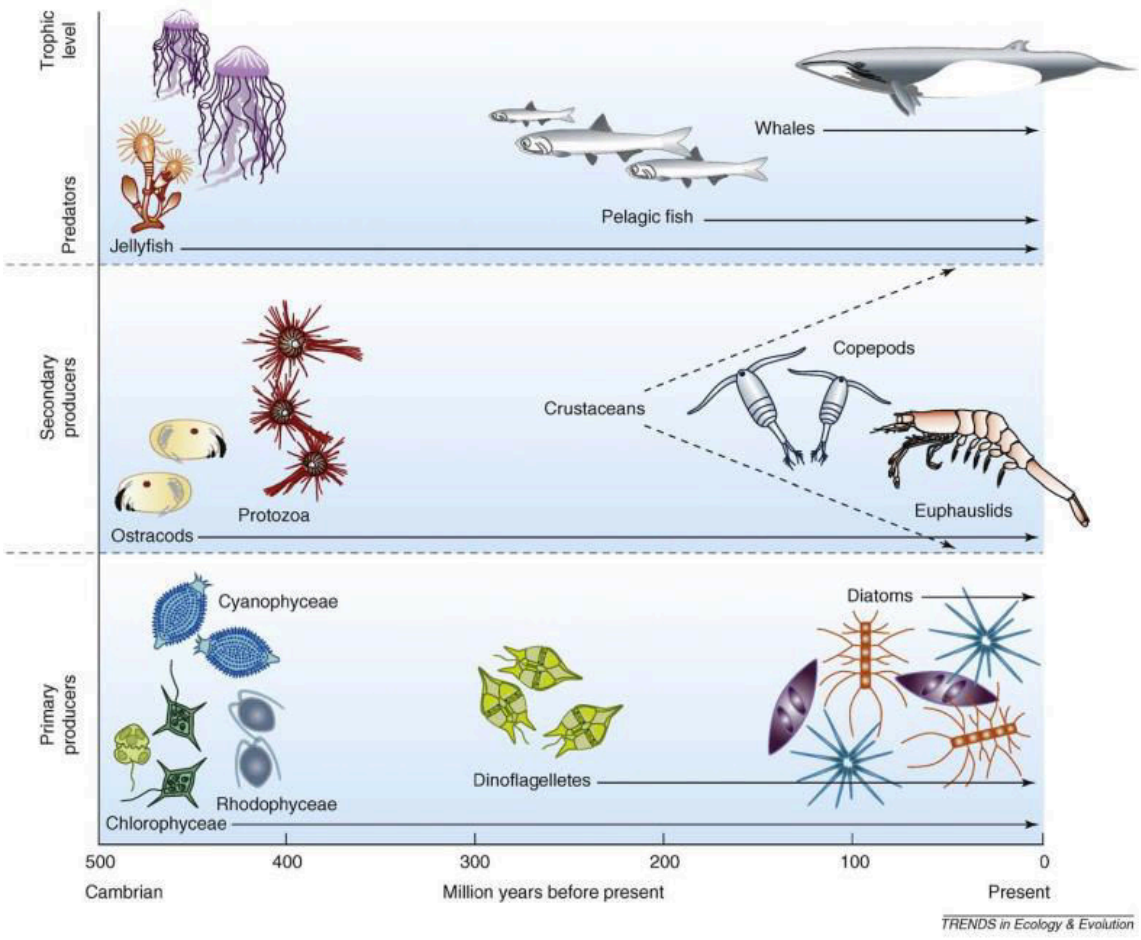

Figure 1.2. Schematization of the evolution of the main representatives of the marine trophic levels. The transition of cnidarians as a major predator to sharing that trophic niche with competitors and more complex predators is represented. It shows the survival of jellyfish and how the withdrawal of competitors and predators can create the ideal scenario for their return to dominance. Obtained from Richardson et al. (2009) as modified from Parsons 1979.

Within the anthropocentric perspective of human beings, problems associated with jellyfish have also been documented outside of competition and predation on species of economic interest. Purcell et al. (2007) compile many of these impacts on human activity. In relation to fishing, in addition to the above, large blooms of jellyfish can complicate the use of nets. In addition, many of the cnidarians share the characteristic of having stinging cells, called cnidocytes, which they use to capture their prey and for defensive purposes. The stings can be more or less severe depending on the species, but they complicate the management of by-catches and can sting fishermen. When they invade near-shore recreation areas, stings can harm bathers and affect local tourism. These stings can also have a negative impact on aquaculture. Cases of blockage of seawater intakes from power plants and desalination plants have also been documented. All these situations occur or are aggravated when jellyfish form large aggregations i.e. jellyfish blooms.

Conversely, there is also much discussion about how anthropogenic activity impacts jellyfish populations by producing an overall increase in the population of gelatinous plankton (Purcell et al., 2007; Richardson et al., 2009; Duarte et al., 2013). In summary, the human activities that could lead to this increase are: the removal of predators and

competitors, eutrophication, the transport of invasive species and the increase in substrate on the coast for the settlement of sessile life phases (polyps) such as boats, structures for aquaculture, buoys, platforms and in general coastal urban and port development. While other authors argue that these are increases in regional jellyfish populations or are specific cases without global impact and therefore cannot be connected with human activities. They argue that there is not enough evidence to confirm any connection (Condon et al., 2012; Pitt et al., 2018). To remedy this impasse, continuous monitoring of the appearance or periodicity of jellyfish blooms and their study in multiple regions of the planet would foster better understanding of the evolution of jellyfish populations as well as our impact on them.

However, to think of gelatinous plankton only as a competitor, predator, or problem would limit our understanding of its role in the ecosystem. Doyle et al. (2014) invites reflection on the many positive ecological, social and cultural aspects of jellyfish. In relation to humans, gelatinous plankton has been the source of numerous investigations for the extraction of products with medical, cosmetic and industrial applications (Ohta et al., 2009; Kim et al., 2012; Avila Rodríguez et al., 2018; Verdes & Holford, 2018; Steinberger et al., 2019). It is worth mentioning the discovery of a green fluorescent protein (GFP) in the genus *Aequorea*, which led to a Nobel Prize for its discoverers and allowed the development of a "revolution in biotechnology" (Zimmer, 2009). Doyle et al. (2014) also propose that we value the enjoyment, curiosity and interest in nature that jellyfish aquariums arouse, which are becoming increasingly common; and the knowledge we obtain from such diverse life strategies as those presented by gelatinous plankton. He even states that the collective participation necessary for the detection of jellyfish strandings and documentation of their distribution is an opportunity to involve the population and create a more participatory science around this sometimes neglected resident of the ocean.

But apart from human applications, at an ecological level gelatinous plankton is an important member of the plankton community. As such, many of its species fulfil enriching functions for the systems they inhabit. Currently, jellyfish are considered to be important as a source of carbon transport through the so-called "jellyfalls" (Lebrato et al., 2019), as indicators of marine pollution (Macali & Bergami, 2020) and as commensal zones for fish and crustaceans serving as both nursery and food (Mansueti, 1963; Purcell & Arai, 2001; Griffin et al., 2019). The latter is important, since, as many authors have stated in the literature for a long time, plankton has been studied in a crustaceocentric way and jellyfish have been relegated to secondary roles in the food chains (Haddock, 2004). However, an increasing number of species are described as feeding on gelatinous plankton: gastropods, cephalopods, crustaceans, fish, sharks, turtles, birds, mammals, (Arai, 2005; Pauly et al., 2009; Choy et al., 2017), including humans, with current estimates of up to 900,000 tons of captures per year (Brotz & Pauly, 2017). The motivation behind this natural predation is currently being discussed (Hays et al., 2018; Thiebot & McInnes, 2020). Investigating the composition of gelatinous plankton members could be key to understanding the role of gelatinous plankton as prey and not only as predators.

In this work we will mainly focus on exploring these two faces (predator and prey) of some members of the gelatinous plankton. We will do this through the study of their metabolism and biochemical composition. In the process, we will learn about physiological aspects of these organisms that will allow us to make estimates and develop hypotheses at an ecological level.

1.3. Respiration: function and study in jellyfish

Understanding the metabolic needs of organisms provides information about the associated demand for food. The food consumed by the organism can be used for various processes such as growth and reproduction, but some of this food will undoubtedly be used for respiration (Schneider, 1989; Uye & Shimauchi, 2005). During respiration, organic substances from the digested food are used to release the energy contained in them to produce adenosine triphosphate (ATP) and heat. During this reaction, oxygen is also needed. The organic substances are oxidised and also produce water (H_2O) and carbon dioxide (CO_2). This is a RedOx reaction very similar to that which takes place during combustion. However, biologically, it proceeds in a much more controlled way. It is mediated by enzymes, which allows energy to be transformed and processed into ATP (Lane, 2015). The role of oxygen in this process is fundamental and explains why its increase in the atmosphere, described above, was so important for the development of complex life on earth. Thanks to it, organisms have an efficient biochemical mechanism with which to release and use the energy from their food. Except for certain specific cases, most metazoans perform some kind of aerobic respiration. Some of these organisms have developed organs specialised in capturing oxygen from the environment, such as gills or lungs. In the case of cnidarians, including jellyfish, they lack these specialised organs but obtain oxygen through diffusion (Fig. 1.3). That is, the cellular layers exposed to the external environment have a sufficient exchange of oxygen with the surrounding environment that is capable of supplying the oxygen demand of the whole organism. Despite having this simple respiratory system, they can reach large sizes thanks their having a low number of cells, each with a high water content (Acuña et al., 2011).

In addition to oxygen, organisms need a source of organic carbon. This can be obtained from organic-rich particles outside the organism (heterotrophy) or can be produced in the body from an inorganic carbon source, such as CO_2 and energy source (autotrophy). Photosynthetic organisms such as algae, phytoplankton and plants are photoautrophic and produce their own food from light and CO_2 through photosynthesis (Benedict, 1978). Some cnidarians have endosymbiotic microalgae, i.e. unicellular algae that live inside the body in an indispensable way (Verde & McCloskey, 1998; Fransolet et al., 2012). A well known the case is that of the hermatypical corals, but other cnidarians such as anemones, jellyfish, or hydras also have this type of symbiosis with microalgae. In this association, the micro algae are zoochlorella or zooxanthellae. One of the organisms that will be introduced later, the jellyfish, *Velella velella,* also has zooxanthellae. In this type of complex symbiosis, oxygen is once again involved. Without going into much detail, during the photosynthetic process oxygen is produced in addition to organic carbon, which serves as food, from H_2O, CO_2 and the energy source which in this case is the sun. Photosynthetic organisms will also have a respiratory process to transformed the energy stored in their self-produced organic substances. In the case of heterotrophs, like most metazoa, the food is obtained from the digestion of prey; these can be animal, vegetable or organic material. In the case of cnidarians most have a simple digestion system called the gastrovascular cavity. This has only one entrance and exit orifice unlike more complex digestive systems (Yonge, 1937).

From the surrounding seawater, oxygen diffused inside the jellyfish and organic substances, the products of food digestion or symbiotic algae, are transported throughout its interior to all the cells. Once in the cell, a series of enzyme-regulated mechanisms transform these organic substances into energy. This is what we know as cellular respiration. Based on Nelson et al. (2008) and Gnaiger et al. (2019) we will break down respiration into 5 steps, developing further those most relevant to this work:

catabolism of macronutrients, generation of acetyl CoA, Krebs- or tricarboxylic acid cycle, Electron Transport System, ATP synthesis.

The catabolism of macronutrients will depend on whether the organic substance from which energy is to be obtained is a carbohydrate, a lipid or a protein. This would be the transformations that take place before entry into the mitochondria, the organelle responsible for transforming most of the energy in the form of ATP, the. During this catabolism, some ATP molecules and some nucleotide nicotinamides, such as NADH, are produced, the function of which will be explained later. The participation of peroxisomes in these catabolic processes can lead to extra-mitochondrial oxygen consumption. After this processing the organic substances derived would enter the mitochondrion; if it was a carbohydrate, pyruvate molecules would enter the mitochondrion; if it was a fatty acid, minor lipid chains; and if it was a protein, amino acids. The pyruvate is oxidised to produce acetyl-CoA and in the process CO_2 is also produced and the pyridine nucleotide NAD^+ is reduced to form NADH. The lipids are oxidized in a process called β-oxidation which also ends with the formation of acetyl-CoA and the reduction of NAD^+ to NADH and flavin nucleotides such as FAD to $FADH_2$. In the case of amino acids, depending on the amino acid, it can be converted, sometimes with the participation of a deamination process (Fernandez-Urruzola 2015), into acetyl-CoA or it will enter as an intermediate molecule of the Krebs cycle which we will talk about next (Fig. 1.3).

Acetyl-CoA enters into what we will refer to as the beginning of the Krebs cycle. Some enzymes in this cycle succeed in oxidising the acetyl-CoA molecule and the resulting forms, producing CO_2 along with NADH, $FADH_2$ and some guanine triphosphate (GTP) molecules. Specifically, 3 molecules of NADH and 1 molecule of $FADH_2$ are produced for each acetyl-CoA. This cycle takes place inside the mitochondrion. The NADH and $FADH_2$ molecules produced are then used in the internal mitochondrial membrane in what is called the Electron Transport System (ETS) (Fig. 1.3). The enzymes of the ETS oxidise the NADH and $FADH_2$ molecules, releasing electrons in the process, which are transported across the membrane through various standard reduction potential-dependent and enzyme-regulated reactions. The process begins in complex I, or NADH dehydrogenase, where the electron is released while NADH is oxidised and NAD^+ is regenerated inside the mitochondrial matrix. Similarly, in complex II, also known as succinate dehydrogenase, $FADH_2$ is oxidized regenerating FADH+ and also releasing an electron to ETS. The transport of the electron ends up in complex IV or cytochrome oxidase where the electron reacts with oxygen reducing it and forming water using protons (H^+) from the surroundings. For every 4 electrons transported, 2 water molecules are produced from 1 O_2 molecule. The transport of these electrons causes a pumping of H^+ to the intermembrane region between the inner and outer mitochondrial membrane. Complex I, III and IV are the ones that contribute to this pumping of H^+ during the flow of electrons. 10 H^+ are pumped for each pair of electrons that pass through the system (Fig. 1.3).

The pumping of H^+ to the intermembrane region and the consumption of H^+ for the formation of H_2O in the interior generates a pH gradient between that intermembrane region and the mitochondrial matrix. During the last step in the synthesis of ATP, the mitochondrion converts the potential energy of this gradient into ATP using enzymes called ATP synthases and sometimes called complex V. This complex facilitates the return of the H^+ accumulated in the intermembrane region to the mitochondrial matrix taking advantage of the process to phosphorylate adenosine diphosphate (ADP) and forming ATP. The mechanism was first described by Mitchell (1961). In this way, oxidative phosphorylation (OXPHOS) stores energy in ATP that will be transported for use in other cellular regions. Approximately 3 molecules of ATP are generated for every

10 H^+ transported. Depending on the level of coupling, the return of H^+ will be used to transform energy in the form of ATP or in the form of heat. This will depend on the level of participation of the decoupling proteins in the return of the H^+ (Nelson et al., 2008; Hill et al., 2016).

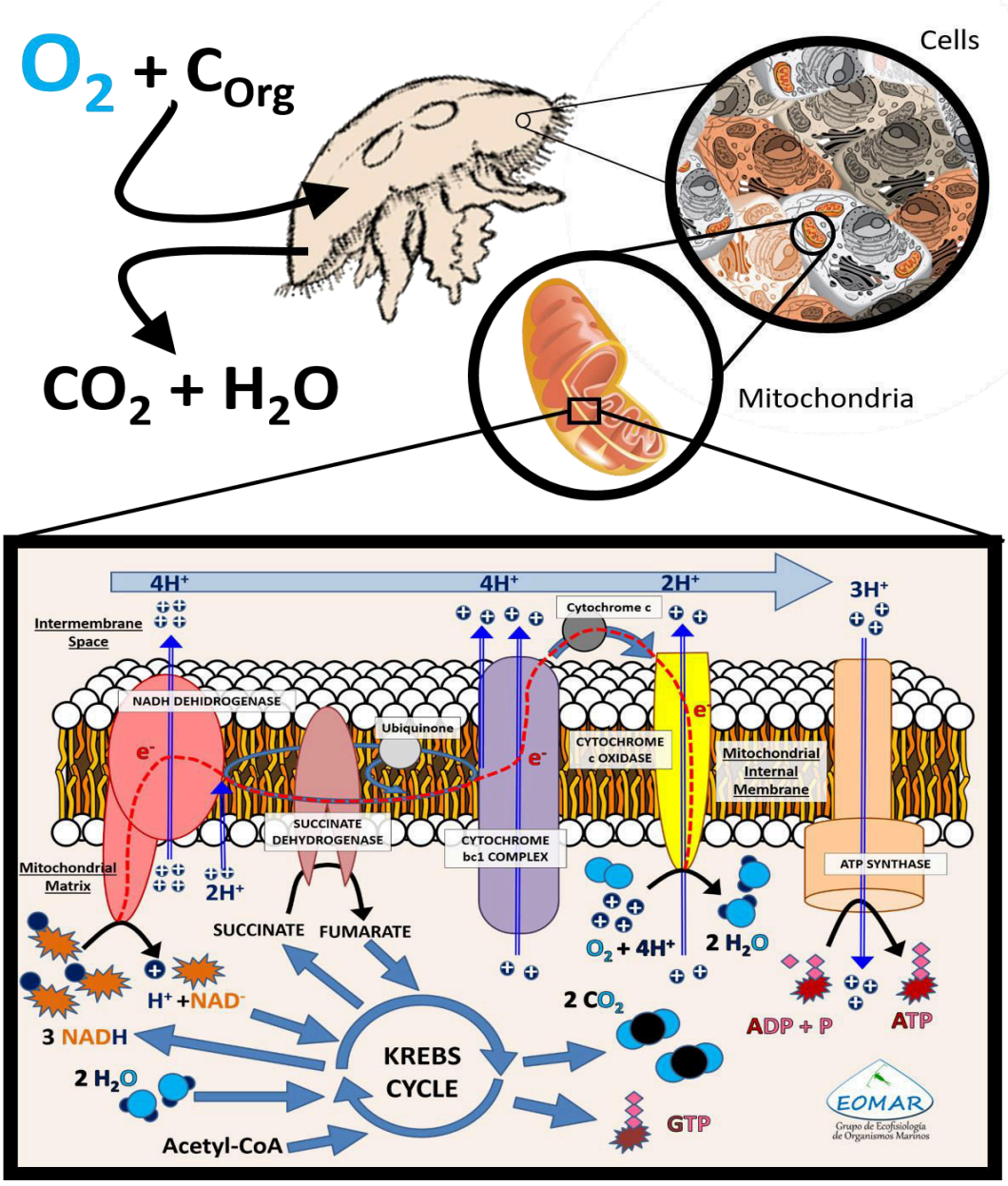

Figure 1.3. Simplified diagram of physiological respiration (R) and its control at the molecular level in the electron transport system (ETS) Above, jellyfish and the respiratory reaction with the corresponding oxygen consumption. Upper circle, cells forming the tissue of the jellyfish Lower circle, illustration of a mitochondrion. Lower rectangle, illustration of the ETS present in the internal mitochondrial membrane. It starts with the production of CO_2 and NADH in the Krebs cycle from acetyl-CoA, continues with the oxidation of NADH and the generation of the electron flow and ends with the reduction of O_2 forming H_2O. During the process, proton pumping (H^+) and ATP generation are also observed.

The release of energy in the form of ATP is in many organisms controlled by an electron transport system and a proton gradient. This cellular respiration is not exclusively aerobic as there are organisms that use final electron acceptors other than O_2, such as NO_3^-, NO_2^- or SO_4^{2-}, especially in areas with low O_2 concentrations (Vosjan, 1982). However, the appearance of these oxidized forms was related to the increase of O_2 in a similar way it required oxygen, which is why the increase of oxygen from the Proterozoic, described above, was so revolutionary for life on our planet. These molecular processes regulate the energy of the organism and condition the demand for the main substrates of this process: organic matter (food), which will be oxidised, and oxygen, which will be reduced. Understanding better this vital process we can understand the mechanisms that control the consumption of oxygen and carbon with its macroscopic consequences and we can study the flow of energy through the members of the ocean trophic system.

These vital reactions, as already introduced, are controlled by the mitochondria. Their environmental impact at the organism level is food intake, O_2 consumption and CO_2 production. In the case of intake (from the environment), all heterotrophic organisms have means for the intake of prey or organic matter from the outside that will serve as a substrate for the functioning of the metabolic mechanisms described above. Also, many organisms have specialized organs for the capture of oxygen and release of carbon dioxide that depend on the structural complexity of these organisms. In the case of cnidarians, their simple tissue structure means that they do not have specialised respiratory organs and oxygen is taken up by integumentary “breathing”, that is, by diffusion through their scarce, aqueous body tissues (Graham, 1988). Thuesen et al. (2005) have observed that, in the mesoglea of scyphozoa, what they refer to as intragel, these jellyfish are able to retain high concentrations of oxygen. This gives them the ability to maintain high respiratory activity and survive in suboxic or hypoxic concentrations not suitable for many organisms. Curiously, the relationship between phylum cnidaria and respiration has been the object of study for many years, being one of the representatives used by Vernon (1895) in his exhaustive study of respiratory exchange in marine invertebrates, even appearing in his diagram of the technique used (Fig. 1.4). More recently, a parasitic type of myxozoan cnidarian has been described that lacks parts of the mitochondrial genome related to aerobic respiration and therefore could be the first animal with a metabolism without ETS (Fig. 1.4) (Yahalomi et al., 2020).

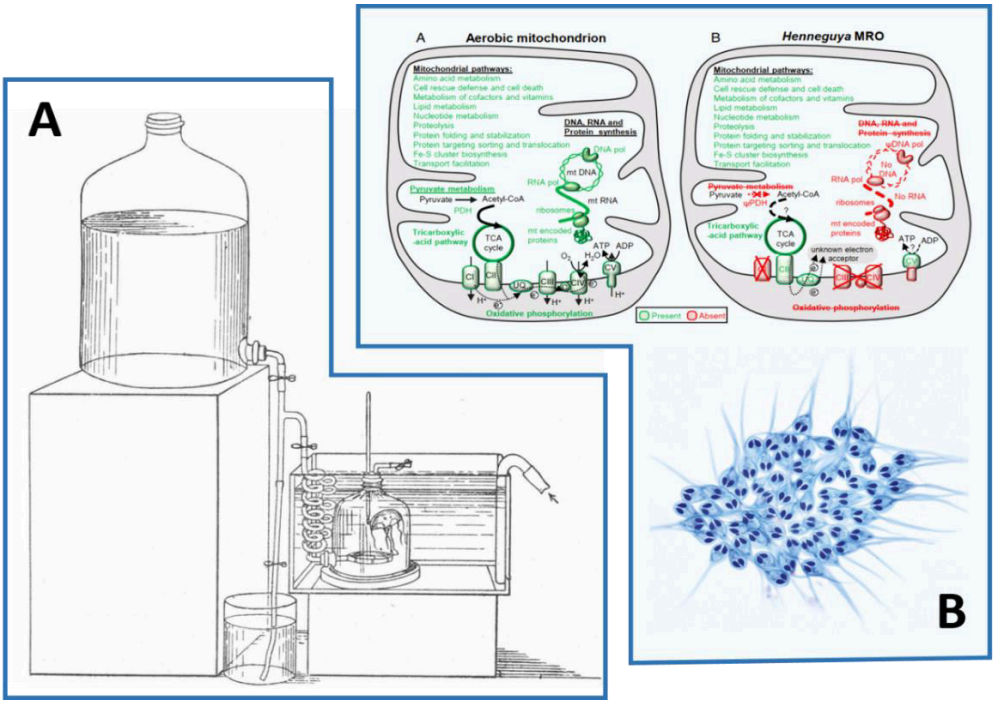

Figure 1.4. Visualisation of seminal work in the study of breathing in cnidarians. **A.** Diagram of the instrumentation for the measurement of oxygen consumption in invertebrates used by Vernon (1895). It can be observed that among the invertebrates of the study he used the jellyfish in his diagram. **B.** Image of Yahalomi et al. (2020) representing a mitochondrion and traditional electron transport system of a metazoan organism together with the theoretical mitochondrion of the parasitic cnidaria of the studied Myxozoa class that evinces, according to its genetic analysis, the absence of the processes in red. Below is the cover image of the PNAS publication of the organism in question, *Henneguya salmonicola*, represented in many media as "the first animal that does not need to breathe".

Therefore, perhaps the respiratory physiology of this phylum holds more keys to understanding this vital process. Purcell et al. (2010) advocated measuring respiration in jellyfish to approximate their prey consumption in order to estimate potential impacts on under nourished populations through their associated carbon demand (Uye & Shimauchi, 2005). Methodologies such as ingestion rate experiments or stomach content analysis allowed for the identification of which prey were consumed, but made it more difficult to quantify the impact on such prey. Respiratory measurements in cnidarians offered some advantages in quantifying the impact of blooms on prey by avoiding the complications of ingestion experiments and being faster than stomach content analysis. However, the incubations necessary to determine respiration through oxygen consumption carry with them a problem that is shared with the other techniques: the sampling of these fragile living organisms. This sampling entails a number of logistical difficulties, especially when the organism is large, as is the case with several species of scyphozoa, some of which are “bloomers” (Purcell et al., 2010; Iguchi et al., 2017). In general, many members of gelatinous plankton have this difficulty and that is why Purcell et al. (2010) encouraged the development of enzymatic techniques such as those described by Packard (1985). These enzymatic techniques study the activity associated with respiratory enzymes and therefore only require the tissue of the organism to determine the activity of the enzymes associated with these cells, which are not necessarily those of the living organism. This will be explained in more detail in the next section.

1.4. The study of O_2 consumption and ETS activity

As it has already been introduced, the study of respiration is key to understanding the functioning of living beings to the extent that it allows for the understanding of food requirements. Generally, respiratory metabolism is studied by measuring oxygen consumption, but we could also work with CO_2 production, although this is more complex to measure when dissolved in seawater (Mayzaud et al., 2005).

The measurement of oxygen consumption in zooplankton can be done through two main modes of incubation (Hernández-León & Ikeda, 2005). One consists of a known constant flow of water and measuring the oxygen concentration at the entrance and exit of the closed chamber containing the organism. This technique is very effective for observing momentary changes in oxygen consumption. However, it is more complicated to prepare and requires rigorous calibration incorporating flow rate among other parameters. In the case of gelatinous plankton, the design of device and flow control require further study to avoid damaging the organism during incubation. The other technique consists in incubating organisms in closed containers without exchanging oxygen with the outside and avoiding the presence of air inside the incubation. In this case the oxygen concentration inside the incubation chamber is monitored and the variation of the oxygen concentration will be a result of the metabolism of the incubated organism. However, this type of incubation requires hours and in some cases days depending on the volume of the chamber and the size of the organism being incubated. Incubations are carried out at a specific temperature in the dark to avoid photosynthetic processes that could produce oxygen. Knowing the variation in oxygen concentration and the time elapsed during incubation, we can determine the rate of oxygen consumption ($\Delta[O_2]/\Delta t$) which we will refer to as physiological respiration (R). Various techniques can be used to monitor O_2 concentration during incubation (Ikeda et al., 2000). The most traditional is the measurement of oxygen by means of the chemical reaction described by (Winkler, 1888). As this is a chemical reaction, it requires the removal of part of the incubation volume, which makes the process difficult or sometimes implies that it is only measured at the beginning and end of incubation. This is why other less invasive continous methods of detecting oxygen concentration through sensors were developed. In order to monitor the O_2 concentration without the need to extract water, there are polarographic instruments, which use an electrochemical reaction by means of electrodes, and photochemical methods such as optodes, which are based on the deactivation ("quenching") of the fluorescence caused by the O_2 concentration. Of these techniques, electrodes are more common and optodes more recent. All the techniques described above have been used to determine respiration in zooplankton, including jellyfish (Fig. 1.5) (Larson, 1987; Uye & Shimauchi, 2005; Lilley & Lombard, 2015).

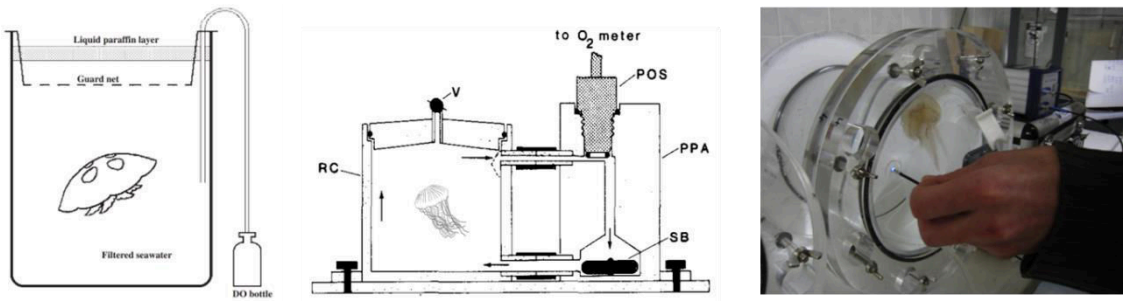

Figure 1.5. Compilation of images depicting examples of the variety of common techniques for measuring oxygen consumption. Left, Uye & Shimauchi (2005) diagram of their arrangement for incubation and water extraction for O_2 analysis by the Winkler method. Centre, diagram of Larson (1987)'s design for an incubation chamber with a polarographic system for measuring O_2 without water stagnation using an electrode (modified to include the jellyfish). Right, Lilley & Lombard (2015) image of the photochemical measurement process with the fibre optic and spot to determine O_2 concentration using an optode during an incubation.

However, incubations are exposed to containment effects on the organism that can affect its metabolic performance (Frandsen & Riisgård, 1997; Purcell et al., 2010). Incubations require time, require obtaining the living organism from the environment and require large containers for large organisms. Therefore, respiration proxies are also used, such as measuring the activity of enzymes that control these respiratory processes. There are numerous enzymes that are studied for this purpose: succinate dehydrogenase (SD) and NADH dehydrogenase from ETS, lactate dehydrogenase (LDH), pyruvate kinase (PK) and citrate synthase (CS) and malate dehydrogenase (MDH) (Hernández-León & Ikeda, 2005). In this paper we focus only on the ETS enzymes; which have a closer relation to O_2 reduction. In particular, the second chapter of this work compares the different techniques for measuring oxygen consumption and determining R and the measurement of ETS. With this information we then study its application in gelatinous plankton.

From the measurement of the enzymatic activity of the ETS, the maximum capacity of oxygen consumption by the cells of the organism is obtained, which we will refer to as potential respiration (Φ) (Packard & Gómez, 2008). To determine this specific enzyme activity, methodologies based on those developed and modified by numerous authors were used (Packard, 1971, 1985; Kenner & Ahmed, 1975; Owens & King, 1975; Gómez et al., 1996; Packard & Christensen, 2004; Maldonado et al., 2012). We begin with the homogenisation of the studied tissue in order to release the enzymes into the medium. The homogenisation medium consists of a 0.1M phosphate buffer solution. To homogenize, instruments such as a sonicator or a Potter-Elvehjem type homogenizer were used. Then this homogenate is centrifuged and the supernatant with the enzymes is mixed with the substrate of the targeted enzymes in concentrations that achieve maximum enzyme-activity. In the case of the ETS study, identical buffered solutions of NADH and NADPH are used. In addition, in the case of this analysis, a solution of the iodonitrotetrazolium salt (INT) is added (Nachlas et al., 1960; Caravelli et al., 2004). The latter acts as an artificial electron acceptor and reaction indicator since, when reduced, the INT generates formazan which stains the solution red. The reaction can therefore be monitored with a spectrophotometer. The resulting rate of formazan production indicates the activity rate of the electron transport system enzymes and allows stoichiometric estimation of the potential rate at which O_2 would be reduced in the system instead of the formazan, i.e. the Φ. The activity observed in the absence of the enzyme and also in the absence of the substrate must be subtracted from this measure in order to correct for any activity that is not strictly that of the enzymatic reaction of interest at known substrate concentrations. Chapter 3 describes the assays performed to test the application on jellyfish and the establishment of optimum assay conditions for the ETS analysis in jellyfish based on Bisswanger (2014).

1.5. Study of the biochemical composition of jellyfish and their role as prey

As described in previous sections, the study of respiration informs us about the metabolic needs of an organism and brings us closer to understanding its demand for food and therefore its impact on prey. Hundreds of millions of years ago demand for food might have been enough to position jellyfish as the top predator in ocean ecosystems (Parsons, 1979). However, with the emergence of new large predators, the role of jellyfish seemed to change. Jellyfish appeared to be free from predation except special cases (Verity & Smetacek, 1996; Sommer et al., 2002). However, many authors have questioned this "stigma" of gelatinous plankton as being "trophic dead-ends" (Doyle et al., 2007; Hamilton, 2016). Furthermore, multiple trophic relationships argue that gelatinous organisms form a vital part of the trophic systems and the explanation behind observed predation is of current interest (Thiebot & McInnes, 2020). Ates (1991) defended the role of jellyfish as prey. He suggested that jellyfish are undervalued as prey because certain observers are reluctant to accept their utility as food and to the difficulties in identifying traces of jellyfish in the digestive system of their predators. The increase in film evidence, nematocyte analysis, genetic analysis of stomach contents and the continuous review of the relationship of many animals with gelatinous plankton have strengthen their role as food (Lamb et al., 2017; Thiebot & McInnes, 2020). The term gelativore seems to be used for the first time in a publication by Drazen & Robison (2004). This term includes all organisms that consume gelatinous plankton. The appearance of this new term is relevant since gelatinous organisms are not conventional prey as it will be explored in this section. There are organisms described in the literature with a high consumption and dependence on gelatinous prey such as: the sunfish, *Mola mola*; the butterfish, *Peprilus triacanthus*; or the leatherback turtle, *Dermochelys coriácea* (Arai, 2005; Heaslip et al., 2012). Although it should be noted that in many cases the diet on jellyfish has not been extensively documented and is usually combined with other prey (Pope et al., 2010). Leaving aside the famous large consumers, there are a multitude of predators of gelatinous plankton (Fig. 1.6).

Probably one of the best collections of gelatinous plankton predators is that of Arai (2005) where predation by other gelatinous organisms, turtles, fish, chondrichthyans (rays and sharks), molluscs, crustaceans, birds and humans are documented. Pauly et al. (2009) also collected evidence of predation on gelatinous plankton by birds, reptiles and a wide variety of fish and crustaceans. Choy et al. (2017) described the structure of the food web that took place at great depths based on videos and included a huge variety of gelatinous plankton that preyed on each other and also interacted with fish, crustaceans and cephalopods, among others. In addition to those organisms that capture jellyfish alive, there are many fish and crustaceans that live in symbiosis with gelatinous organism, at least during part of their life cycle, and there is evidence that they sometimes feed on pieces of their host (Mansueti, 1963; Rorke et al., 2015; Griffin et al., 2019). There is also a whole benthic community that feeds on the remains of dead jellyfish when they are stranded or sink. This community includes crustaceans, echinoderms and other benthic cnidarians (Fig. 1.6) (Alamaru et al., 2009; Ates, 2017). This does not include the added predation suffered by some gelatinous organisms during their sessile life phases, for example, when they are polyps (Fig. 1.6) (Takao et al., 2014).

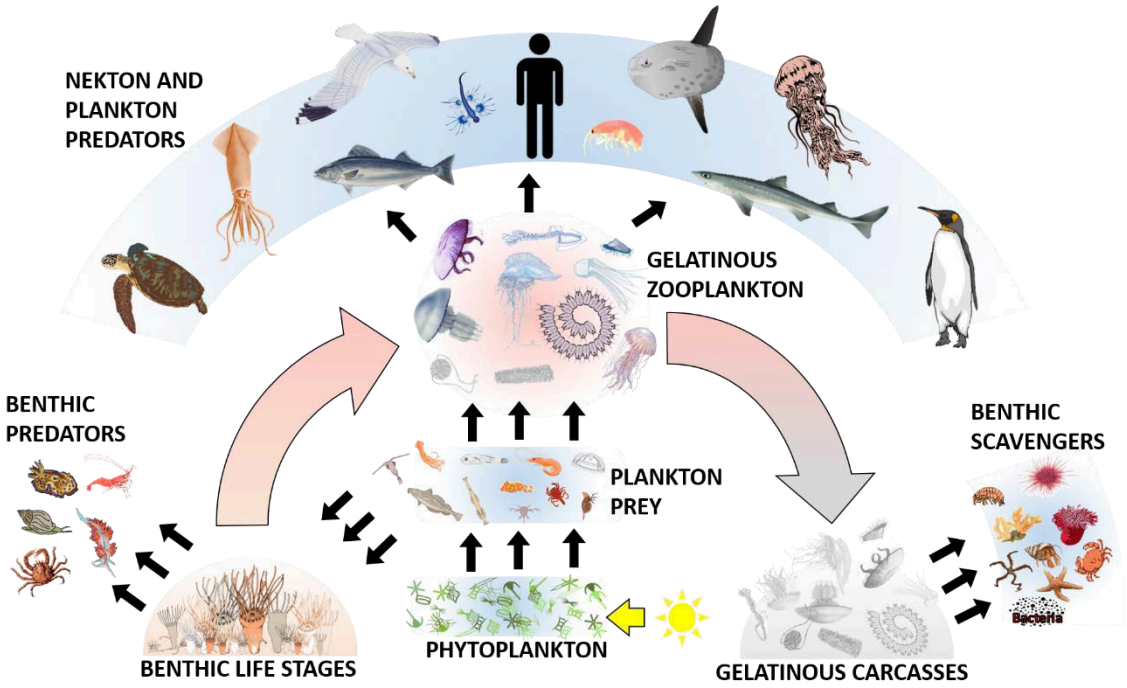

Figure 1.6. Simplified scheme compiling the trophic interactions described for gelatinous plankton. Black arrows symbolize predation flows. Coloured arrows represent possible transitions between life stages of the gelatinous plankton. The groups represented describe the energetic input to seawater through the primary producers, a very simplified version of the planktonic prey that includes eggs, larvae and small individuals of the nekton, a series of representatives of the gelatinous plankton, some of the predators of the gelatinous plankton from different groups, the benthic sessile life phase represented by some members of the gelatinous plankton with predator-prey interaction represented, the sinking of dead gelatinous plankton plus its consequent predation by benthic decomposers and scavengers. The members of the groups are based on various works (Arai, 2005; Pauly et al., 2009; Riascos et al., 2012; Takao et al., 2014; Ates, 2017; Choy et al., 2017). They have been omitted, but there are many symbiotic interactions between gelatinous plankton and many other individuals where there is sometimes mutual predation. There are also additional interactions between group members that have not been shown such as internal predation between planktonic prey and between planktonic and nektonic predators, primary production of endosymbiotic algae within gelatinous plankton, and microbial loops. For the sake of simplification and to focus the scheme on the demand for gelatinous prey, these have been omitted. The organisms are not to scale.

However, all this predation baffles the scientific community as the high water-content of jellyfish is considered to make them a poor food-source. It was thought that the explanation might lie in the fact that gelatinous plankton only served as food in the absence of more nutritious alternatives, but studies such as Marques et al. (2016) show that the demand for jellyfish is not merely circumstantial and benefits many opportunistic species that are able to take advantage of the formation of large jellyfish blooms. Penguins have also been observed feeding on jellyfish on a regular basis regardless of the abundance of jellyfish and alternative prey (Thiebot et al., 2017). In addition, fish at the top of the food chain, even though there are more energy rich nectonic prey, will feed on jellyfish (Cardona et al., 2012). Therefore, the reasons for the demand for jellyfish are still investigated. The main hypotheses have been put forward by Hays et al. (2018) and Thiebot & McInnes (2020). They offer various hypotheses that are related to the ease of capture and their rapid digestion compared to other prey. Other

hypotheses rely more on the enormous abundance of jellyfish in many regions. Others suggest that the motivation behind their predation might be the strategic intake of higher energy content parts such as gonads. A paradigm shift in research is also suggested in which jellyfish have moved from being non-food, to being poor food, and now to being a nutritional source of vital substances. This last hypothesis seems to be gaining strength and encourages research into the composition of underappreciated jellyfish (Stenvers et al., 2020). To increase our understanding of this aspect of the marine food chain, we must better understand the biochemical composition and energy content of more members of the gelatinous plankton and throughout their life cycles. For this reason, this research explores the proteins, lipids and carbohydrates of jellyfish species whose composition has been less studied in chapter 5, and chapter 6.

For the determination of protein, lipids and carbohydrates (biochemical composition), various techniques were used on homogenates that had been prepared for enzymatic analysis. The techniques are described in more detail in each chapter where they are applied. They can be summarised as controlled chemical reactions that produce coloured end-products that can be analysed spectrophotometrically. The concentration is determined from a standard curve. Figure 1.7 summarizes, in a schematic way, each one of the procedures.

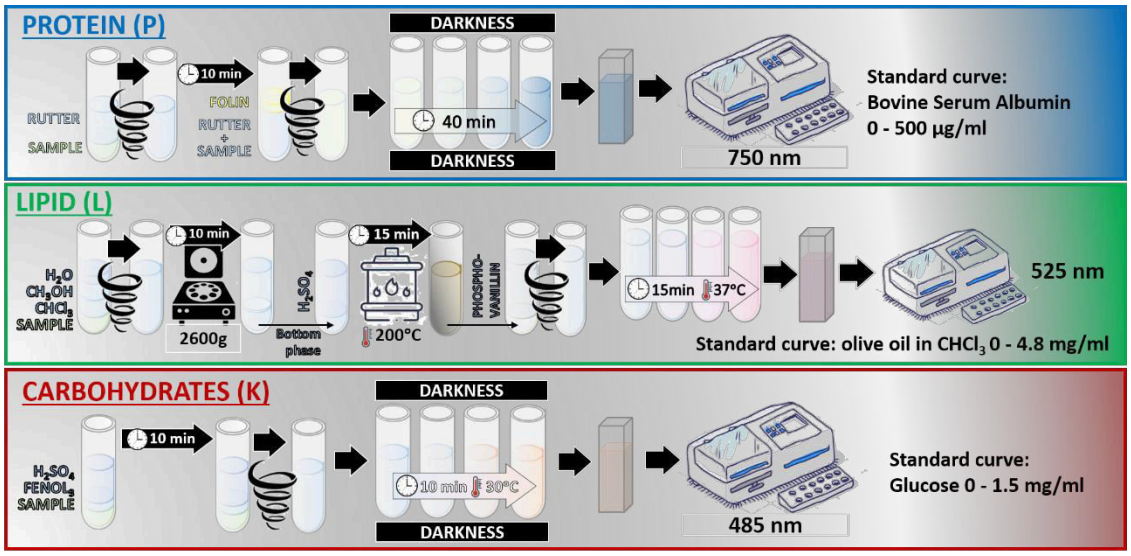

Figure 1.7. Compilation of diagrams of the procedures used for the determination of proteins, lipids and carbohydrates (Lowry, 1951; Bligh & Dryer, 1959; Dubois, 1956, respectively).

With the information from the respiratory metabolism we investigate the motivation behind the predation by the jellyfish while with the biochemical composition we learn about the motivation behind the predation on the jellyfish. We are thus able to study the energy production of these organisms together with their energy content in a novel way by describing systems in terms of energy as well as carbon (Karl, 2014; Chiaverano et al., 2018).

1.6. Summary of the organisms used in this work

A wide variety of organism were employed in this research. In chapter 2 a representative of heterotrophy and a representative of autotrophy were used with the aim of comparing techniques for measuring respiration through oxygen consumption and by ETS analysis. For the heterotrophs, mysids of the species, *Leptomysis lingvura* (G.O. Sars, 1866), were used. *L. lingvura* is an epibenthic crustacean that lives in large aggregations in the subtidal waters north of Gran Canaria where they were sampled with hand nets by scuba-divers. These mysids feed on detritus, phytoplankton, micro and mesozooplankton and are food for a great variety of fish (Mauchline, 1980). In the research group where the thesis was written, extensive work has been carried out to achieve adequate knowledge on the maintenance and measurement of the respiration of these organisms (Herrera-Ulibarri, 2013; Osma et al., 2016). Therefore, *L. lingvura* was a good candidate to compare respiration measurement techniques in heterotrophic organisms. On the other hand, as a representative of autotrophs, a chlorophyte, *Ulva rigida* C. Agardh, 1823, was used. The species of the genus *Ulva* (Sea lettuce) are green intertidal macroalgae common globally (Zou et al., 2007) and in the case of Gran Canaria, they are easy to sample and maintain. In addition, our laboratory has a history of working with this alga (Asensio Elvira, 2013). It is useful to investigate the applicability of these respiratory measuring techniques in autotrophs too and understand possible issues regarding jellyfish with endosymbiotic algae.

Once the technique for measuring oxygen consumption had been selected and the tests described for the application of enzymatic measures had been carried out, the study of respiration in jellyfish was carried out in Chapters 3, 4, 5 and 6. The species dealt with in that work included two scyphozoa and two hydrozoa.

The scyphozoans studied were both of the order Semaeostomeae (***Pelagia noctiluca*** and ***Aurelia aurita***) but with different life cycles. *Pelagia noctiluca* (Forsskål, 1775) is a holoplanktonic jellyfish, that is, it spends its entire life cycle as part of the plankton (Ramondenc et al., 2019) (Fig. 1.8). It has been extensively studied in the Mediterranean (Canepa et al., 2014) where it forms large blooms and preys on zooplankton populations and fish larvae non-selectively (Sabatés et al., 2010; Milisenda et al., 2018). It also has nematocysts for the capture of its prey causing a relatively serious sting when it encounters bathers (Mariottini et al., 2008). So, it impacts on local fauna populations and human activities. Depending on the Mediterranean and Atlantic circulation, periods of high aggregation of *P. noctiluca* are observed in Canary Island waters (Rodríguez et al., 2015). *P. noctiluca* is also prey to fish, turtles and crustaceans (Milisenda et al., 2014; Couce-Montero et al., 2015).

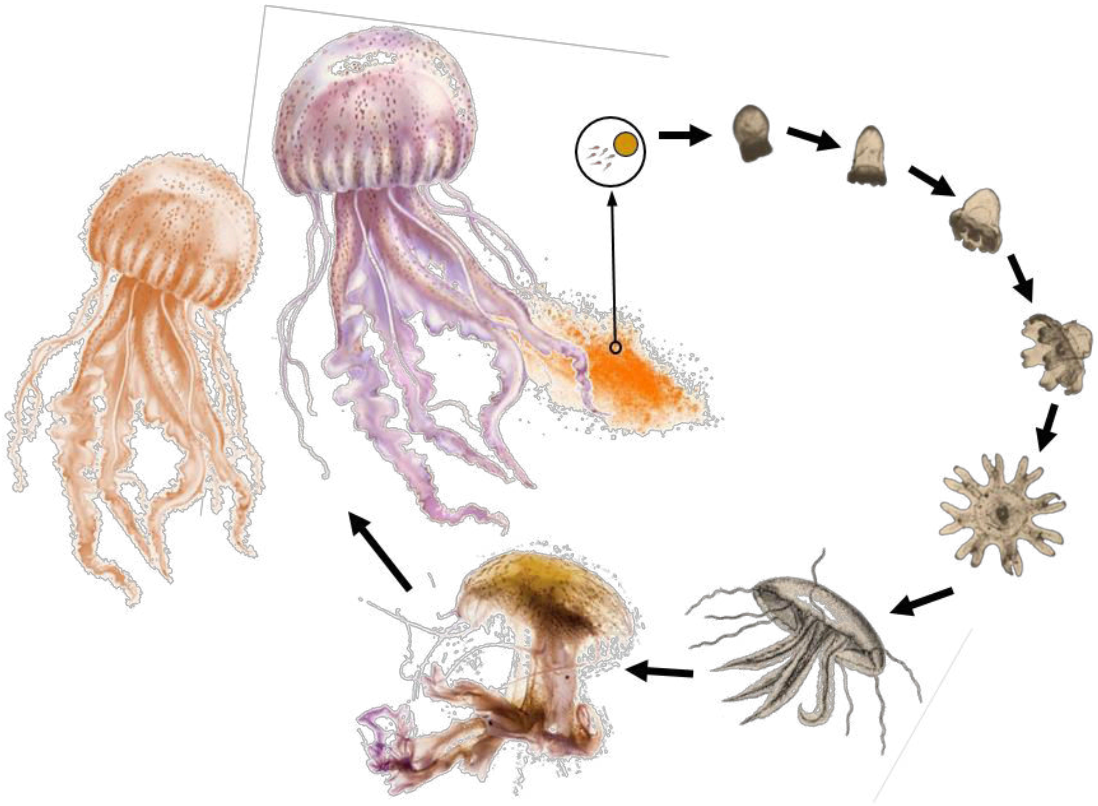

Figure 1.8. Life cycle of *Pelagia noctiluca* (Sandrini et al., 1983; Canepa et al., 2014; Ramondenc et al., 2019).

Aurelia aurita (Linnaeus, 1758) is a very commonly studied species because its cultivation is simple, so it is widely used in aquariums. It is a scyphozoan with a complex life cycle that combines sessile and planktonic life stages, common to other scyphozoans (Ceh et al., 2015) (Fig. 1.9). The life form during the sessile stage is known as scyphistoma or polyp. Polyps can reproduce asexually and form colonies of the same individual. Also, polyps under specific conditions start a metamorphosis called strobilation during which they segment forming a strobila from which the planktonic larval phase called ephyra bud-off (Fig. 1.9) (Fuchs et al., 2014; Sukhoputova & Kraus, 2017). The ephyrae develop into the adult medusoid life stage, the jellyfish. Adult jellyfish reproduce sexually by releasing gametes into their environment. These fertilised gametes develop an embryonic phase that culminates in the formation of a planula larva that finds hard-bottom substrate to form the polyp (Holst & Jarms, 2007). There are descriptions of exceptions to the cycle where the planula develops into an ephyra without an obvious polyp phase and life-cycle reversal phenomena have also been documented (He et al., 2015). Polyps are very versatile; they manage to reproduce by various mechanisms (Gilchrist, 1937; Vagelli, 2007) and during strobilation they can produce up to 20 ephyrae per polyp and survive to continue multiplying and repeating the process (Lucas et al., 2012) (Fig. 1.9). Therefore, they are key steps in controlling the formation of blooms. However, it is the jellyfish that participate in the food chains in the open ocean as predator and prey (Schneider & Behrends, 1998). In addition to its cultivated side and its curious life cycle, *A. aurita* forms large blooms in nature with important consequences (Dong, 2019). It is again a predator of a wide variety of zooplankton members and occasionally fish larvae (Sullivan et al., 1997; Titelman & Hansson, 2006). It is also the

prey of fish, turtles, crustaceans, echinoderms and other cnidarians, including other scyphozoans (Ates, 2017).

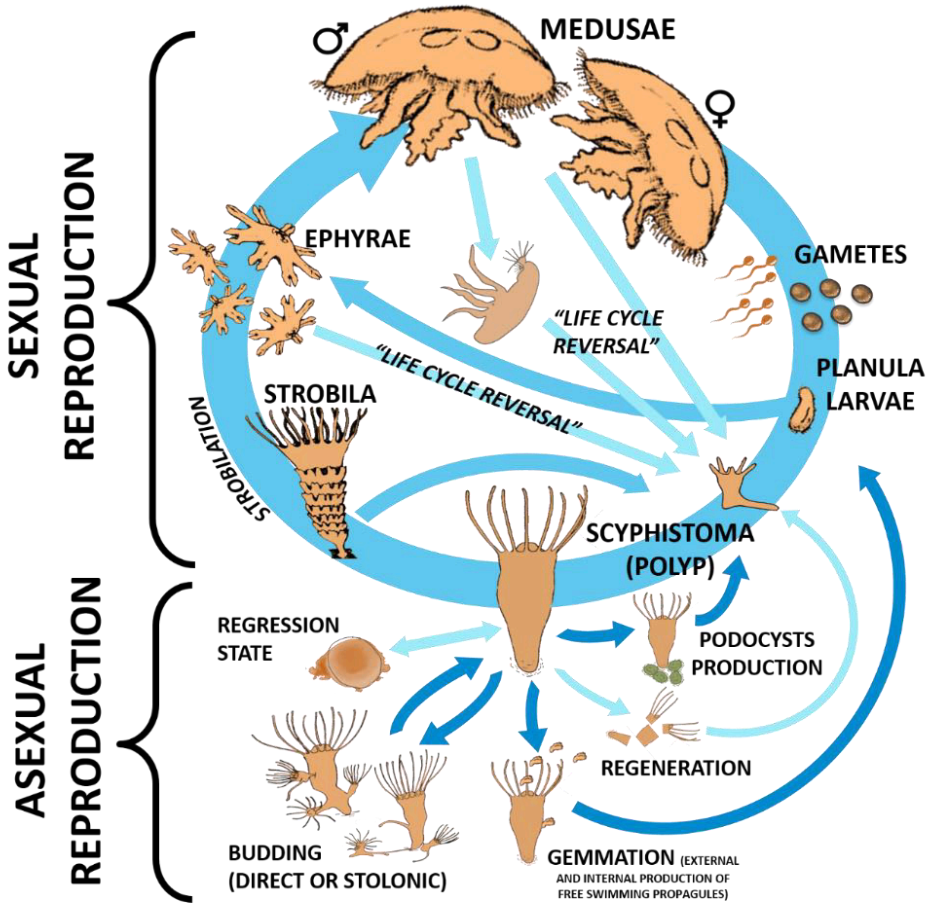

Figura 1.9. Compilation of observed reproductive processes in the genus *Aurelia* (Gilchrist, 1937; Hamner & Jenssen, 1974; H., 2001; Vagelli, 2007; He et al., 2015; Hubot et al., 2017). This diagram brings together the cycle of sexual reproduction that includes the medusa phase, the polyp phase, and the various mechanisms of asexual reproduction of the polyp phase.

The hydrozoans studied were ***Physalia physalis*** and ***Velella velella***. Both are gelatinous members of the pleuston, the ecosystem in the surface layer of the ocean that is in contact with the atmosphere (Zamponi, 2011). Both species form colonies of highly polymorphic individuals where each has a specialized function (Schuchert, 2010; Munro et al., 2019). The colony is attached to a chitinous floating structure called a pneumatophore or float which is a specialized gas-filled colony structure that allows the colony to use the atmospheric wind to propel through the pleuston (Mackie, 1974; Iosilevskii & Weihs, 2009). In addition to their exceptional oceanic niche, both hydrozoans have several unique characteristics. They have a blue colouring due to the presence of a specific chromatoprotein (Mackie et al., 1988). Also, as it has already been mentioned, they form free floating polymorphic colonies where individuals, or zooids, perform specific functions. This type of conformation led Mackie & Boag (1963) to state that it was a way to escape from the limitations of a diploblastic body; where other organisms have evolved triploblastic bodies and form organs from the mesoderm, these colonial individuals have built organ-like structures from the specialization of individuals in the colony. This is the maximum use of the physiological-anatomical

possibilities of coloniality (Mackie et al., 1988). Wilson (1975) described it as one of the great stories of evolution. Interestingly, these species were some of the first described due to their floating nature and large strandings that early on brought them into contact with humans (Araya et al., 2016; Betti et al., 2019). However, their physiology and role in ecosystems is not as frequently studied as with other more easily cultured gelatinous plankton (Pierce, 2006).

Physalia physalis (Linnaeus, 1758) is of the order siphonophorae. Unlike other siphonophores it is found in its adult state propelled by the wind through surface water, travelling long distances thanks to its large pneumatophore (Ferrer & Pastor, 2017). From their floating situation they extend long zooids with tentacular palpons specialized in defense and capture using nematocysts (Munro et al., 2019) (Fig. 1.10). Its toxin is powerful and thus poses a risk for humans and negatively impacts tourism (Edwards & Hessinger, 2000; Prieto et al., 2015), especially when they form large blooms. These toxins are normally used to catch their traditional prey: small fish, fish larvae and more exceptionally cephalopods and chaetognaths (Purcell, 1984). As observed in many jellyfish species, *P. physalis* also serves as a host for some commensal fish species such as species of the genus *Nomeus* (Jenkins, 1983). In addition, they are prey for predators in the pleuston such as nudibranchs in the genus *Glaucus* or barnacles such as *Lepas anserifera*, but they are also prey for turtles and fish and when stranded for crabs (Jones, 1968; Wenner, 1977; Frick et al., 2009). Their life cycle and anatomy have recently been reviewed by Munro et al. (2019). This review highlights the need for further studies on the functioning of this species.

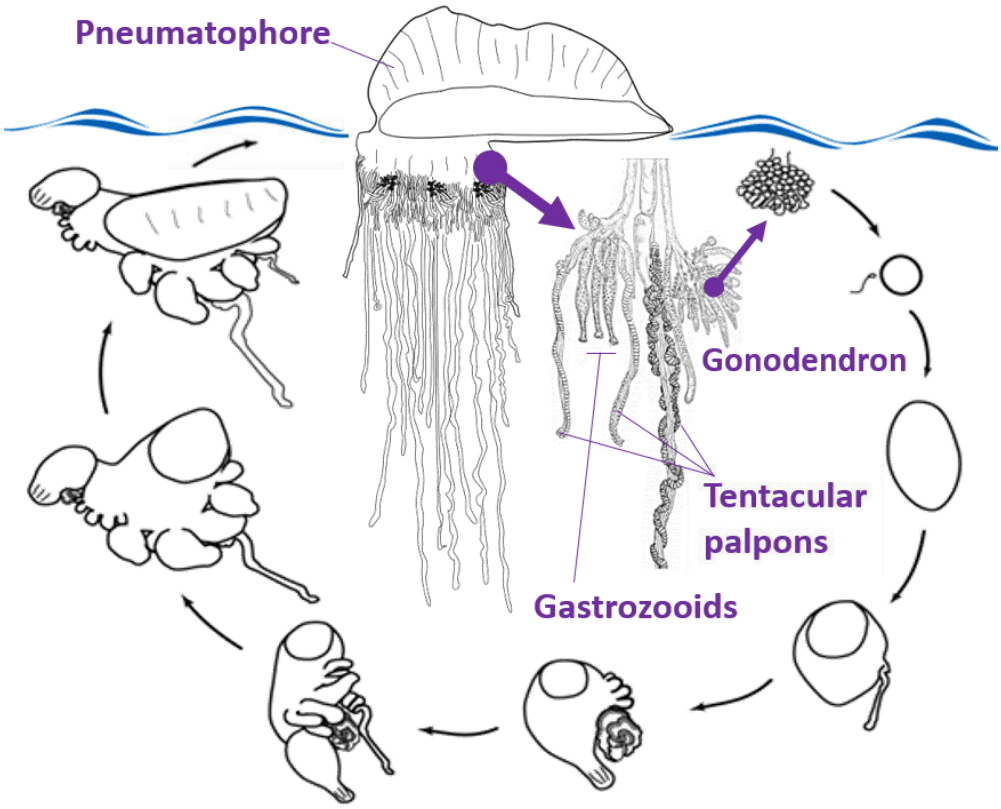

Figure 1.10. Colony anatomy and life cycle of *Physalia physalis*. (Lane, 1960; Munro et al., 2019)

Velella velella (Linnaeus, 1758), on the other hand, is a species of the order Anthoathecata and its life cycle is not completely known, but two life phases have been described. A "polyp" phase during the formation of the colony of zooids that float suspended from the float. From the zooids in charge of reproduction, smaller medusa life phases are generated (Duarte et al., 2018) (Fig. 1.11). This jellyfish phase generates gametes, but fertilization and embryonic development has not been well described. However, reproduction culminates with the formation of the conaria and rataria larval stages (Schuchert, 2010). The subsequent development of the *V. velella* adult colony, unlike *P. physalis*, has endosymbiotic microalgae, zooxanthellae, which are also present in the medusa phase. In addition, the nematocysts have also been documented so that *V. velella* can prey on zooplankton and fish eggs (Zeman et al., 2018). On the other hand, *V. velella* colonies have multiple predators. Some cohabit in the pleuston, such as the holoplanktonic sea snail (*Janthina pallida)* and the gooseneck barnacle (*Lepas anserifera,* Bieri, 1966), others do not, such as turtles, fish, sharks (chondrichthyans) or birds (Phillips et al., 2017). In addition, some crustaceans, during their stage as megalopa larvae, parasitize these floating colonies to travel long distances establishing a symbiotic temporal relationship (Purcell et al., 2012).

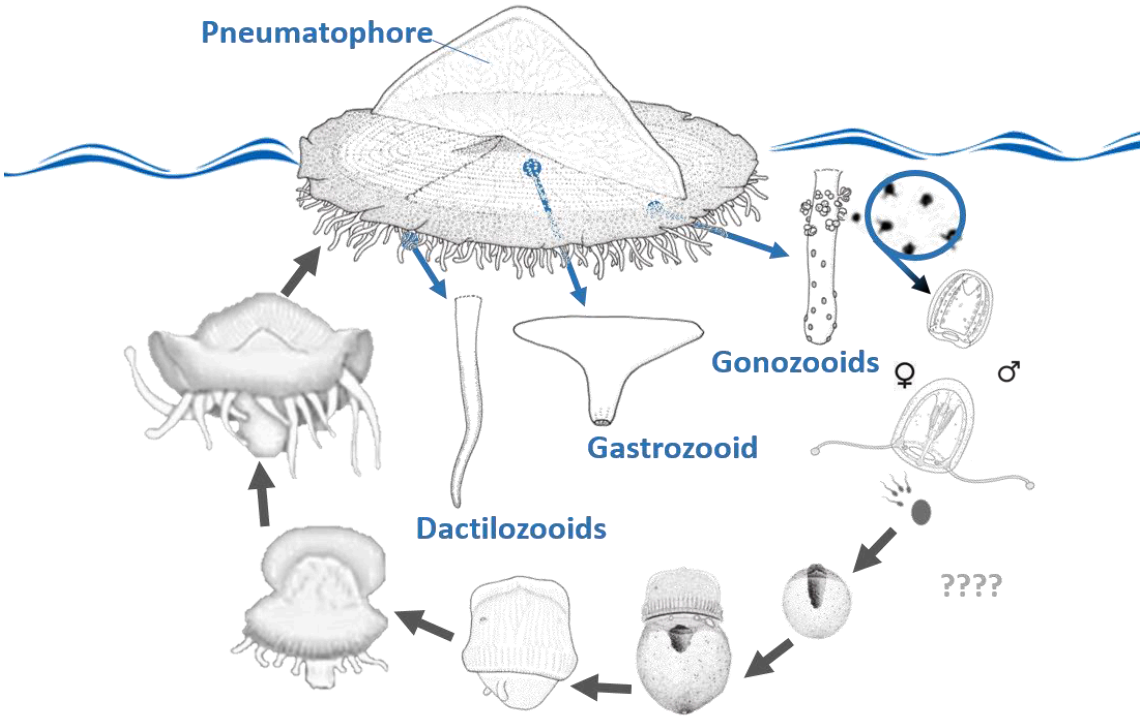

Figure 1.11. Anatomy of the colony and life cycle of *Velella velella* (Schuchert, 2010; Pires et al., 2018).

In none of these gelatinous plankton members have the physiological processes been studied with the enzymatic techniques used here. Nor have any respiration data been used to study the contributions of these individuals from the energetic point of view. Furthermore, in the case of *Physalia* and *Velella,* in particular, respiration and composition have rarely been measured. This research reports some of the first respiration measurements on these species. Also, in the case of *Aurelia*, the monitoring of its metabolism and composition throughout its development from ephyra to jellyfish have revealed aspects never before documented about the changes it experiences during the first days in the plankton.

1.7. Objectives of the thesis

The overall aim of this work is to improve the understanding of the role of jellyfish as both predator and prey. To study their impact as a predator, techniques for the study of respiratory activity were developed and tested. They allow, for example, the estimation of carbon demand, and therefore the food needed to sustain this demand. For the study of their demand as prey, the proteins, lipids and carbohydrates of these gelatinous plankton were analysed. This permitted calculation of their energy content and helped to quantitatively explain why they were preyed upon. In addition, these same methodologies were used to detect changes in respiratory metabolism and composition during growth and starvation periods.

The objectives addressed in:

- Chapter 2:
    - Compare results of different techniques for measuring oxygen consumption. In addition, to compare results from O_2 consumption and enzymatic rates in autotrophic and heterotrophic organisms in order to further understand respiration in gelatinous plankton.

- Chapter 3 and 4:
    - To develop and apply new methodology to perform traditional respiratory and enzymatic measurements on *A. aurita* and *P. noctiluca*.

- Chapter 4 and 5:
    - Compare the respiration obtained by oxygen consumption methods and enzymatic techniques in different organisms of the gelatinous plankton. Then, compare jellyfish results with results from other zooplankton.
    - Study the implications of this respiratory metabolism on carbon demand and energy flow associated with gelatinous plankton in order to estimate the impact on prey populations
    - Analyse the relationship between energy release associated with respiration and energy content as a new tool for studying metabolism.

- Chapter 5 (alone):
    - Measure for the first time the biochemical composition and energy content of pleuston members, *V. velella* and *P. physalis* and to compare results with the rest of the gelatinous plankton.

- Chapter 6:
    - Monitor *A. aurita*'s respiration as a response to prolonged starvation to explain why *A. aurita* is highly resistant to starvation.
    - To use the techniques developed here to observe the changes experienced at the level of respiratory metabolism and biochemical composition during the transition period from 24h ephyra larva to 54d juvenile jellyfish.

REFERENCES

Acuña, J. L., Á. López-Urrutia, & S. Colin, 2011. Faking giants: the evolution of high prey clearance rates in jellyfishes. science American Association for the Advancement of Science 333: 1627–1629.

Alamaru, A., O. Bronstein, G. Dishon, & Y. Loya, 2009. Opportunistic feeding by the fungiid coral *Fungia scruposa* on the moon jellyfish *Aurelia aurita*. Coral Reefs 28: 865–865.

Arai, M. N., 2005. Predation on pelagic coelenterates: A review. Journal of the Marine Biological Association of the United Kingdom 85: 523–536.

Araya, J. F., J. A. Aliaga, & M. E. Araya, 2016. On the distribution of *Physalia physalis* (Hydrozoa: Physaliidae) in Chile. Marine Biodiversity 46: 731–735, http://dx.doi.org/10.1007/s12526-015-0417-6.

Archibald, J. M., 2015. Endosymbiosis and eukaryotic cell evolution. Current Biology Elsevier Ltd 25: R911–R921, http://dx.doi.org/10.1016/j.cub.2015.07.055.

Asensio Elvira, M. T., 2013. Respiratory metabolism in the marine macroalga, Ulva spp: exploratory studies with the respiratory electron transport system (ETS). Universidad de Las Palmas de Gran Canaria.

Ates, R. M. L., 1991. Predation on Cnidaria by vertebrates other than fishes. Hydrobiologia 216–217: 305–307.

Ates, R. M. L., 2017. Benthic scavengers and predators of jellyfish, material for a review. Plankton and Benthos Research 12: 71–77.

Avila Rodríguez, M. I., L. G. Rodríguez Barroso, & M. L. Sánchez, 2018. Collagen: A review on its sources and potential cosmetic applications. Journal of Cosmetic Dermatology 17: 20–26.

Benedict, C. R., 1978. Nature of Obligate Photoautotrophy. Annual Review of Plant Physiology 29: 67–93.

Betti, F., M. Bo, F. Enrichetti, M. Manuele, R. Cattaneo-Vietti, & G. Bavestrello, 2019. Massive strandings of *Velella velella* (Hydrozoa: Anthoathecata: Porpitidae) in the Ligurian Sea (North-western Mediterranean Sea). European Zoological Journal 86: 343–353.

Bieri, R., 1966. Feeding Preferences and Rates of the Snail, *Ianthina prolongata*, the Barnacle, *Lepas anserifera*, the Nudibranchs, *Glaucus atlanticus* and *Fiona pinnata*, and the Food Web in the Marine Neuston. Publications of the Seto Marine Biological Laboratory 14: 161–170.

Bisswanger, H., 2014. Enzyme assays. Perspectives in Science Elsevier 1: 41–55, http://dx.doi.org/10.1016/j.pisc.2014.02.005.

Bligh, E. G., & W. J. Dyer, 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology NRC Research Press 37: 911–917.

Brochier-Armanet, C., E. Talla, & S. Gribaldo, 2009. The multiple evolutionary histories of dioxygen reductases: Implications for the origin and evolution of aerobic respiration. Molecular Biology and Evolution 26: 285–297.

Brotz, L., & D. Pauly, 2017. Studying jellyfish fisheries: Toward accurate national catch reports and appropriate methods for stock asssessments In Mariottini, G. L. (ed), Jellyfish. : 313–329.

Canepa, A., V. Fuentes, A. Sabatés, S. Piriano, F. Boero, & J. M. Gili, 2014. *Pelagia noctiluca* in the Mediterranean In Pitt, K. A., & C. H. Lucas (eds), Jellyfish Blooms. Springer Science, Dordrecht: 1–304.

Canfield, D. E., 2005. The early history of atmospheric oxygen: Homage to Robert M. Garrels. Annual Review of Earth and Planetary Sciences 33: 1–36.

Caravelli, A., L. Giannuzzi, & N. Zaritzky, 2004. Effect of chlorine on filamentous microorganisms present in activated sludge as evaluated by respirometry and INT-dehydrogenase activity. Water Research 38: 2395–2405.

Cardona, L., I. Á. de Quevedo, A. Borrell, & A. Aguilar, 2012. Massive consumption of gelatinous plankton by mediterranean apex predators. PLoS ONE 7: e31329-1–14.

Cartwright, P., S. L. Halgedahl, J. R. Hendricks, R. D. Jarrard, A. C. Marques, A. G. Collins, & B. S. Lieberman, 2007. Exceptionally preserved jellyfishes from the Middle Cambria. PLoS ONE 2: e1121-1–7.

Ceh, J., J. Gonzalez, A. S. Pacheco, & J. M. Riascos, 2015. The elusive life cycle of scyphozoan jellyfish - Metagenesis revisited. Scientific Reports Nature Publishing Group 5: 1–13, http://dx.doi.org/10.1038/srep12037.

Chiaverano, L. M., K. L. Robinson, J. Tam, J. J. Ruzicka, J. Quiñones, K. T. Aleksa, F. J. Hernandez, R. D. Brodeur, R. Leaf, S. Uye, M. B. Decker, M. Acha, H. W. Mianzan, & W. M. Graham, 2018. Evaluating the role of large jellyfish and forage fishes as energy pathways, and their interplay with fisheries, in the Northern Humboldt Current System. Progress in Oceanography 164: 28–36.

Choy, C. A., S. H. D. Haddock, & B. H. Robison, 2017. Deep pelagic food web structure as revealed by in situ feeding observations. Proceedings. Biological sciences 284:.

Collins, A. G., 2002. Phylogeny of the Medusozoa and the evolution of cnidarian life cycles. Journal of Evolutionary Biology 15: 418–432.

Condon, R. H., W. M. Graham, C. M. Duarte, K. A. Pitt, C. H. Lucas, S. H. D. Haddock, K. R. Sutherland, K. L. Robinson, M. N. Dawson, M. B. Decker, C. E. Mills, J. E. Purcell, A. Malej, H. Mianzan, S. Uye, S. Gelcich, & L. P. Madin, 2012. Questioning the Rise of

Gelatinous Zooplankton in the World's Oceans. BioScience 62: 160–169.

Conway Morris, S., 2000. The Cambrian "explosion": Slow-fuse or megatonnage? Proceedings of the National Academy of Sciences of the United States of America 97: 4426–4429.

Couce-Montero, L., V. Christensen, & J. J. Castro, 2015. Effects of small-scale and recreational fisheries on the Gran Canaria ecosystem. Ecological Modelling Elsevier B.V. 312: 61–76, http://dx.doi.org/10.1016/j.ecolmodel.2015.05.021.

Cunningham, J. A., A. G. Liu, S. Bengtson, & P. C. J. Donoghue, 2017. The origin of animals: Can molecular clocks and the fossil record be reconciled?. BioEssays 39: 1–12.

Dong, Z., 2019. Blooms of the Moon Jellyfish Aurelia : Causes, Consequences and Controls World Seas: an Environmental Evaluation. Elsevier-Academic Press: 163–171, https://doi.org/10.1016/B978-0-12-805052-1.00008-5.

Doyle, T. K., G. C. Hays, C. Harrod, & J. D. R. Houghton, 2014. Ecological and societal benefits of jellyfish In Pitt, K. A., & C. H. Lucas (eds), Jellyfish blooms. Springer: 105–127.

Doyle, T. K., J. D. R. Houghton, R. McDevitt, J. Davenport, & G. C. Hays, 2007. The energy density of jellyfish: Estimates from bomb-calorimetry and proximate-composition. Journal of Experimental Marine Biology and Ecology 343: 239–252.

Drazen, J. C., & B. H. Robison, 2004. Direct observations of the association between a deep-sea fish and a giant scyphomedusa. Marine and Freshwater Behaviour and Physiology 37: 209–214.

Duarte, C. M., K. A. Pitt, C. H. Lucas, J. E. Purcell, S. Uye, K. L. Robinson, L. Brotz, M. B. Decker, K. R. Sutherland, A. Malej, L. Madin, H. Mianzan, J. M. Gili, V. Fuentes, D. Atienza, F. Pagès, D. Breitburg, J. Malek, W. M. Graham, & R. H. Condon, 2013. Is global ocean sprawl a cause of jellyfish blooms?. Frontiers in Ecology and the Environment 11: 91–97.

Duarte, I., S. Leandro, M. Ferreira, H. Pinto, J. Falcão, J. Reis, S. Maia, & S. Marques, 2019. Early development of Velella velella medusae in laboratory. Frontiers in Marine Science | International Meeting on Marine Research .

Dubois, M., K. Gilles, J. K. Hamilton, P. A. Rebers, & F. Smith, 1956. A colorimetric method for the determination of sugars. Nature 168: 350–356.

Edwards, L., & D. A. Hessinger, 2000. Portuguese Man-of-war (*Physalia physalis*) venom induces calcium influx into cells by permeabilizing plasma membranes. Toxicon 38: 1015–1028.

Fernández-Urruzola, I., 2015. Understanding the zooplankton ammonium excretion: from biogeochemical implications to intracellular regulatory mechanisms. Universidad de Las Palmas de Gran Canaria.

Ferrer, L., & A. Pastor, 2017. The Portuguese man-of-war: Gone with the wind. Regional Studies in Marine Science Elsevier B.V. 14: 53–62, http://dx.doi.org/10.1016/j.rsma.2017.05.004.

Frandsen, K. T., & H. U. Riisgård, 1997. Size dependent respiration and growth of jellyfish, *Aurelia aurita*. Sarsia 82: 307–312.

Fransolet, D., S. Roberty, & J. C. Plumier, 2012. Establishment of endosymbiosis: The case of cnidarians and Symbiodinium. Journal of Experimental Marine Biology and Ecology Elsevier B.V. 420–421: 1–7, http://dx.doi.org/10.1016/j.jembe.2012.03.015.

Frick, M. G., K. L. Williams, A. B. Bolten, K. A. Bjorndal, & H. R. Martins, 2009. Foraging ecology of oceanic-stage loggerhead turtles *Caretta caretta*. Endangered Species Research 9: 91–97.

Fuchs, B., W. Wang, S. Graspeuntner, Y. Li, S. Insua, E. M. Herbst, P. Dirksen, A. M. Böhm, G. Hemmrich, F. Sommer, T. Domazet-Lošo, U. C. Klostermeier, F. Anton-Erxleben, P. Rosenstiel, T. C. G. Bosch, & K. Khalturin, 2014. Regulation of polyp-to-jellyfish transition in *Aurelia aurita*. Current Biology 24: 263–273.

Gabaldón, T., 2018. Relative timing of mitochondrial endosymbiosis and the "pre-mitochondrial symbioses" hypothesis. IUBMB Life 70: 1188–1196.

Gilchrist, F. G., 1937. Budding and Locomotion in the Scyphistomas of *Aurelia*. The Biological Bulletin 72: 99–124.

Gnaiger, E., N. Aasander Frostner, E. Abdul Karim, N. A. Abumrad, D. Acuna-Castroviejo, R. C. Adiele, B. Ahn, S. S. Ali, L. Alton, M. G. Alves, F. Amati, N. D. Amoedo, I. Andreadou, M. Arago, C. Aral, O. Arandarcikaite, E. Al., & L. Zvejniece, 2019. Mitochondrial respiratory states and rate. .

Gómez, M., S. Torres, & S. Hernzández-León, 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. South African Journal of Marine Science 17: 15–20.

Graham, J. B., 1988. Ecological and evolutionary aspects of integumentary respiration: Body size, diffusion, and the invertebrata. Integrative and Comparative Biology 28: 1031–1045.

Gray, M. W., 2017. Lynn Margulis and the endosymbiont hypothesis: 50 years later. Molecular Biology of the Cell 28: 1285–1287.

Griffin, D. C., C. Harrod, J. D. R. Houghton, & I. Capellini, 2019. Unravelling the macro-evolutionary ecology of fish-jellyfish associations: life in the "gingerbread house." Proceedings. Biological sciences 286: 1–9.

Haddock, S. H. D., 2004. A golden age of gelata: past and future research on planktonic ctenophores and cnidarians. Hydrobiologia 530: 549−556.

Hamilton, G., 2016. The secret lives of JELLYFISH.

Nature 531: 432–434.

Hamner, W. M., & R. M. Jenssen, 1974. Growth , Degrowth , and Irreversible Cell Differentiation in *Aurelia aurita*. American Zoologist 14: 833–849.

Hays, G. C., T. K. Doyle, & J. D. R. Houghton, 2018. A Paradigm Shift in the Trophic Importance of Jellyfish?. Trends in Ecology and Evolution 33: 874–884.

He, J., L. Zheng, W. Zhang, & Y. Lin, 2015. Life cycle reversal in *Aurelia sp.1* (Cnidaria, Scyphozoa). PLoS ONE 10: 1–14.

Heaslip, S. G., S. J. Iverson, W. D. Bowen, & M. C. James, 2012. Jellyfish support high energy intake of leatherback sea turtles (dermochelys coriacea): Video evidence from animal-borne cameras. PLoS ONE 7: 1–7.

Hedges, S. B., J. E. Blair, M. L. Venturi, & J. L. Shoe, 2004. Size and complexity among multicellular organisms. BMC Evolutionary Biology 4: 1–9.

Hernández-León, S., & T. Ikeda, 2005. Zooplankton respiration. Respiration in Aquatic Systems. Oxford University Press, New York 57–82.

Herrera-Ulibarri, A., 2013. Identification, Abundance and Rearing of Mysids of Gran Canaria: aplication to laboratory and oceanographic respiratory metabolism studies. .

Hill, R. W., G. A. Wyse, & M. Anderson, 2016. Fisiologia Animal-2. Artmed Editora.

Holland, H. D., 2006. The oxygenation of the atmosphere and oceans. Philosophical Transactions of the Royal Society B: Biological Sciences 361: 903–915.

Holst, S., & G. Jarms, 2007. Substrate choice and settlement preferences of planula larvae of five Scyphozoa (Cnidaria) from German Bight, North Sea. Marine Biology 151: 863–871.

Hubot, N., C. H. Lucas, & S. Piraino, 2017. Environmental control of asexual reproduction and somatic growth of *Aurelia spp.* (Cnidaria, Scyphozoa) polyps from the Adriatic Sea. PLoS ONE 12: 1–16.

Iguchi, N., H. Iwatani, K. Sugimoto, S. Kitajima, N. Honda, & O. Katoh, 2017. Biomass, body elemental composition, and carbon requirement of *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) in the southwestern Japan Sea. Plankton and Benthos Research 12: 104–114.

Ikeda, T., J. J. Torres, S. Hernández-León, & S. P. Geiger, 2000. Metabolism. In 'ICES Zooplankton Methodology Manual'.(Eds ME Harris, RP Wiebe, PH Lenz, J. Skjoldal and HR Huntley.) pp. 455–532. Academic Press: London.

Iosilevskii, G., & D. Weihs, 2009. Hydrodynamics of sailing of the Portuguese man-of-war *Physalia physalis*. Journal of the Royal Society Interface 6: 613–626.

Jenkins, R. L., 1983. Observations on the Commensal Relationship of *Nomeus gronovii* with *Physalia physalis* . American Society of Ichthyologists and Herpetologists 1983: 250–252.

Jones, E. C., 1968. *Lepas anserifera* Linné (Cirripedia Lapadomorpha) feeding on fish and *Physalia*. Crustaceana 14: 312–313.

Karl, D. M., 2014. Solar energy capture and transformation in the sea. Elementa: Science of the Anthropocene 2: 1–6.

Kenner, R. A., & S. I. Ahmed, 1975. Correlation between oxygen utilization and electron transport activity in marine phytoplankton. Marine biology Springer 33: 129–133.

Kim, J. Y., S. M. Lee, & J. H. Lee, 2012. Biogas production from moon jellyfish (*Aurelia aurita*) using of the anaerobic digestion. Journal of Industrial and Engineering Chemistry The Korean Society of Industrial and Engineering Chemistry 18: 2147–2150, http://dx.doi.org/10.1016/j.jiec.2012.06.010.

Knoll, A. H., 1991. End of the Proterozoic Eon. Scientific American 265: 64–73.

Knoll, A. H., 2003. The geological consequences of evolution. Geobiology 1: 3–14.

Knoll, A. H., K. D. Bergmann, & J. V. Strauss, 2016. Life: The first two billion years. Philosophical Transactions of the Royal Society B: Biological Sciences 371: 1–13.

Lamb, P. D., E. Hunter, J. K. Pinnegar, S. Creer, R. G. Davies, & M. I. Taylor, 2017. Jellyfish on the menu: MtDNA assay reveals scyphozoan predation in the Irish sea. Royal Society Open Science 4:.

Lane, C. E., 1960. The Portuguese Man -of-War. Scientific American 202: 158–171.

Lane, N., 2015. The vital question: energy, evolution, and the origins of complex life. WW Norton & Company.

Lane, N., 2017. Serial endosymbiosis or singular event at the origin of eukaryotes?. Journal of Theoretical Biology Elsevier Ltd 434: 58–67.

Lane, N., & W. F. Martin, 2015. Eukaryotes really are special, and mitochondria are why. Proceedings of the National Academy of Sciences of the United States of America 112: E4823.

Larson, R. J., 1987. Respiration and Carbon Turnover Rates of Medusae from the NE Pacific. Comparative Biochemistry and Physiology Part A: Physiology 87: 93–100, https://www.sciencedirect.com/science/article/pii/0300962987904300.

Lebrato, M., M. Pahlow, J. R. Frost, M. Küter, P. de Jesus Mendes, J. C. Molinero, & A. Oschlies, 2019. Sinking of Gelatinous Zooplankton Biomass Increases Deep Carbon Transfer Efficiency Globally. Global Biogeochemical Cycles 33: 1764–1783.

Lilley, M. K. S., & F. Lombard, 2015. Respiration of fragile planktonic zooplankton: Extending the

possibilities with a single method. Journal of Experimental Marine Biology and Ecology 471: 226–231.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, & R. J. Randall, 1951. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry 193: 265–275.

Lucas, C. H., 2001. Reproduction and life history strategies of the common jellyfish, Aurelia aurita, in relation to its ambient environment. Hydrobiologia 451: 229–246.

Lucas, C. H., W. M. Graham, & C. Widmer, 2012. Jellyfish Life Histories: Role of Polyps in Forming and Maintaining Scyphomedusa Populations Advances in Marine Biology. Elsevier Ltd.: 133–196, http://dx.doi.org/10.1016/B978-0-12-394282-1.00003-X.

Lynam, C. P., M. J. Gibbons, B. E. Axelsen, C. A. J. Sparks, J. Coetzee, B. G. Heywood, & A. S. Brierley, 2007. Jellyfish overtake fish in a heavily fished ecosystem. Current Biology 16: R492.

Macali, A., & E. Bergami, 2020. Jellyfish as innovative bioindicator for plastic pollution. Ecological Indicators 115: 1–8.

Mackie, G. O., 1974. Locomotion, flotation, and dispersal Coelenterate Biology: Reviews and New Perspectives. Academic Press New York: 313–357.

Mackie, G. O., & D. A. Boag, 1963. Fishing, feeding and digestion in siphonophores. Pubbl statz zool Napoli 33: 178–196.

Mackie, G. O., P. R. Pugh, & J. E. Purcell, 1988. Siphonophore Biology. Advances in Marine Biology 24: 97–262.

Maldonado, F., T. T. Packard, & M. Gómez, 2012. Understanding tetrazolium reduction and the importance of substrates in measuring respiratory electron transport activity. Journal of Experimental Marine Biology and Ecology 434–435: 110–118.

Mansueti, R., 1963. Symbiotic behavior between small fishes and jellyfishes, with new data on that between the stromateid, *Peprilus alepidotus*, and the scyphomedusa, *Chrysaora quinquecirrha*. Copeia JSTOR 40–80.

Margulis, L., & C. Sagan, 1995. Microcosmo. , 1–256.

Mariottini, G. L., E. Giacco, & L. Pane, 2008. The mauve stinger *Pelagia noctiluca* (Forsskål, 1775). Distribution, ecology, toxicity and epidemiology of stings. A review. Marine Drugs 6: 496–513.

Marques, R., C. Bouvier, A. M. Darnaude, J. C. Molinero, C. Przybyla, S. Soriano, J. A. Tomasini, & D. Bonnet, 2016. Jellyfish as an alternative source of food for opportunistic fishes. Journal of Experimental Marine Biology and Ecology Elsevier B.V. 485: 1–7, http://dx.doi.org/10.1016/j.jembe.2016.08.008.

Martijn, J., J. Vosseberg, L. Guy, P. Offre, & T. J. G. Ettema, 2018. Deep mitochondrial origin outside the sampled alphaproteobacteria. Nature 557: 101–105.

Mauchline, J., 1980. The biology of mysids and euphausiids. Advances in Marine Biology 18: 1–680.

Mayzaud, P., M. Boutoute, S. Gasparini, L. Mousseau, & D. Lefevre, 2005. Respiration in marine zooplankton - The other side of the coin: CO_2 production. Limnology and Oceanography 50: 291–298.

Milisenda, G., S. Rosa, V. L. Fuentes, F. Boero, L. Guglielmo, J. E. Purcell, & S. Piraino, 2014. Jellyfish as prey: Frequency of predation and selective foraging of *Boops boops* (vertebrata, actinopterygii) on the mauve stinger *Pelagia noctiluca* (cnidaria, scyphozoa). PLoS ONE 9: 1–10.

Milisenda, G., S. Rossi, S. Vizzini, V. L. Fuentes, J. E. Purcell, U. Tilves, & S. Piraino, 2018. Seasonal variability of diet and trophic level of the gelatinous predator *Pelagia noctiluca* (Scyphozoa). Scientific Reports 8: 12140.

Mills, D. B., & D. E. Canfield, 2014. Oxygen and animal evolution: Did a rise of atmospheric oxygen "trigger" the origin of animals? BioEssays 36: 1145–1155.

Mitchell, P., 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 189: 291–292.

Munro, C., Z. Vue, R. R. Behringer, & C. W. Dunn, 2019. Morphology and development of the Portuguese man of war, *Physalia physalis*. Scientific Reports Springer US 9: 1–12, http://dx.doi.org/10.1038/s41598-019-51842-1.

Nachlas, M. M., I. Margulies, & A. M. Seligman, 1960. A Calorimetric Succinic Method for Dehydrogenase the Estimation Activity *. The Journal of Biological Chemistry 235: 499–503.

Nelson, D. L., A. L. Lehninger, & M. M. Cox, 2008. Lehninger principles of biochemistry. Macmillan.

Nutman, A. P., V. C. Bennett, C. R. L. Friend, M. J. Van Kranendonk, & A. R. Chivas, 2016. Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures. Nature Nature Publishing Group 537: 535–538, http://dx.doi.org/10.1038/nature19355.

Ohta, N., M. Sato, K. Ushida, M. Kokubo, T. Baba, K. Taniguchi, M. Urai, K. Kihira, & J. Mochida, 2009. Jellyfish mucin may have potential disease-modifying effects on osteoarthritis. BMC Biotechnology 9: 1–11.

Osma, N., I. Fernández-Urruzola, M. Gómez, S. Montesdeoca-Esponda, & T. T. Packard, 2016. Predicting in vivo oxygen consumption rate from ETS activity and bisubstrate enzyme kinetics in cultured marine zooplankton. Marine Biology 163: 146.

Owens, T. G., & F. D. King, 1975. The measurement of respiratory electron-transport-system activity in marine zooplankton. Marine Biology 30: 27–36.

Packard, T. T., 1971. The Measurement of Respiratory

Electron-transport Activity in Marine Phytoplankton. Journal of Marine Research 29: 235–244.

Packard, T. T., 1985. Measurement of electron transport activity of marine microplankton . In Williams, P. J., & H. W. Jannasch (eds), Advances in Aquatic Microbiology. Academic Press, New York: 207–261.

Packard, T. T., & J. P. Christensen, 2004. Respiration and vertical carbon flux in the Gulf of Maine water column. Journal of Marine Research 62: 93–115.

Packard, T. T., & M. Gómez, 2008. Exploring a first-principles-based model for zooplankton respiration. ICES Journal of Marine Science Oxford University Press 65: 371–378.

Park, E., D. S. Hwang, J. S. Lee, J. I. Song, T. K. Seo, & Y. J. Won, 2012. Estimation of divergence times in cnidarian evolution based on mitochondrial protein-coding genes and the fossil record. Molecular Phylogenetics and Evolution Elsevier Inc. 62: 329–345, http://dx.doi.org/10.1016/j.ympev.2011.10.008.

Parsons, T. R., 1979. Some ecological, experimental and evolutionary aspects of the upwelling ecosystem. South African Journal of Science. 75: 536–540.

Parsons, T. R., & C. M. Lalli, 2002. Jellyfish population explosions : Revisiting a hypothesis of possible causes. Mer. , 111–121.

Pauly, D., W. Graham, S. Libralato, L. Morissette, & M. L. Deng Palomares, 2009. Jellyfish in ecosystems, online databases, and ecosystem models. Hydrobiologia 616: 67–85.

Phillips, N., L. Eagling, C. Harrod, N. Reid, V. Cappanera, & J. Houghton, 2017. Quacks snack on smacks: Mallard ducks (*Anas platyrhynchos*) observed feeding on hydrozoans (*Velella velella*). Plankton and Benthos Research 12: 143–144.

Pierce, J., 2006. Aquarium design for the Portuguese man-of-war *Physalia physalis*. International Zoo Yearbook 40: 221–231.

Pires, R. F. T., N. Cordeiro, J. Dubert, A. Marraccini, P. Relvas, & A. Dos Santos, 2018. Untangling *Velella velella* (Cnidaria: Anthoathecatae) transport: A citizen science and oceanographic approach. Marine Ecology Progress Series 591: 241–251.

Pitt, K. A., C. H. Lucas, R. H. Condon, C. M. Duarte, & B. Stewart-Koster, 2018. Claims That Anthropogenic Stressors Facilitate Jellyfish Blooms Have Been Amplified Beyond the Available Evidence: A Systematic Review. Frontiers in Marine Science 5: 1–11.

Pope, E. C., G. C. Hays, T. M. Thys, T. K. Doyle, D. W. Sims, N. Queiroz, V. J. Hobson, L. Kubicek, & J. D. R. Houghton, 2010. The biology and ecology of the ocean sunfish *Mola mola*: A review of current knowledge and future research perspectives. Reviews in Fish Biology and Fisheries 20: 471–487.

Prieto, L., D. MacÍas, A. Peliz, & J. Ruiz, 2015. Portuguese Man-of-War (*Physalia physalis*) in the Mediterranean: A permanent invasion or a casual appearance? Scientific Reports Nature Publishing Group 5: 1–7.

Purcell, J. E., 1984. Predation on fish larvae by *Physalia physalis*, the Portuguese man of. Marine Ecology Progress Series 19: 189–191.

Purcell, J. E., 2009. Extension of methods for jellyfish and ctenophore trophic ecology to large-scale research. Hydrobiologia 616: 23–50.

Purcell, J. E., & M. N. Arai, 2001. Interactions of pelagic cnidarians and ctenophores with fish: a review. Hydrobiologia 451: 27–44.

Purcell, J. E., E. Clarkin, & T. K. Doyle, 2012. Foods of *Velella velella* (Cnidaria: Hydrozoa) in algal rafts and its distribution in Irish seas. Hydrobiologia 690: 47–55.

Purcell, J. E., V. Fuentes, D. Atienza, U. Tilves, D. Astorga, M. Kawahara, & G. C. Hays, 2010. Use of respiration rates of scyphozoan jellyfish to estimate their effects on the food web. Hydrobiologia 645: 135–152.

Purcell, J. E., A. Malej, & A. Benović, 1999. Potential Links of Jellyfish to Eutrophication and. Coastal and estuarine studies Wiley Online Library 55: 241–263.

Purcell, J. E., S. I. Uye, & W. T. Lo, 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: A review. Marine Ecology Progress Series 350: 153–174.

Ramondenc, S., M. Ferrieux, S. Collet, F. Benedetti, L. Guidi, & F. Lombard, 2019. From egg to maturity: A closed system for complete life cycle studies of the holopelagic jellyfish *Pelagia noctiluca*. Journal of Plankton Research 41: 207–217.

Riascos, J. M., M. Vergara, J. Fajardo, V. Villegas, & A. S. Pacheco, 2012. The role of hyperiid parasites as a trophic link between jellyfish and fishes. Journal of Fish Biology 81: 1686–1695.

Richardson, A. J., A. Bakun, G. C. Hays, & M. J. Gibbons, 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends in Ecology and Evolution 24: 312–322.

Rodríguez, G., G. Clarindo, & L. McKnight, 2015. Jellyfish outbreaks in coastal city beaches from a management perspective. Coastal Cities and their Sustainable Future 1: 277–288.

Roger, A. J., S. A. Muñoz-Gómez, & R. Kamikawa, 2017. The Origin and Diversification of Mitochondria. Current Biology 27: R1177–R1192.

Rokas, A., 2008. The origins of multicellularity and the early history of the genetic toolkit for animal development. Annual Review of Genetics 42: 235–251.

Rorke, O., S. D. Lavery, M. Wang, A. M. Waite, L. E. Beckley, P. A. Thompson, & A. G. Jeffs, 2015. Hitching Rides and Stealing Bites. ICES Journal of

Marine Science 72: 124–127.

Ruiz-Trillo, I., G. Burger, P. W. H. Holland, N. King, B. F. Lang, A. J. Roger, & M. W. Gray, 2007. The origins of multicellularity: a multi-taxon genome initiative. Trends in Genetics 23: 113–118.

Sabatés, A., F. Pagès, D. Atienza, V. Fuentes, J. E. Purcell, & J. M. Gili, 2010. Planktonic cnidarian distribution and feeding of *Pelagia noctiluca* in the NW Mediterranean Sea. Hydrobiologia 645: 153–165.

Sagan, L., 1967. On the origin of mitosing cells. Journal of Theoretical Biology 14: 225–274.

Sánchez-Baracaldo, P., 2015. Origin of marine planktonic cyanobacteria. Scientific Reports Nature Publishing Group 5: 14–17.

Sandrini, L. R., M. Avian, & C. Anatomy, 1983. Biological cycle of *Pelagia noctiluca*: morphological aspects of the development. Comparative and General Pharmacology 174: 169–174.

Schneider, G., 1989. Estimation of food demands of *Aurelia aurita* medusae populations in the kiel bight/western baltic. Ophelia 31: 17–27.

Schneider, G., & G. Behrends, 1998. Top-down control in a neritic plankton system by *Aurelia aurita* medusae — a summary. Ophelia 48: 71–82.

Schuchert, P., 2010. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Capitata Part 2. Revue Suisse de Zoologie 117: 337–555.

Sommer, U., H. Stibor, A. Katechakis, F. Sommer, & T. Hansen, 2002. Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production:primary production. Hydrobiologia 484: 11–20.

Sperling, E. A., C. A. Frieder, A. V. Raman, P. R. Girguis, L. A. Levin, & A. H. Knoll, 2013. Oxygen, ecology, and the Cambrian radiation of animals. Proceedings of the National Academy of Sciences of the United States of America 110: 13446–13451.

Steinberger, L. R., T. Gulakhmedova, Z. Barkay, M. Gozin, & S. Richter, 2019. Jellyfish-Based Plastic. Advanced Sustainable Systems 3: 1–6.

Stenvers, V., X. Chi, & J. Javidpour, 2020. Seasonal variability of the fatty acid composition in *Aurelia aurita* (Cnidaria: Scyphozoa): implications for gelativore food web studies. Journal Of Plankton Research 00: 1–13.

Sukhoputova, A. V., & Y. A. Kraus, 2017. Environmental factors inducing the transformation of polyp into medusae in *Aurelia aurita* (Scyphozoa). Russian Journal of Developmental Biology 48: 106–116.

Sullivan, B. K., C. L. Suchman, & J. H. Costello, 1997. Mechanics of prey selection by ephyrae of the scyphomedusa *Aurelia aurita*. Marine Biology 130: 213–222.

Takao, M., H. Okawachi, S. I. Uye, & H. Okawachi, 2014. Natural predators of polyps of *Aurelia aurita* s.l. (Cnidaria: Scyphozoa: Semaeostomeae) and their predation rates. Plankton and Benthos Research 9: 105–113.

Thiebot, J. B., J. P. Y. Arnould, A. Gómez-Laich, K. Ito, A. Kato, T. Mattern, H. Mitamura, T. Noda, T. Poupart, F. Quintana, T. Raclot, Y. Ropert-Coudert, J. E. Sala, P. J. Seddon, G. J. Sutton, K. Yoda, & A. Takahashi, 2017. Jellyfish and other gelata as food for four penguin species – insights from predator-borne videos. Frontiers in Ecology and the Environment 15: 437–441.

Thiebot, J. B., & J. C. McInnes, 2020. Why do marine endotherms eat gelatinous prey?. ICES Journal of Marine Science 77: 58–71.

Thuesen, E. V., L. D. Rutherford, P. L. Brommer, K. Garrison, M. A. Gutowska, & T. Towanda, 2005. Intragel oxygen promotes hypoxia tolerance of scyphomedusae. Journal of Experimental Biology 208: 2475–2482.

Titelman, J., & L. J. Hansson, 2006. Feeding rates of the jellyfish *Aurelia aurita* on fish larvae. Marine Biology 149: 297–306.

Uye, S., & H. Shimauchi, 2005. Population biomass, feeding, respiration and growth rates, and carbon budget of the scyphomedusa *Aurelia aurita* in the Inland Sea of Japan. Journal of Plankton Research 27: 237–248.

Vagelli, A., 2007. New observations on the asexual reproduction of *Aurelia aurita* (Cnidaria , Scyphozoa) with comments on its life cycle and adaptive significance. Invertebrate Zoology 4: 111–127.

Verde, E. A., & L. R. McCloskey, 1998. Production, respiration, and photophysiology of the mangrove jellyfish *Cassiopea xamachana* symbiotic with zooxanthellae: Effect of jellyfish size and season. Marine Ecology Progress Series 168: 147–162.

Verdes, A., & M. Holford, 2018. Beach to Bench to Bedside: Marine Invertebrate Biochemical Adaptations and Their Applications in Biotechnology and Biomedicine. Results and Problems in Cell Differentiation 65: 359–376.

Verity, P. G., & V. Smetacek, 1996. Organism life cycles, predation, and the structure of marine pelagic ecosystems. Marine Ecology Progress Series 130: 277–293.

Vernon, H. M., 1895. The Respiratory Exchange of the Lower Marine Invertebrates. The Journal of Physiology 19: 18–70.

Vosjan, J. H., 1982. Respiratory Electron Transport System Activities in Marine Environments. Hydrobiological Bulletin 16: 61–68.

Wenner, A., 1977. Food Supply, Feeding Habits, and Egg Production in Pacific Mole Crabs (*Hippa pacifica* Dana). 31: 39–47.

Williams, T. A., P. G. Foster, C. J. Cox, & T. M. Embley, 2013. An archaeal origin of eukaryotes supports only two primary domains of life. Nature

Nature Publishing Group 504: 231–236, http://dx.doi.org/10.1038/nature12779.

Wilson, E. O., 1975. Sociobiology: The new synthesis. Harvard university Press.

Winkler, L. W., 1888. Die Bestimmung des im Wasser gelösten Sauerstoffes, Ber. Dtsch. Chem. Ges., 21, 2843–2854. .

Yahalomi, D., S. D. Atkinson, M. Neuhof, E. Sally Chang, H. Philippe, P. Cartwright, J. L. Bartholomew, & D. Huchon, 2020. A cnidarian parasite of salmon (Myxozoa: *Henneguya*) lacks a mitochondrial genome. Proceedings of the National Academy of Sciences of the United States of America 117: 5358–5363.

Yonge, C. M., 1937. Evolution and Adaptation in the Digestive System of the Metazoa. Biological Reviews 12: 87–114.

Young, G. A., & J. W. Hagadorn, 2010. The fossil record of cnidarian medusae. Palaeoworld 19: 212–221, http://dx.doi.org/10.1016/j.palwor.2010.09.014.

Zamponi, M., 2011. Dispersión de Cnidaria: comunidad marina pleuston y comunidad marina bentos. Revista Real Academia Galega de Ciencias 30: 91–112.

Zeman, S. M., M. Corrales-Ugalde, R. D. Brodeur, & K. R. Sutherland, 2018. Trophic ecology of the neustonic cnidarian *Velella velella* in the northern California Current during an extensive bloom year: insights from gut contents and stable isotope analysis. Marine Biology Springer Berlin Heidelberg 165: 1–13, https://doi.org/10.1007/s00227-018-3404-1.

Zimmer, M., 2009. GFP: from jellyfish to the Nobel prize and beyond. Chemical 38: 2823–2832.

Zou, D., K. Gao, J. Xia, Z. Xu, X. Zhang, & S. Liu, 2007. Responses of dark respiration in the light to desiccation and temperature in the intertidal macroalga, *Ulva lactuca* (Chorophyta) during emersion. Phycologia 46: 363–370.

CHAPTER 2

RESPIRATION: COMPARISON OF THE WINKLER TECHNIQUE, O_2 ELECTRODES, O_2 OPTODES AND THE RESPIRATORY ELECTRON TRANSPORT SYSTEM ASSAY

ABSTRACT

Aerobic respiration is a biological energy transformation process that consumes organic carbon and oxygen. In the ocean, the balance between photosynthesis and respiration is recognized as critical to understanding the ocean's impact on the hydrospheric and atmospheric CO_2. Techniques to determine respiration can be based on inorganic chemistry, electrochemistry, photochemistry, and enzymology. Here, for method comparison, physiological respiration was simultaneously measured by the Winkler method (W), O_2 electrodes (E), and O_2 optodes (O). These techniques detected respiratory O_2 consumption (R), *in situ*, in dark incubation chambers. Respiratory electron transport system (ETS) activity measurements detected potential respiration (Φ), biochemically. *Leptomysis lingvura*, a marine mysid, and *Ulva rigida*, a species of green algal sea-lettuce were the two organisms tested.

Physiological respiration results from each technique were not statistically significantly different (Multiple paired Student's-t tests, p-value > 0.05) and were inside the range of similar published measurements. The application of Passing-Bablok regression analysis evidenced the high correlation between the results and the Bland-Altman analysis examined the average difference ("bias") and limits of agreement between the methods. The mean dry-mass-specific respiration in *L. lingvura* and *U. rigida* was 0.147 ± 0.037 and 0.023 ± 0.008 µmol O_2 h^{-1} (mg Dry Mass)$^{-1}$, n=9, respectively. The R to Φ ratios were different in the two organisms. However, linear regression between R and Φ for *L. lingvura* and *U. rigida* was stronger (r^2 = 0.814 and 0.313) than the linear regression between R and dry biomass (r^2 = 0.643 and 0.213).

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2.1. INTRODUCTION

Respiration is measured for many reasons: investigating physiology, quantifying the carbon cycle, determining primary and secondary production, calculating carbon flux, studying the biological pump, calculating CO_2 emission and O_2 depletion rates, and understanding oceanic metabolic balance, amongst others. Here, the respiration measurements by four different techniques were compared while working with two different types of organisms. The respiration methodologies used were: The Winkler method (W), O_2 electrodes (E), O_2 optodes (O) and the kinetic assay for the respiratory electron transport system (ETS). The first three methods allow the determination of physiological respiration; the last, the determination of its enzymatic basis. The organisms chosen for these experiments were a coastal pericardian crustacean, the mysid, *Leptomysis lingvura* (G.O. Sars, 1866), and a littoral-zone macroalgae, the chlorophyte, *Ulva rigida* (C. Agardh, 1823).

Aerobic respiration is the focus of this research. It is the catabolic process by which eukaryotes and many prokaryotes obtain vital energy using a series of redox reactions that end with the reduction of O_2 as a final electron acceptor. Respiration has been extensively studied, but in the aquatic sciences, photosynthesis had long been considered more important for understanding oceanic metabolism. This assumption began to be challenged at the turn of the millennium when the relative roles of oceanic anabolism, catabolism, and their complex coupling started to be assessed (Dortch & Packard, 1989; Arístegui & Harrison, 2002; Williams & del Giorgio, 2005; Duarte et al., 2013). During aerobic respiration organic matter is oxidized and O_2 is reduced producing carbon dioxide and water. The rate of O_2 consumption in darkness is used as a measure of physiological respiration (R). Darkness ensures that O_2 production by photosynthesis is prevented. During respiration the O_2 consumed by the organism is then used, at a cellular level, as a final electron acceptor in the mitochondrial electron transport system. This produces water from the O_2 reduction and a pH gradient across the mitochondrial inner membrane that will power the generation of the energy-rich molecule, adenosine triphosphate (ATP) (Green, 1964; Nelson et al., 2008).

To measure respiration via O_2 consumption, the organism of interest was incubated in a sealed container which prevented O_2 exchange with the exterior. There should be a decline in the O_2 concentration with time as the organism respires. From among various methods to measure dissolved O_2 three were chosen (Atwood et al., 1977; Friedrich et al., 2013). The first, and the most common, is the chemical detection of O_2 in water, the Winkler Method (Conover, 1960; Carpenter, 1965). This technique is based on the measurement of O_2 at the start and end of an incubation (Marshall et al., 1935). The difference (ΔO_2) is interpreted as the amount of O_2 consumed by the organism in the time interval (Δt) and it is used to calculate a rate of O_2 consumption ($\Delta O_2/\Delta t$). This calculation assumes that respiration was constant throughout the experiment (Omori & Ikeda, 1984).

The second method uses an O_2 electrode to measure O_2 concentration continuously via an electrochemical reaction. The most common electrode is the Clark- type (Kanwisher, 1959) where the anode and cathode are made of silver-silver chloride and platinum respectively. In the presence of O_2 a flux of electrons is transferred from the Ag-AgCl anode to the Pt cathode. In the cathode a small amount of O_2 is reduced in the process. With adequate calibration the electron flux is related to the partial pressure of O_2 and hence, O_2 concentration.

The third is the O_2 optode that measures oxygen continuously without consuming it in the process. It determines O_2 concentration through fluorescence quenching, oxygen's ability to alter fluorescence emitted by certain dyes (oxygen quenchable flourophores). This property was exploited by exciting the fluorophore with emitted light at a precise wave length and recording the resulting fluorescence (Opitz, 1986; Opitz & Lübbers, 1986a, 1986b). Both excitation and emission were accomplished by a fiber optics probe. The timing or wave length of the fluorescence emitted by the fluorophore is altered quantitatively by the O_2 concentration. So with proper calibration the O_2 can be determined continuously without stirring and without consuming O_2 (Klimant et al., 1995; Gatti et al., 2002; Warkentin et al., 2007).

These three techniques normally require prolonged incubations to observe the decrease in O_2. The underlying assumption is that the respiration of an organism inside a container is the same as it is *in situ*. Unfortunately, for all organisms, this assumption is tenuous. Sampling, undoubtably, and likely, entrapment, will cause sufficient stress to alter respiratory behavior. In addition, during the incubation period, starvation will depress the organism's physiology, reducing its respiration (Ikeda et al., 2000). Care must be taken to minimize these factors.

Indirect methods, designed to eliminate the problems of incubation based on the enzymology behind respiration, have been under development since the 1960s (Nachlas et al., 1960; Curl & Sandberg, 1961; Packard, 1971). Instead of observing how dissolved O_2 is removed from the water these methods measure the maximum reaction rate of the enzymes involved in cellular respiration. Here the focus is on the ETS (Packard, 1971; Kenner & Ahmed, 1975a; King & Packard, 1975; Owens & King, 1975; Båmstedt, 1980; Packard & Williams, 1981; Gómez et al., 1996; Maldonado et al., 2012) and its measurement by kinetic analysis (Packard & Christensen, 2004; Osma et al., 2016b).

This ETS kinetic analysis is based on adding the appropriate substrates to activate the enzymes of the ETS and on using an indicator, a redox dye, to observe and quantify the reaction taking place. In the technique designed by Packard (1971) the indicator, INT, is a tetrazolium salt (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride) which acts as an artificial electron acceptor (Smith & McFeters, 1997) replacing oxygen. When INT is reduced, it forms a red formazan dye that, in solution, can be quantified spectrophotometrically via the Beer-Lambert Law. The rate of formazan production can be used to calculate stoichiometrically the rate of O_2 consumption at the cellular level. The result is the theoretical maximum respiration of the organism, the potential respiration (Φ). From Φ, one can calculate the actual respiration using first-principles models based on the internal concentration of the substrates of the enzymes and the enzyme kinetics (Packard et al., 1996; Packard & Gómez, 2008; Aguiar-González et al., 2012; Osma et al., 2016a) or by using a model based on a statistical relationship to respiration (King & Packard, 1975; Packard & Williams, 1981; Hernández-León & Gómez, 1996; Ikeda et al., 2000; Packard & Christensen, 2004; Hernández-León & Ikeda, 2005).

Given that comparison of the direct respiration methods is rarely published, it was decided to make this comparison on a marine crustacean and marine macroalgae. This will improve our understanding, not only of the ways respiration is now measured, but it will also be useful to determine the level of agreement in many oceanographic and marine biological investigations.

2.2. MATERIALS AND METHODS

2.2.1. Sampling and culturing the organisms

Leptomysis lingvura and *Ulva rigida* were sampled, respectively, in October at the northwest and northeast coast of the island of Gran Canaria, Canary Islands, Spain. The mysids were captured at 10 m depth in the subtidal zone via scuba diving with a 500 µm mesh hand-net, while *U. rigida* was obtained by hand picking in a rocky intertidal area during low tide. Later, in the laboratory, both organisms were microscopically analyzed to the species level (Afonso Carrillo & Sansón, 1999; Gómez Cabrera, 2000; Herrera-Ulibarri, 2013; Meland et al., 2015). The mysids were cultured in 35 L glass tanks at 18ºC ± 0.5ºC with a recirculation system based on Lussier et al. (1988) under conditions described by Herrera-Ulibarri (2013). *Artemia* nauplii (48 h old) were used to feed them twice daily and their tanks were cleaned every 2 days according to Herrera-Ulibarri (2013). The mysids were left 7 days to acclimate under these conditions before they were used in experiments.

U. rigida was cultured at 18ºC in 2 L Florence flasks containing 1 L of filtered seawater enriched with 10 µM NO_3^- and PO_4^{-3}. The seawater and nutrients were renewed every morning. The culture had a photoperiod of 12 hours in an incubator chamber where the photosynthetic photon flux density was 100 µmoles m^{-2} s^{-1} from cool white TL-D 36W/840 fluorescent lamps. The conditions were similar to the ones described by Cabello-Pasini et al. (2011). The *U. rigida* samples were cultured for 3 days before using them in an experiment.

2.2.2. Respiration

The Winkler reagents were standardized and the electrodes and the optodes were calibrated before any measurement of physiological respiration. Both *L. lingvura* and *U. rigida* were kept in the dark at 18ºC for an hour to acclimate before the experiment. This acclimatization process was an attempt to minimize stress and its associated increase in O_2 consumption and remnant photosynthesis. Finally, 2ml plastic vials were readied to store the ETS samples at -80ºC.

The three physiological determinations required sealed bottle incubations to measure respiration by detecting the depletion of O_2 (O_2 consumption). Glass bottles of 60 ml were filled with seawater and kept in the dark, at 18ºC ± 0.5ºC with a thermostatic bath and constant magnetic stirring. This was necessary for the proper functioning of the electrodes, but it also precluded stratification which would distort the measurements (Ikeda et al., 2000). This stirring was not aggressive and the stirring-bar was separated from the organism by a plastic net to prevent injury.

2.2.2.1. Winkler method

This method was based on Winkler (1888) method to measure dissolved oxygen in water. The technique described for this study comes from Ikeda et al. (2000) which largely follows Omori and Ikeda (1984). As mentioned previously this was an end-point chemical analysis in contrast to a kinetic (time-course) measurement so 3 water samples were taken at the start to determine the starting oxygen concentration. The filtered seawater inside each 60ml glass incubation bottle was distributed in three 10ml flasks

allowing some overflow. These flasks were the triplicates for the Winkler test. Once the flasks were filled, the reagents were added to fix the oxygen. Then the analysis continued with the addition of acid and immediate titration. In each case, an Eppendorf multipipette®-plus was used to add the titrant solution while stirring the mixture. Starch was added to determine the reaction end-point. The first measurement was discarded as an outlier.

The volume of the titrant solution was used to calculate the dissolved O_2 concentration in the incubation bottle (Ikeda et al., 2000). Also, every day before the Winkler analysis the reagents were standardized and blanks were determined using a KIO_3 solution (0.00167 M).

2.2.2.2. Electrodes

The electrodes used in this study were part of a Strathkelvin 928 6-Channel Oxygen System.

They were sealed in the top part of the 60 ml glass bottle that required stirring to prevent O_2 depletion at the electrode surfaces as a result of the electrochemical reaction at the cathode (Ikeda et al., 2000). The electrodes measured O_2 concentration continuously and simultaneously in four incubation bottles during five to seven hours.

2.2.2.3. Optodes

In this study the optode was a 1-Channel Fibox 4 from Presens with type PSt3 sensors (Detection limit 15ppb, approximately 0.47µM). The sensor consisted of a circular O_2-sensing spot containing the photoluminiscent quenching dye. It had to be stuck inside the incubation bottle to sense the O_2 present in the media. A polymer fiber-optic probe connected to the control-box shined a beam of light, at a precise wave-length, on the optode spot from the outside. The fluorescence of the spot passed back through the same fiber-optic tube to the detector, and was then processed by the apparatus software and converted to an O_2 concentration (Warkentin et al., 2007). The optode was calibrated manually to match the calibration conditions of the electrode using the same two-point calibration solutions. Thus both sensing systems were calibrated simultaneously. This optode system was limited to only one measurement at a time, so it was used to measure O_2 concentration periodically, every 15 minutes, approximately, for five to seven hours, moving the probe from optode spot to optode spot in the different bottles.

2.2.2.4. ETS analysis

The ETS analysis (Packard, 1971; Packard & Christensen, 2004) measures the enzymatic activity of the respiratory electron transport system using INT. The formazan produced from the reduction of the INT has an intense red colour that can be measured according to the Beers-Lambert law. Using a spectrophotometer to measure the absorbance of formazan at 490 nm during 8 minutes, the rate of formation can be determined. To carry out this kinetic analysis the substrate must saturate the ETS enzymes. Accordingly, NADH, succinate and NADPH are added at optimum concentrations. *L. lingvura*'s ETS activity was measured using the reagents described in Owens and King (1975) for zooplankton; while *U. rigida´*s ETS activity was measured using the reagents described in Kenner and Ahmed (1975) for phytoplankton. Both measurements incorporated modifications described in Gómez et al. (1996). The methods differ in the substrates and

the phosphate buffered solutions used to homogenate the samples and to free the enzymes. *L. lingvura* samples were homogenized using sonication with a vibra-cell ultrasonic processor VCX 130 from SONICS. *U. rigida* samples were homogenized with a motor-driven teflon-glass tissue grinder using a disintegrated glass microfibre filter (GF/C Circle, 25 mm) as an abrasive. Each type of sample was homogenized in their respective buffered solutions. Crude homogenates were centrifuged for 10 minutes at 4000 revolutions per minute to keep the enzymes of interest free in the medium and to reduce the turbidity caused by other material. Then the sample enzymes in the supernatant fluid, substrates and INT were mixed in a cuvette in a 1:3:1 proportion that was then used for the spectrophotometric kinetic analysis. The kinetic analyses were made at 18ºC using a Cary100 UV-Vis Spectrophotometer from Agilent Technologies.

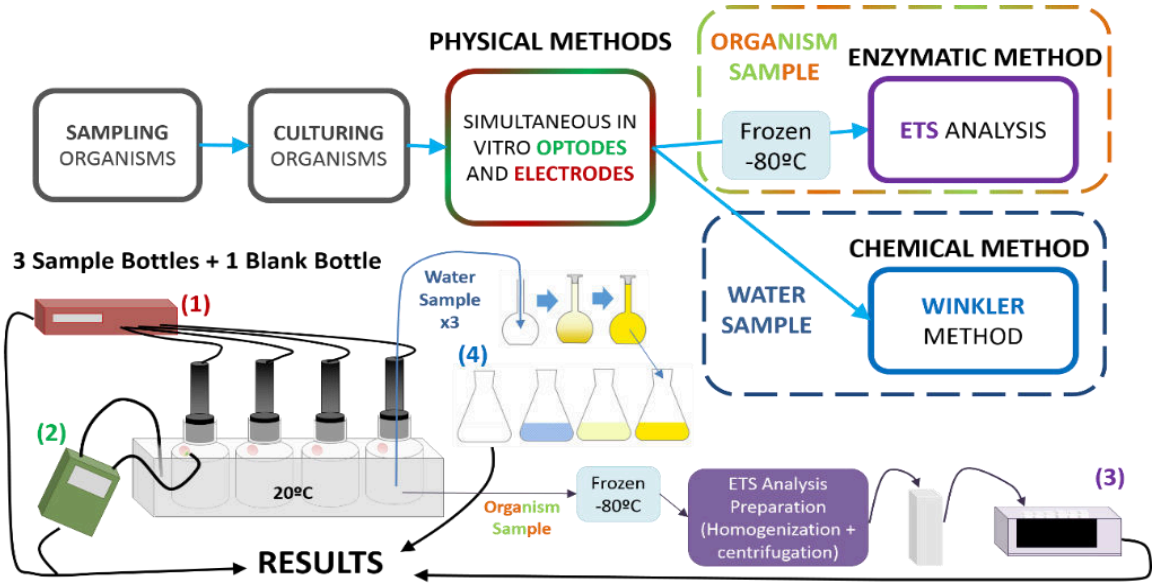


Figure 2.1. Diagram and scheme of the steps taken during the experiment when using multiple methodologies. **(1)** Electrode, **(2)** Optode, **(3)** Spectrophotometer for ETS analysis and protein measurement, **(4)** Winkler determination

2.2.2.5. Combined routine

Fig. 2.1 depicts the different parts of the combined analysis routine. The measurements employing the three physiological methods were made simultaneously and then the organisms were frozen and subsequently analysed for ETS activity.

The Winkler method required a chemical reaction with the water, so it was only used to measure start and end point concentrations. This way the sensor-based measurements monitoring the O_2 in continuum, were not impacted. Both electrodes and optodes measured O_2 concentration simultaneously during the incubation. The respiration measurements were performed in groups of three using three bottles incubating the organism and 1 bottle used as a blank as shown in Fig. 2.1.

This routine continued for 5 to 7 hours. Afterwards the electrode was taken out of the bottle and the water was carefully siphoned into three 10ml flasks until it displaced some of the volume. These water samples were used for the Winkler titration. After fixing the O_2 in these flasks, the organisms left in the bottles were filtered from the remaining water and stored in a labelled 2ml plastic vial at -80ºC for ETS and protein analyses. After

finishing all the physiological respiration measurements, the ETS activity was analysed as described above. The homogenate remaining from the ETS analysis was then used to measure the protein.

In total, respiration was measured by the four methods in 18 samples, 9 *Leptomysis lingvura* samples and 9 *Ulva rigida* samples. The output from the optode and electrodes were already in units of O_2 concentration and time, therefore the rate calculations were easily made. However, some results had to be discarded in order to select an adequate time range to correctly estimate the respiration. Original Winkler results were in sodium thiosulphate volume units which had to be converted to O_2 concentration. These results consisted of one outlier and duplicate initial and end-point measurements.

Physiological O_2 consumption was calculated from the difference between the slope of O_2 depletion inside each incubation bottle and the slope from the control bottle (the blank). Then, this slope, in $\mu M\ h^{-1}$, was multiplied by the volume of each corresponding bottle to obtain the result in $\mu mol\ of\ O_2\ h^{-1}$ (Fig. 2.2A, B and 2.3).

To be useful, ecologically and oceanographically, ETS activity has to be stoichiometrically converted from a formazan-formation rate to an O_2 reduction rate which is the potential respiration (Φ, Owens and King 1975; Packard et al. 1983; Packard and Christensen 2004).

2.2.3. Biomass determination

The biomass, in milligrams of protein, was measured using the protein method described by Lowry et al. (1951) as modified by Rutter (1967). Analyses were made spectrophotometrically on homogenates used for the ETS analysis described above. The results in milligrams of protein were converted to milligrams of dry mass (DM) using the relationships of (Herrera et al., 2011a) for *L. lingvura* (74.19% ± 5.22%) and of Satpati and Pal (2011) for *U. rigida* (6.64%).

2.2.4. Data analysis

Microsoft excel and R software were used for the calculations, conversion and graphic design.

Statistical analyses of the results were performed with R software. To determine if there was a significant difference between the physiological respiration determined by each method, even though the measurements were performed in the same bottle, multiple t-tests for dependent samples (McDonald, 2009) were run. To do this, the normality of the differences between the results was examined (Shapiro & Wilk, 1965; Posten, 1984) as was the correlation between the methods' results. After testing the assumptions of normality and correlation, multiple t-tests for paired data were used to test the significance of the differences between the methods' measurements. In these tests the null hypothesis was that the results from each method were not significantly different.

Since the use of correlation and t-tests for method comparison could be questioned, the results were also compared using Passing-Bablock regression analysis and Bland-Altman analysis (Passing & Bablok, 1983; Bland & Altman, 1986). These new analyses served to compensate for the limitations in the correlation test and paired t-test (Linnet, 1999; Dewitte et al., 2002; Giavarina, 2015).

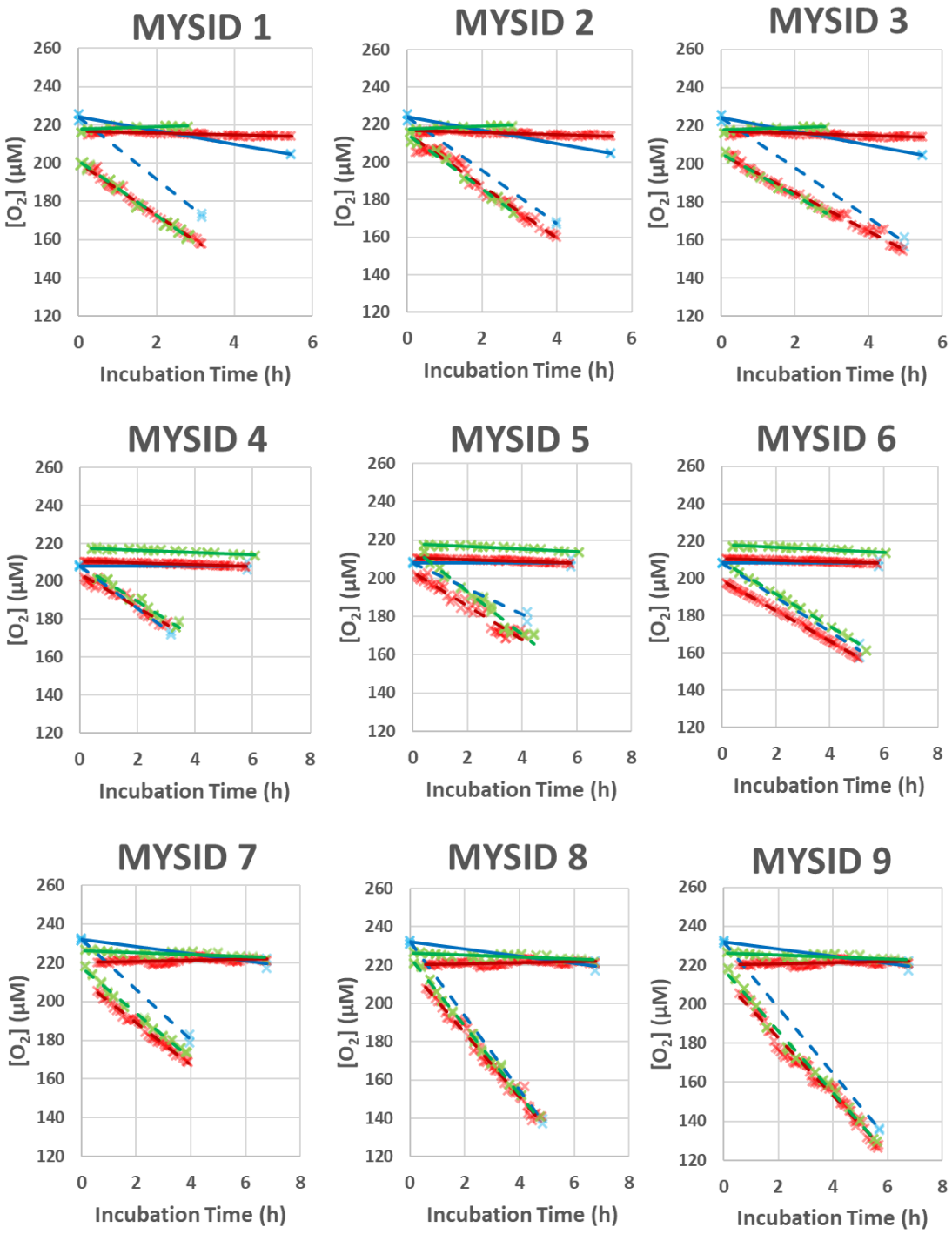


Figure 2.2A. *Leptomysis lingvura* physiological respiration time-courses. Winkler: blue, electrodes: red, optodes: green. Dashed line: Oxygen decrease in sample bottle. Full line: Oxygen decrease in blank bottle.

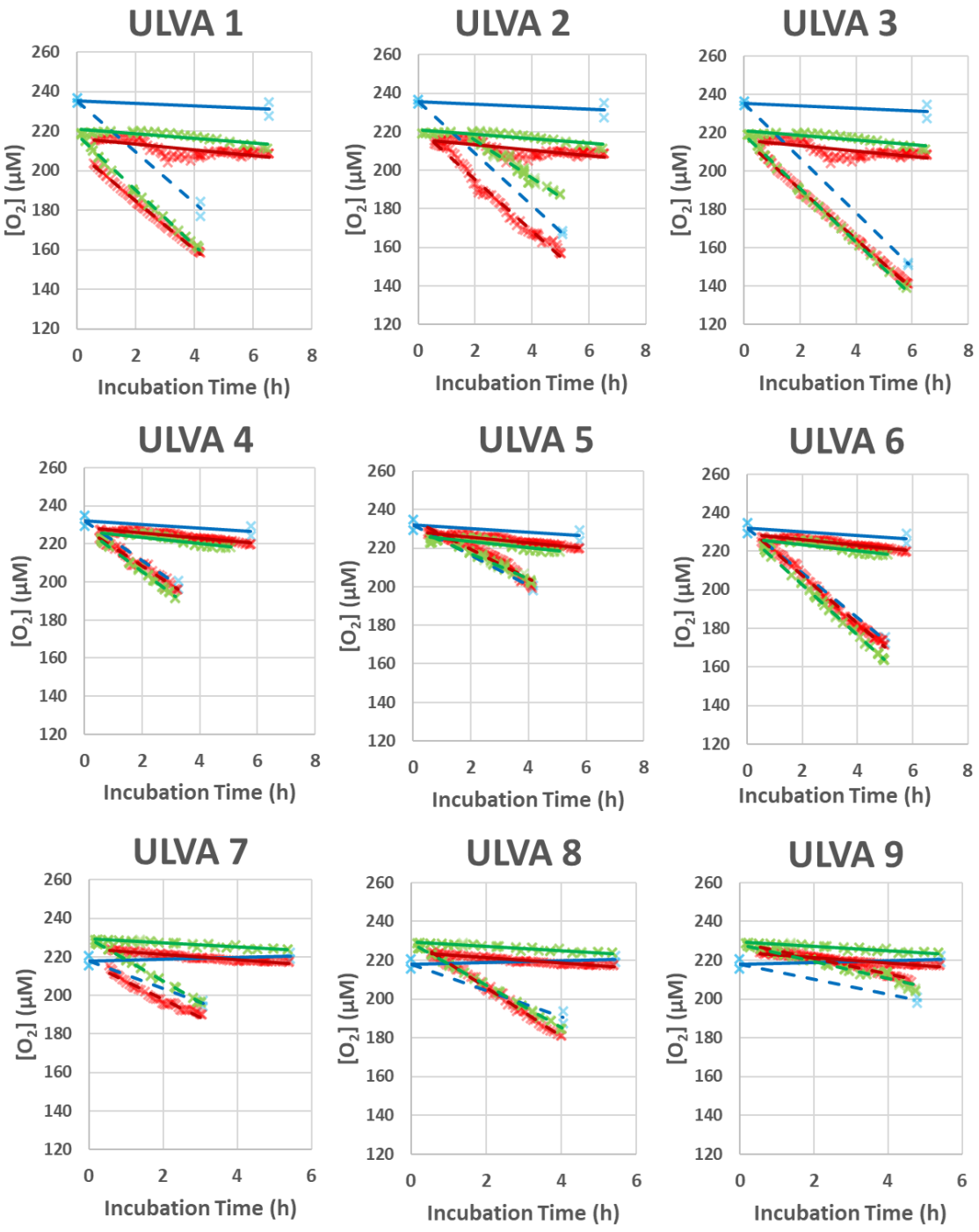


Figure 2.2B. *Ulva rigida* physiological respiration time-courses. Winkler: blue, electrodes: red, optodes: green. Dashed line: Oxygen decrease in sample bottle. Full line: Oxygen decrease in blank bottle.

2.3. RESULTS

2.3.1. Method comparison

Prior to conducting the statistical evaluation described in the data analysis section, the assumption of normally distributed differences was examined. The assumption was satisfied by the estimation of skew and kurtosis levels of the differences between the results (-1.030 and 1.184; 0.155 and -0.102; 1.432 and 2.366; skew and kurtosis of W-E, W-O and E-O respectively which were less than the maximum allowable values for a t-test (i.e. skew < |2.0| and kurtosis < |9.0| (Posten, 1984)). In some cases, the above assumption was satisfied also by the shapiro-wilk test p>0.05 (0.1549, 0.99, 0.019; p-value of W-E, W-O, E-O respectively). It will also be noted that the correlation (r) between the methods' results were 0.875, 0.793, 0.914 for W-E, W-O and E-O respectively, $p < 0.001$, suggesting that the dependent samples t-test was appropriate in this case. The results of the multiple dependent t-tests showed for every comparison that there is not enough evidence to reject the null hypothesis (W-E: $t(17) = 0.399$, $p = 0.695$; W-O: $t(17) = -1.054$, $p = 0.307$; E-O: $t(17) = -2.069$, $p = 0.054$). Thus, the results of the methods do not show significant statistical difference. However, since this paired t-test suffers from certain limitations when comparing methods, Passing –Bablok analysis and Bland-Altman analysis were also applied to the results (Fig. 2.3 and 2.4) (Passing & Bablok, 1983; Bland & Altman, 1986; Linnet, 1999; Dewitte et al., 2002; Giavarina, 2015).

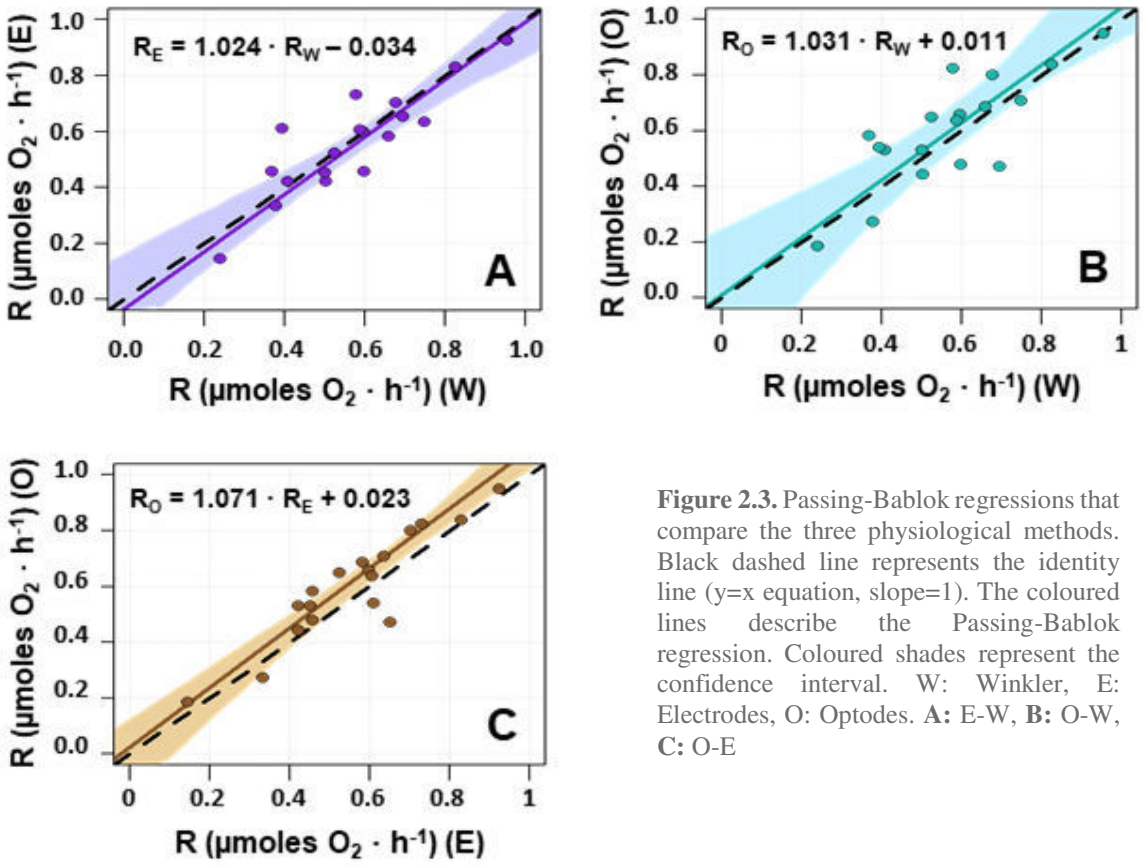


Figure 2.3. Passing-Bablok regressions that compare the three physiological methods. Black dashed line represents the identity line (y=x equation, slope=1). The coloured lines describe the Passing-Bablok regression. Coloured shades represent the confidence interval. W: Winkler, E: Electrodes, O: Optodes. **A:** E-W, **B:** O-W, **C:** O-E

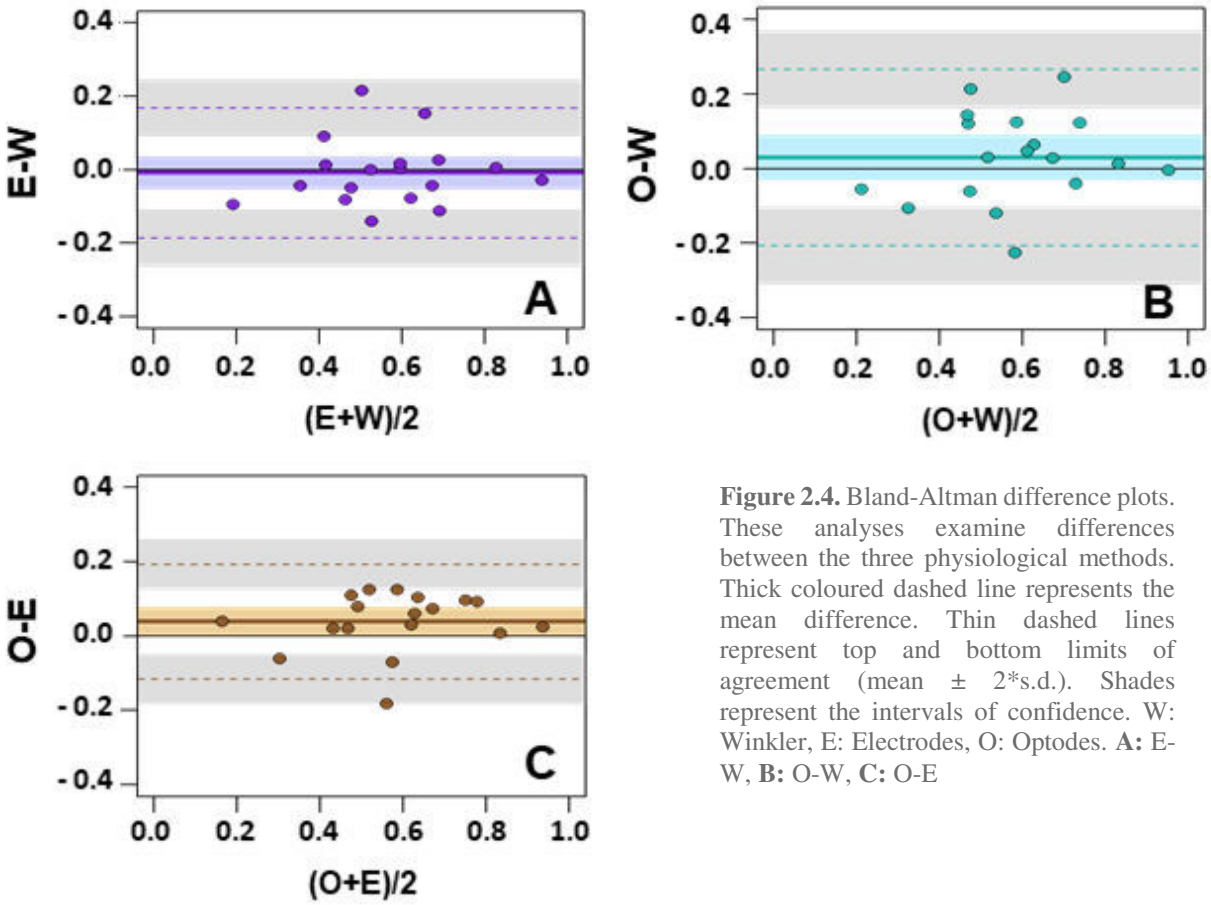


Figure 2.4. Bland-Altman difference plots. These analyses examine differences between the three physiological methods. Thick coloured dashed line represents the mean difference. Thin dashed lines represent top and bottom limits of agreement (mean ± 2*s.d.). Shades represent the intervals of confidence. W: Winkler, E: Electrodes, O: Optodes. **A:** E-W, **B:** O-W, **C:** O-E

2.3.2. Analysis of R, Φ and DM

The regression and correlation (r) between the average R results, in µmoles of O_2 h^{-1}, were analysed from each organism and from each respective Φ and DM (Fig. 2.5). These results were scattered, as evidenced by the low r^2s due to the small size range of the samples. Nevertheless, the correlation between R and Φ was similar to the correlation between R and DM, both in the mysids ($r = 0.902$, $p = 0.002$ and $r = 0.802$, $p = 0.009$) and in the ulva ($r = 0.559$, $p = 0.117$ and $r = 0.462$, $p = 0.211$). The correlation in *L. lingvura* was higher than in *U. rigida* which had lower p-values. This information agrees with the coefficients of determination (r^2). The r^2 between R and Φ and the r^2 between R and biomass were higher in *L. lingvura* than in *U. rigida* results (Fig. 2.5). To view these results in a different light, the R and Φ were normalized by dry mass and plotted in a boxplot, in units of µmoles O_2 h^{-1} (mg DM)$^{-1}$. This way of depicting the variables made it easier to visualize the similarity between R and Φ in *L. lingvura* and the disparity between the two variables in *U. rigida* (Fig. 2.6).

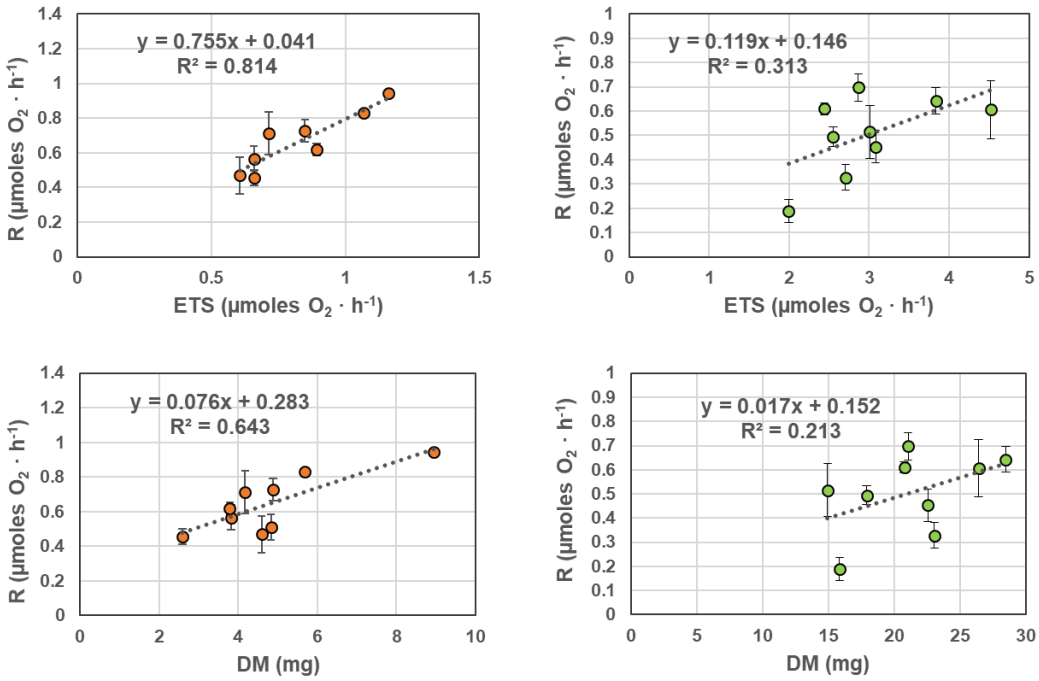


Figure 2.5. Top: Linear regression between ETS and R for *Leptomysis lingvura* (left, orange) and *Ulva rigida* (right, light green). Standard deviation error bars from averaging the 3 methods (n=3). Left graph for *L. lingvura* (n = 8, p = 0.002) and right graph for *U. rigida* (n = 9, p = 0.117). Bottom: Linear regression between DM and average R for *Leptomysis lingvura* (left, orange) and *Ulva rigida* (right, light green). Standard deviation error bars from averaging the 3 methods (n=3). Left graph for *L. lingvura* (n = 8, p = 0.009) and right graph for *U. rigida* (n = 9, p = 0.211).

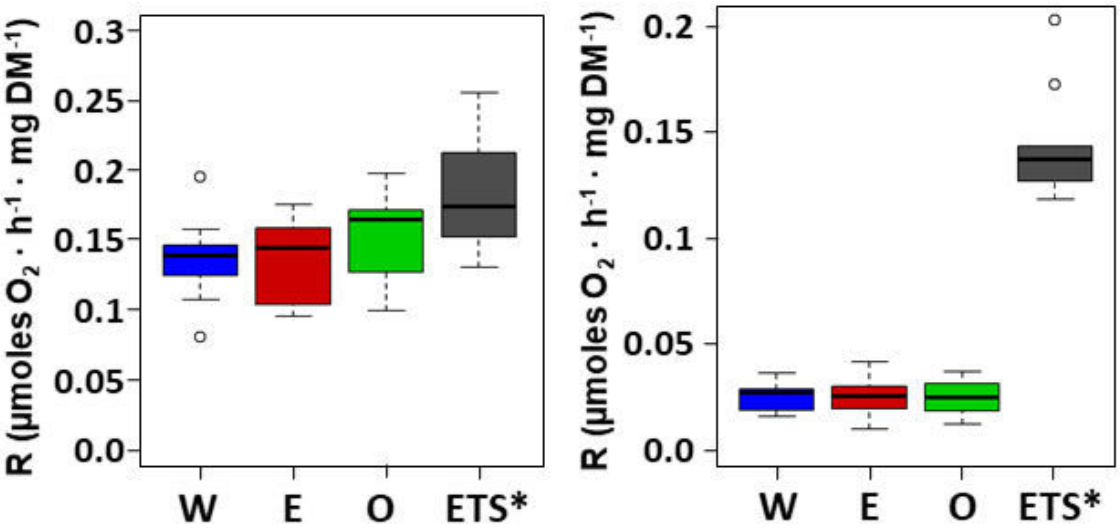


Figure 2.6. Boxplot of the dry mass specific respiration results obtained by the different methods for *L. lingvura* (left), n=9, and *U. rigida* (right), n=9. * ETS results are Potential Respiration.

2.4. DISCUSSION

The DM specific respiration results, here, for *L. lingvura* and *U. rigida,* were examined with comparable respiration measurements in the literature. The mysid respiration values used in the comparison (Fig. 2.6) pertain to coastal species living at a similar temperature to *L. lingvura* because both temperature and depth have an impact on respiration (Ikeda, 2013). The physiological respiration results, presented in µmol of O_2 h^{-1} (mg DM)$^{-1}$ (*L. lingvura*: 0.141 ± 0.029, n=9, *U. rigida*: 0.024 ± 0.008, n=9) (Fig. 2.6), fall within the range of previous measurements by multiple authors (Coastal Mysidae Family members: 0.071 – 0.182 (Ikeda, 2013), Genus Ulva: 0.014 – 0.052 (Cabello-Pasini & Figueroa, 2005; Cabello-Pasini et al., 2011) and support our findings for the three respiration techniques used here (Fig. 2.7).

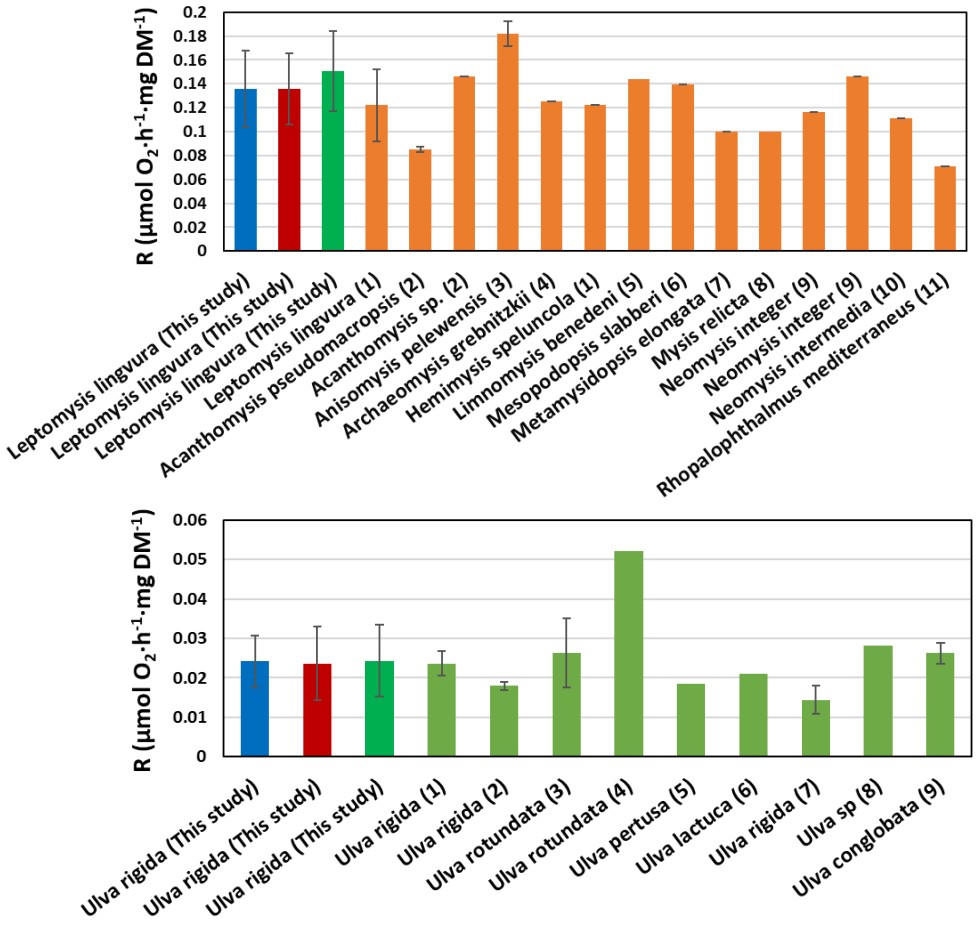


Figure 2.7. Top: Comparative barchart of this study's results of respiration with other coastal mysid species of the Mysidae family in the literature. Most were obtained from Ikeda (2013). **(1):** Gaudy et al. 1980; **(2):** Ikeda 1974; **(3):** Morioka et al. 1987; **(4):** Szalontai et al. 2003; **(5):** Vilas et al. 2006; **(6):** Clutter and Theilacker 1971; **(7):** Weisse and Rudstam 1989; **(8):** Ogonowski et al. 2012; **(9):** Omori 1969. Orange bars: literature results, blue: winkler method, red: electrodes, green: optodes. Bottom: Comparative bar-chart of this study's results of respiration with other studies with Ulva species. **(1):** Cabello-Pasini and Figueroa 2005; **(2):** Cabello-Pasini et al. 2011; **(3):** Figueroa et al. 2003; **(4):** Henley and Ramus 1989; **(5):** Ogata and Takada 1967; **(6):** Newell and Pye 1968; **(7):** Viaroli et al. 2005; **(8):** Zambrano and Carballeira 1999; **(9):** Zou and Gao 2014. Light green bars: literature results, blue: winkler method, red: electrodes, green: optodes.

The physiological results using the three different techniques (Figs. 2.3, 2.4) were not statistically different (Multiple paired Student's-t tests, p-value > 0.05). This was supported by the correlation (r) between the different techniques and by the Passing-Bablok regression (Fig. 2.3). The Bland-Altman analysis yielded a more detailed interpretation of the agreement between the methods. None of the Bland-Altman difference plots (Fig. 2.4) revealed a trend between the differences and the magnitudes of the methods' outputs. This was expected if the methods were comparable. The average differences between the methods (here called "biases") were: between Winkler and electrodes (E-W), -0.008 ± 0.09 (Fig. 2.4A); between Winkler and optodes (O-W), 0.030 ± 0.121 (Fig. 2.4B); and between electrodes and optodes (O-E), 0.039 ± 0.079 (Fig. 2.4C). The limits of agreement, as defined by Bland & Altman (1986), were: for E-W, -0.189 and 0.172 (Fig. 2.4A); for O-W, -0.212 and 0.272 (Fig. 2.4B); and for O-E, -0.119 and 0.197 (Fig. 2.4C). The bias was close to 0, especially when considering the confidence interval (Fig. 2.4). From this analysis one can say that the results from the different methods were not significantly different.

Considering the Winkler measurements, compromising the oxygen during siphoning is a source of error. Oxygen can diffuse into both the original incubation bottle and into the subsample bottle during the siphoning step. In both cases, the apparent O_2 utilization would be reduced. Here, the Winkler measurements did not demonstrate this diffusion effect. With only an initial and an end point, the Winkler method gives less information than the continuous, time-course, readings of the electrodes and optodes. These two methods gave more information about the evolution of O_2 inside the bottle, were better correlated, had the closest limits of agreement, and were easier to use. Between the two, the optodes have the advantage of not consuming O_2 (Klimant et al., 1995; Ikeda et al., 2000; Warkentin et al., 2007; Friedrich et al., 2013; Lilley & Lombard, 2015) (Table 2.1).

Table 2.1. Advantages and disadvantages of this work's methods (W refers to the Winkler method; E, the O_2 electrode; O, the O_2 optode; and ETS, the mitochondrial respiratory electron transport system. ETS does not require incubation nor an oxygen consumption measurement so some characteristics cannot be compared. This table refers to the methods and instrumentation cited in the Methods section. New or different instruments and techniques may have improvements or defects that are not considered here.

METHODS' CHARACTERISTICS COMPARISON				
DISADVANTAGES	**W**	**E**	**O**	**ETS**
Needs Bottle Incubations	✓	✓	✓	×
Needs living samples	✓	✓	✓	×
Requires adapting incubation bottles	×	×	✓	-
Requires killing the sample	×	×	×	✓
Chemical reagent preparation needed for reactions	✓	×	×	✓
Requires siphoning water	✓	×	×	-
The results obtain show potential respiration	×	×	×	✓
Disturbs incubation conditions	✓	✓	×	-
ADVANTAGES	**W**	**E**	**O**	**ETS**
Easy to use in unconventional incubation bottles	×	×	✓	-
Easily portable	×	×	✓	×
Allows autonomous measuring	×	✓	✓	×
Allows continuous measuring of oxygen variation	×	✓	✓	-
Free from electronic sources of error	✓	×	×	×
Easy and fewer calibrations needed	×	×	✓	✓
Higher precision in respiration measurements	×	×	✓	✓
Measurements are not affected by physiological state	×	×	×	✓
The results obtain are actual physiological respiration	✓	✓	✓	×
Simple to use/fewer steps	×	✓	✓	×
Weakly affected by changes in temperature and pressure	×	×	✓	✓
Higher data acquisition rate	×	×	×	✓
Most accurate oxygen measurement	✓	×	×	-
Can measure multiple samples at once	×	✓	×	✓

The three physiological techniques (Fig. 2.1) required *in vitro* (in glass) incubations that enzymatic methods (ETS, etc.,) obviate. As a result, the ETS method permitted samples to be transported and stored at temperatures below -80°C for later analysis. Thus, all the difficulties associated with keeping organisms healthy as well as minimizing "bottle effects" were avoided (Table 2.1). This increases the reproducibility of experimental results (Båmstedt, 1980) and allows respiration to be assessed synoptically for oceanographic sections and mapping (Packard, 1985). However, it assesses the capacity of an organism to respire and from this capacity the actual respiration has to be calculated. At the ecosystem level of organization, this is usually done using an empirically determined calibration factor ((R:Φ = 0.26), Packard & Codispoti 2007) or using an enzyme-kinetics based model (Osma et al., 2016b). Here, at the species level of biological organization, the factor (R:Φ), differed remarkably between *L. lingvura* (0.81) and *U. rigida* (0.17) The r (0.902, p = 0.002) and r^2 (0.814, p = 0.002) between R and Φ for *L. lingvura* (Fig. 2.5) were similar to the r , described in the results section, between the other physiological methods (0.793-0.914, p < 0.001); showcasing equivalent correlation between the enzymological technique and the other methods.

In any case, the r^2 between the R and Φ (0.814) was higher than the r^2 between R and DM (0.643) (Fig. 2.5) (Martínez et al., 2010).

Further analysis of Φ in the mysids, revealed that, as to be expected, it was significantly higher than the average R (Multiple Paired Student's t-test, p-value > 0.05) (Fig. 4). The ratio between R and Φ (R:Φ) averaged 0.807 ± 0.099, n=8, indicating that the mysids were using 81% of their respiratory capacity. If one equates the R:Φ ratio with the $v:V_{max}$ ratio, in Michaelis-Menten parlance, a value of around 0.5 is to be expected (Cleland, 1967; Nelson et al., 2008) because that ratio positions an organism's metabolism at an optimum efficiency for regulating its respiration rate. It is the position dictated by the Michaelis-Menten constant (k_m), i.e., $v = ½ V_{max}$. Here, the incubation conditions could have stimulated the R of the mysids since more than one individual was inside the incubation bottle and the water around them was stirred to allow proper electrode measurement. This could have elevated R and hence the R:Φ ratio. Other factors impacting this ratio are the nourishment and physiological states of the organisms (Hernández-León & Gómez, 1996; Packard et al., 1996; Herrera et al., 2011b; Osma et al., 2016a, 2016b).

In general, ETS activity is more stable than respiration and not susceptible to short-term environmental changes caused by bottle effects, stress or underfeeding (Båmstedt, 1980; Savenkoff et al., 1995; Hernández-León & Gómez, 1996; Hernández-León & Ikeda, 2005; Herrera et al., 2011b; Osma et al., 2016a). As mentioned above, this potential respiration can be used in an enzyme-kinetic model to calculate the physiological respiration if adequate substrate measurements in the sample can be made (Packard et al., 1996; Packard & Gómez, 2008; Aguiar-González et al., 2012; Osma et al., 2016b, 2016c).

Here, the relationship between R and Φ was very different for *U. rigida*. The Φ was an order of magnitude higher than the physiological respiration. This led to a particularly low R:Φ ratio (0.169 ± 0.053, n=9), similar to the ratio described before for phytoplankton and other macroalgae (Kenner & Ahmed, 1975b; Peñuelas et al., 1988; Mazej & Gaberščik, 1999). Considering the numerous enzymatic redox reactions that occur uniquely in autotroph cells, it is possible that there are reactions in algae that undesirably increase formazan formation. This could cause an overestimation of Φ in phytoplankton and macro-algae. One possible cause of an over-production of formazan is the variable presence of mitochondria NAD(P)H dehydrogenase (Møller, 2001). Also,

chloroplastic chlororespiration, because it uses similar electron transport enzymes could also be a factor (Goedheer, 1963; Peñuelas et al., 1988; Guedeney et al., 1996; Burrows et al., 1998; Peltier & Cournac, 2002; Beardall et al., 2003; Kuntz, 2004; Raven & Beardall, 2005; Rumeau et al., 2007). It would be fruitful to study these metabolic processes using INT and other types of tetrazolium reduction.

2.5. CONCLUSIONS

Respiration (R) results obtained from the Winkler, the electrode and the optode methods were in agreement and fell into the range expected from the literature (Fig. 2.6). This confirms that respiratory measurements made with any of these methods are comparable. Furthermore, the limits of agreement between the techniques are fairly high (Fig. 2.4) and demand more comparisons with other instruments and methodologies to determine the acceptable agreement.

The potential respiration (Φ) from the enzyme analysis was expectedly higher than the R and better correlated with R than either measurement with dry biomass.

The R:Φ ratio for *L. lingvura* was 0.807 ± 0.099, n=8, and for *U. rigida,* 0.169 ± 0.052, n=9. This was expected from the literature, but the difference begs investigation.

REFERENCES

Afonso Carrillo, J., & M. Sansón, 1999. Algas, hongos y fanérogamas marinas de las Islas Canarias. Servicio de Publicaciones, Universidad de La Laguna.

Aguiar-González, B., T. T. Packard, E. Berdalet, S. Roy, & M. Gómez, 2012. Respiration predicted from an enzyme kinetic model and the metabolic theory of ecology in two species of marine bacteria. Journal of Experimental Marine Biology and Ecology Elsevier 412: 1–12.

Arístegui, J., & W. G. Harrison, 2002. Decoupling of primary production and community respiration in the ocean: Implications for regional carbon studies. Aquatic Microbial Ecology 29: 199–209.

Atwood, D. K., W. Frank Kinard, M. J. Barcelona, & E. C. Johnson, 1977. Comparison of polarographic electrode and winkler titration determinations of dissolved oxygen in oceanographic samples. Deep Sea Research Pergamon Press Ltd. 24: 311–313, http://dx.doi.org/10.1016/0146-6291(77)90000-5.

Båmstedt, U., 1980. ETS activity as an estimator of respiratory rate of zooplankton populations. The significance of variations in environmental factors. Journal of Experimental Marine Biology and Ecology 42: 267–283.

Beardall, J., A. Quigg, & J. A. Raven, 2003. Oxygen consumption: photorespiration and chlororespiration In Larkum, A., S. Douglas, & J. A. Raven (eds), Photosynthesis in algae. Springer: 157–181.

Bland, J. M., & D. Altman, 1986. Statistical methods for assessing agreement between two methods of clinical measurement. The lancet Elsevier 327: 307–310.

Burrows, P. A., L. A. Sazanov, Z. Svab, P. Maliga, & P. J. Nixon, 1998. Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid ndh genes. The EMBO Journal John Wiley & Sons, Ltd 17: 868–876.

Cabello-Pasini, A., & F. L. Figueroa, 2005. Effect of nitrate concentration on the relationship between photosynthetic oxygen evolution and electron transport rate in Ulva rigida (Chlorophyta). Journal of Phycology 41: 1169–1177.

Cabello-Pasini, A., V. Macías-Carranza, R. Abdala, N. Korbee, & F. L. Figueroa, 2011. Effect of nitrate concentration and UVR on photosynthesis, respiration, nitrate reductase activity, and phenolic compounds in Ulva rigida (Chlorophyta). Journal of Applied Phycology 23: 363–369.

Carpenter, J. H., 1965. the Accuracy of the Winkler Method for Dissolved Oxygen Analysis. Limnology and Oceanography 10: 135–140.

Cleland, W. W., 1967. Enzyme kinetics. Annual review of biochemistry 36: 77–112.

Conover, R. J., 1960. The feeding behavior and respiration of some marine planktonic Crustacea. The Biological Bulletin 119: 399–415.

Curl, H., & J. Sandberg, 1961. The measurement of dehydrogenase activity in marine organisms. Journal of Marine Research 19: 123–138.

Dewitte, K., C. Fierens, D. Stöckl, & L. M. Thienpont, 2002. Application of the Bland-Altman plot for interpretation of method-comparison studies: A critical investigation of its practice. Clinical Chemistry 48: 799–801.

Dortch, Q., & T. T. Packard, 1989. Differences in biomass structure between oligotrophic and eutrophic marine ecosystems. Deep Sea Research Part A. Oceanographic Research Papers Elsevier 36: 223–240.

Duarte, C. M., A. Regaudie-de-Gioux, J. M. Arrieta, A. Delgado-Huertas, & S. Agustí, 2013. The oligotrophic ocean is heterotrophic. Annual Review of Marine Science 5: 551–569.

Friedrich, J., A. Längin, & K. Kümmerer, 2013. Comparison of an Electrochemical and Luminescence-Based Oxygen Measuring System for Use in the Biodegradability Testing According to Closed Bottle Test (OECD 301D). Clean - Soil, Air, Water 41: 251–257.

Gatti, S., T. Brey, W. E. G. Müller, O. Heilmayer, & G. Holst, 2002. Oxygen microoptodes: A new tool for oxygen measurements in aquatic animal ecology. Marine Biology 140: 1075–1085.

Giavarina, D., 2015. Understanding Bland Altman Analysis. Biochemia Medica 25: 141–151.

Goedheer, J. C., 1963. A cooperation of two pigment systems and respiration in photosynthetic luminescence. Biochimica et Biophysica acta Elsevier 66: 61–71.

Gómez Cabrera, M. M., 2000. Manual de prácticas de Zoología Marina. Universidad de Las Palmas de Gran Canaria, Servicio de Publicaciones y Producción Documental. Departamento de Biología, 155p .

Gómez, M., S. Torres, & S. Hernzández-León, 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. South African Journal of Marine Science 17: 15–20.

Green, D. E., 1964. The Mitochondrion. Scientific American 210: 67–74.

Guedeney, G., S. Corneille, S. Cuiné, & G. Peltier, 1996. Evidence for an association of ndh B, ndh J gene products and ferredoxin-NADP-reductase as components of a chloroplastic NAD(P)H dehydrogenase complex. FEBS letters Wiley Online Library 378: 277–280.

Hernández-León, S., & M. Gómez, 1996. Factors affecting the respiration/ETS ratio in marine zooplankton. Journal of Plankton Research 18: 239–255.

Hernández-León, S., & T. Ikeda, 2005. A global assessment of mesozooplankton respiration in the ocean. Journal of Plankton Research 27: 153–158.

Herrera-Ulibarri, A., 2013. Identification, Abundance and Rearing of Mysids of Gran Canaria: aplication to laboratory and oceanographic respiratory metabolism studies. Universidad de Las Palmas de Gran Canaria.

Herrera, A., M. Gómez, L. Molina, F. Otero, & T. Packard, 2011a. Rearing techniques and nutritional quality of two mysids from Gran Canaria (Spain). Aquaculture Research 42: 677–683.

Herrera, A., T. Packard, A. Santana, & M. Gómez, 2011b. Effect of starvation and feeding on respiratory metabolism in *Leptomysis lingvura* (G.O. Sars, 1866). Journal of Experimental Marine Biology and Ecology Elsevier B.V. 409: 154–159, http://dx.doi.org/10.1016/j.jembe.2011.08.016.

Ikeda, T., 2013. Synthesis towards a global-bathymetric model of metabolism and chemical composition of mysid crustaceans. Journal of Experimental Marine Biology and Ecology Elsevier B.V. 445: 79–87, http://dx.doi.org/10.1016/j.jembe.2013.04.001.

Ikeda, T., J. J. Torres, S. Hernández-León, & S. P. Geiger, 2000. Metabolism. In 'ICES Zooplankton Methodology Manual'.(Eds ME Harris, RP Wiebe, PH Lenz, J. Skjoldal and HR Huntley.) pp. 455–532. Academic Press: London.

Kanwisher, J., 1959. Oxygen Electrode. Limnology and Oceanography 4: 210–217.

Kenner, R. A., & S. I. Ahmed, 1975a. Measurements of electron transport activities in marine phytoplankton. Marine Biology 33: 119–127.

Kenner, R. A., & S. I. Ahmed, 1975b. Correlation between oxygen utilization and electron transport activity in marine phytoplankton. Marine biology Springer 33: 129–133.

King, F. D., & T. T. Packard, 1975. Respiration and the activity of the respiratory electron transport system in marine zooplankton. Limnology and Oceanography 20: 849–854.

Klimant, I., V. Meyer, & M. Kühl, 1995. Fiber-optic oxygen microsensors, a new tool in aquatic biology. Limnology and Oceanography 40: 1159–1165.

Kuntz, M., 2004. Plastid terminal oxidase and its biological significance. Planta Springer 218: 896–899.

Lilley, M. K. S., & F. Lombard, 2015. Respiration of fragile planktonic zooplankton: Extending the possibilities with a single method. Journal of Experimental Marine Biology and Ecology 471: 226–231.

Linnet, K., 1999. Limitations of the paired t-test for evaluation of method comparison data. Clinical Chemistry 45: 314–315.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, & R. J. Randall, 1951. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry 193: 265–275.

Lussier, S. M., A. Kuhn, M. J. Chammas, & J. Sewall, 1988. Techniques for the laboratory culture of *Mysidopsis* species (Crustacea: Mysidacea). Environmental Toxicology and Chemistry: An International Journal Wiley Online Library 7: 969–977.

Maldonado, F., T. T. Packard, & M. Gómez, 2012. Understanding tetrazolium reduction and the importance of substrates in measuring respiratory electron transport activity. Journal of Experimental Marine Biology and Ecology 434–435: 110–118.

Marshall, S. M., A. G. Nicholls, & A. P. Orr, 1935. On the Biology of *Calanus finmarchicus*. Part VI. Oxygen Consumption in Relation to Environmental Conditions. Journal of the Marine Biological Association of the United Kingdom 20: 1–27.

Martínez, I., M. Gómez, & T. T. Packard, 2010. Potential respiration is a better respiratory predictor than biomass in young *Artemia salina*. Journal of Experimental Marine Biology and Ecology Elsevier B.V. 390: 78–83, http://dx.doi.org/10.1016/j.jembe.2010.05.011.

Mazej, Z., & A. Gaberščik, 1999. ETS-activity as a measure of vitality of different macrophyte species. Phyton - Annales Rei Botanicae 39: 181–185.

McDonald, J. H., 2009. Handbook of biological statistics. sparky house publishing Baltimore, MD.

Meland, K., J. Mees, M. Porter, & K. J. Wittmann, 2015. Taxonomic review of the orders Mysida and Stygiomysida (Crustacea, Peracarida). PLoS ONES 10: e0124656.

Møller, I. M., 2001. Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annual Review of Plant Biology 52: 561–591.

Nachlas, M. M., I. Margulies, & A. M. Seligman, 1960. A Calorimetric Succinic Method for Dehydrogenase the Estimation Activity *. The Journal of Biological Chemistry 235: 499–503.

Nelson, D. L., A. L. Lehninger, & M. M. Cox, 2008. Lehninger principles of biochemistry. Macmillan.

Omori, M., & T. Ikeda, 1984. Methods in marine zooplankton ecology. John-Wiely and Sone. Inc. New York, .

Opitz, N., 1986. O_2 Optodes for analyzing micro blood samples using thin sensor layers with small O_2 capacities and special reflection properties for optical decoupling of sensor and sample Oxygen Transport to Tissue VIII. Springer: 137–142.

Opitz, N., & D. W. Lübbers, 1986a. Theory and Development of Fluorescence-Based Optochemical Oxygen Sensors Oxygen Optodes.pdf. International anesthesiology clinics 25: 177–197.

Opitz, N., & D. W. Lübbers, 1986b. Arrangement for

measuring diffusing particles. Google Patents.

Osma, N., M. Aristizabal, I. Fernández-Urruzola, T. T. Packard, & M. Gómez, 2016a. Influence of Starvation on Respiratory Metabolism and Pyridine Nucleotide Levels in the Marine Dinoflagellate *Oxyrrhis marina*. Protist Elsevier GmbH. 167: 136–147.

Osma, N., I. Fernández-Urruzola, M. Gómez, S. Montesdeoca-Esponda, & T. T. Packard, 2016b. Predicting in vivo oxygen consumption rate from ETS activity and bisubstrate enzyme kinetics in cultured marine zooplankton. Marine Biology 163: 146.

Osma, N., F. Maldonado, I. Fernández-Urruzola, T. T. Packard, & M. Gómez, 2016c. Variability of respiration and pyridine nucleotides concentration in oceanic zooplankton. Journal of Plankton Research Oxford University Press 38: 537–550.

Owens, T. G., & F. D. King, 1975. The measurement of respiratory electron-transport-system activity in marine zooplankton. Marine Biology 30: 27–36.

Packard, T., E. Berdalet, D. Blasco, S. O. Roy, L. St-Amand, B. Lagacé, K. Lee, & J. P. Gagné, 1996. CO_2 production predicted from isocitrate dehydrogenase activity and bisubstrate enzyme kinetics in the marine bacterium Pseudomonas nautica. Aquatic Microbial Ecology 11: 11–19.

Packard, T. T., 1971. The Measurement of Respiratory Electron-transport Activity in Marine Phytoplankton. Journal of Marine Research 29: 235–244.

Packard, T. T., 1985. Measurement of electron transport activity of marine microplankton . In Williams, P. J., & H. W. Jannasch (eds), Advances in Aquatic Microbiology. Academic Press, New York: 207–261.

Packard, T. T., & J. P. Christensen, 2004. Respiration and vertical carbon flux in the Gulf of Maine water column. Journal of Marine Research 62: 93–115.

Packard, T. T., & L. A. Codispoti, 2007. Respiration, mineralization, and biochemical properties of the particulate matter in the southern Nansen Basin water column in April 1981. Deep Sea Research Part I: Oceanographic Research Papers Elsevier 54: 403–414.

Packard, T. T., P. C. Garfield, & L. A. Codispoti, 1983. Oxygen consumption and denitrification below the Peruvian upwelling Coastal Upwelling Its Sediment Record. Springer: 147–173.

Packard, T. T., & M. Gómez, 2008. Exploring a first-principles-based model for zooplankton respiration. ICES Journal of Marine Science Oxford University Press 65: 371–378.

Packard, T. T., & P. J. Williams, 1981. Rates of respiratory oxygen consumption and electron transport in surface seawater from the northwest Atlantic. Oceanologica Acta 4: 351–358.

Passing, H., & W. Bablok, 1983. A New Biometrical Procedure for Testing the Equality of Measurements from Two Different Analytical Methods. Application of linear regression procedures for method comparison studies in Clinical Chemistry, Part I. Clinical Chemistry and Laboratory Medicine 21: 709–720.

Peltier, G., & L. Cournac, 2002. Chlororespiration. Annual review of plant biology 53: 523–550.

Peñuelas, J., J. Murillo, & J. Azcón-Bieto, 1988. Actual and potential dark respiration rates and different electron transport pathways in freshwater aquatic plants. Aquatic botany Elsevier 30: 353–362.

Posten, H. O., 1984. Robustness of the two-sample t-test Robustness of statistical methods and nonparametric statistics. Springer: 92–99.

Raven, J. A., & J. Beardall, 2005. Respiration in aquatic photolithotrophs In Del Giorgio, P. A., & P. J. Williams (eds), Respiration in aquatic ecosystems. Oxford University Press New York: 36–46.

Rumeau, D., G. Peltier, & L. Cournac, 2007. Chlororespiration and cyclic electron flow around PSI during photosynthesis and plant stress response. Plant, cell & environment Wiley Online Library 30: 1041–1051.

Rutter, W. J., 1967. Protein determinations in embryos In Wilt, F. H., & N. K. Wessels (eds), Methods in developmental biology. New York: 671–683.

Satpati, G. G., & R. Pal, 2011. Biochemical composition and lipid characterization of marine green alga *Ulva rigida*-a nutritional approach. J. Algal Biomass Utln. 2: 10–13.

Savenkoff, C., T. T. Packard, M. Rodier, M. Gérino, D. Lefèvre, & M. Denis, 1995. Relative contribution of dehydrogenases to overall respiratory ETS activity in some marine organisms. Journal of Plankton Research 17: 1593–1604.

Shapiro, S. S., & M. B. Wilk, 1965. An analysis of variance test for normality (complete samples). Biometrika JSTOR 52: 591–611.

Smith, J. J., & G. A. McFeters, 1997. Mechanisms of INT (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride), and CTC (5-cyano-2, 3-ditolyl tetrazolium chloride) reduction in *Escherichia coli* K-12. Journal of Microbiological Methods Elsevier 29: 161–175.

Warkentin, M., H. M. Freese, U. Karsten, & R. Schumann, 2007. New and fast method to quantify respiration rates of bacterial and plankton communities in freshwater ecosystems by using optical oxygen sensor spots. Applied and Environmental Microbiology 73: 6722–6729.

Williams, P. J. le B., & P. A. del Giorgio, 2005. Respiration in aquatic ecosystems: history and background. Respiration in aquatic ecosystems Oxford University Press New York 1–17.

Winkler, L. W., 1888. Die Bestimmung des im Wasser gelösten Sauerstoffes, Ber. Dtsch. Chem. Ges., 21, 2843–2854. .

CHAPTER 3

TESTING AND DEVELOPING A KINETIC ETS ASSAY FOR JELLYFISH

ABSTRACT

Before ETS assays could be made on jellyfish, the optimum conditions for the assay had to be determined. This is standard practice with enzyme analysis. We chose to use *Aurelia aurita* as it was representative of the other jellyfish that we planned to study. The optimum substrate concentration was determined from the substrate dependence of the ETS in the classical Michaelis-Menten manner. Both the mathematics of the Michaelis-Menten curve and the pH dependence of the ETS reaction were determined. From the curve describing the pH dependence [ETS = f(pH)] the optimum pH of the assay was identified. The effect of temperature on the ETS activity in these samples was studied following the Arrhenius equation from which the calculation of the activation energy was made. This, facilitated temperature corrections in the experimental work. In addition, the linearity of the enzymatic reaction rate as a function of enzyme concentration and proteinaceous biomass was verified. This was important because the results facilitated comparison of measurements made at different enzyme concentrations and different biomasses. The results were compared with the characteristics of previous ETS assays and ETS measurements. These optimum conditions were then employed for the ETS analyses on jellyfish reported in the ensuing chapters.

3.1. INTRODUCTION

The basis for the ETS analysis has been described in chapter 1 and 2. This technique is based on the one described in Packard (1971) and Packard et al. (1971) and modified after by Owens & King (1975), Gómez et al. (1996), Packard & Christensen (2004) and Osma et al. (2016a,b). This type of enzymatic analysis using jellyfish extract have previously been used by King & Packard (1975), Thuesen & Childress (1994) and Iguchi et al. (2017) where only the first and last one used this ETS type analysis. These works showcase the usefulness of these type of techniques which Purcell et al. (2010) argues that they are a great alternative to classic respiration methodology. They obviate some of the logistical problems of measuring respiration using incubations with large fragile jellyfish. However, none of the previous applications of the ETS assay replicated the enzymological tests performed by Owens & King (1975). Therefore, it was considered important to verify the assay conditions established by Owens & King (1975) before applying the technique to jellyfish tissue.

The conditions tested were based on a series of tests described in (Bisswanger, 2014)'s recommendations for the development of enzymatic assays. As this is an assay that has been extensively studied in other organisms we focused on establishing optimal conditions of substrate concentration, pH y temperature in jellyfish.

3.2. MATERIALS AND METHODS

3.2.1. The effect of substrate concentration

To determine the optimal concentration of the substrate we had to measure the activity at different concentrations of NADH and NADPH (Brooks et al., 2004). For this purpose, a big sample of jellyfish was homogenized to obtain a large volume of homogenate. Several aliquots of this homogenate were centrifuged into 2ml vials for further analysis. The supernatant liquid from the centrifuged vials was mixed to obtain exact replicas of the supernatant. This supernatant was then mixed in several cuvettes with different NADH concentrations ranging from 0.003 to 1.8 mM. This procedure was then repeated with NADPH.

These results were mathematically analysed using the linearization models of Lineweaver-Burk, Eadie-Hofstee and Hanes-Woolf (Haldane & Stern, 1932; Hanes, 1932; Lineweaver & Burk, 1934; Hofstee, 1952). The Hanes-Woolf model showed the best results (Fig. 3.1) so it was used to determine the Michaelis-Menton constant (K_m) and Maximum velocity (V_{max}) (Segel, 1976) using the linearized Hanes-Woolf equation (Eqn. 3.1):

$$\frac{[S]}{v} = \frac{1}{v_{max}} \cdot [S] + \frac{K_m}{v_{max}} \qquad (3.1),$$

where we have the substrate concentrations ([S]) and the reaction rate (v) of the experiment at different substrate concentrations. Then, the slope ($1/ V_{max}$) will allow us to find out the maximum reaction rate and the y-axis intersection (K_m/ V_{max}) to calculate the K_m related to the affinity of the enzyme to the substrate.

Once these constants are determined, we can better study the affinity between the enzyme and the substrate of the process through the representation of a rectangular hyperbola also called the Michaelis-Menten curve (Fig. 3.1) (Michaelis & Menten, 1913) (Eqn. 3.2):

$$v = \frac{v_{max} \cdot [S]}{K_m + [S]} \qquad (3.2)$$

3.2.2. The effect of pH

The effect of the pH and the optimum pH of the phosphate buffer for the enzyme assay were determined by an experiment in which enzyme measurements were made at different pHs. Samples of similar size and reagents were prepared at different pHs and then the enzymatic reaction was measured as previously explained. The range of pHs tested was between 6.5 and 10.3. These pHs were achieved using by delicate additions of NaOH and H_3PO_4 solutions. The pH was measured using a GLP21 pH meter from Crison Instruments. For comparison, the signal was normalized by the protein content of the homogenates. Most enzymes have an optimum pH around which they have their maximum activity (Fig. 3.2) (Robinson, 2015).

3.2.3. The effect of temperature

The relationship between temperature and enzymatic activity normally exhibits a behaviour described by the Arrhenius equation ($k = Ae^{-(Ea/RgT)}$, Sizer, 1943) before reaching denaturation (Robinson, 2015). In this equation k is the reaction rate, A is the "Frequency factor", but is equivalent to k at a reference temperature and they have the same units. Rg is the universal gas constant in molar units. (It is equivalent to the Boltzman factor times Avogadros Number.) T is in degrees Kelvin not degrees Celsius. The effect of temperature was studied by measuring the reaction at 7 different temperatures between 10 and 40ºC. Knowing the relationship between temperature and enzymatic activity allows us to convert reaction rates from one temperature to another temperature, nearby, using the activation energy (Ea). This thermodynamic parameter is calculated from an Arrhenius Plot using analytical geometry. Ln(k) is plotted against 1/T, with T in Kelvin degrees. The calculation is based on a linear regression of the two variables. The slope is –Ea/Rg and the intercept, on the ordinate, is Ln(A). Eqn 1.3 is the logarithmic regression of the Arrhenius equation (Arrhenius, 1889; Eyring, 1935; Low et al., 1973; Laidler, 1984):

$$\mathrm{Ln}(k) = \mathrm{Ln}(A) - \left(\frac{E_a}{R_g}\right) \cdot \left(\frac{1}{T}\right) \qquad (3.3)$$

In this equation, Ea is the molar activation energy in kJ mol^{-1}, Rg ($8.314 \cdot 10^{-3}$) is in kJ mol^{-1} K^{-1}. In Eqn. 3.3 from our experiment, we have Ln(k) and 1/T as independent variables. Fig. 3.3 shows the determinations of Ln(A) and -Ea/Rg. Ea then equals the slope times Rg.

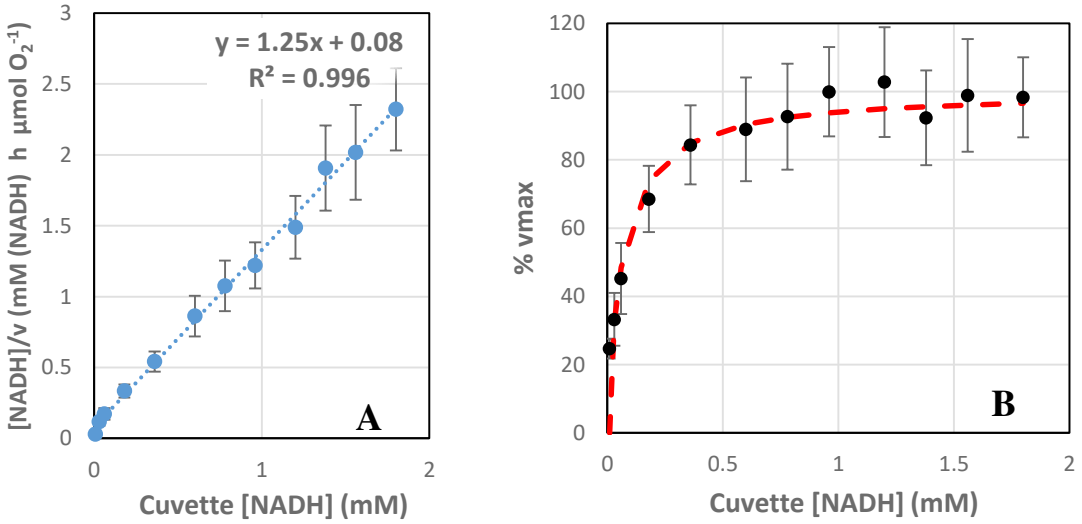
3.2.4. The effect of dilution

The activity of the same homogenate diluted with buffer at different concentrations between 0 and 100% of the original homogenate was analysed. This will demonstrate (or not) that the reaction rate (Vmax) is linearly related to the enzyme concentration. Simultaneously it shows linearity with biomass concentration. (Biomass packages the enzyme (s).) After the homogenate was diluted, the rest of the steps continued as previously explained to check the effect of the dilution (Fig. 3.4). The aim was to verify which was the limit to which the sample could be diluted and to check if it displayed a linear behaviour that allowed converting to the original concentrations.

3.3. RESULTS AND DISCUSSION

The homogenization techniques for jellyfish tissue were also investigated and Teflon grinders and sonifiers were considered valid techniques for homogenization. It must be taken into account that, if excessive, sonication may also detrimentally heat up the homogenate (Christensen & Packard, 1977; Finlay et al., 1983). Hence, very small samples should be sonified with lower intensities and/or intermittently, especially if working with low volumes. On the other hand, if the sample is very large, sonication may be insufficient, so a Teflon-glass tissue-grinder works better for larger samples. In our case, we encourage the addition of a glass-fiber filter-disk as an abrasive during grinding to aid in the homogenization. Timing and dilution will depend on the size and type of jellyfish as is described in further chapters. All the homogenization must be carried out in an ice bath to prevent the decomposition of the enzymes.

Based on the results of the previously described experiments, the conditions under which the enzymatic assay described in this work were carried out, were established. It was determined on the basis of the Michaelis-Menten curves (Fig. 3.1) that an adequate reaction was achieved with a concentration of 1mM NADH and NADPH in the cuvette. With this concentration, the activity was close to V_{max} (>90%) without excessive use of reagent. It was also 10 times higher than the k_m which is the minimum recommended by Bisswanger (2014). To achieve this concentration in the cuvette, the reagents had to have a concentration of 1.7mM before mixing in the cuvette with the homogenate and the INT.



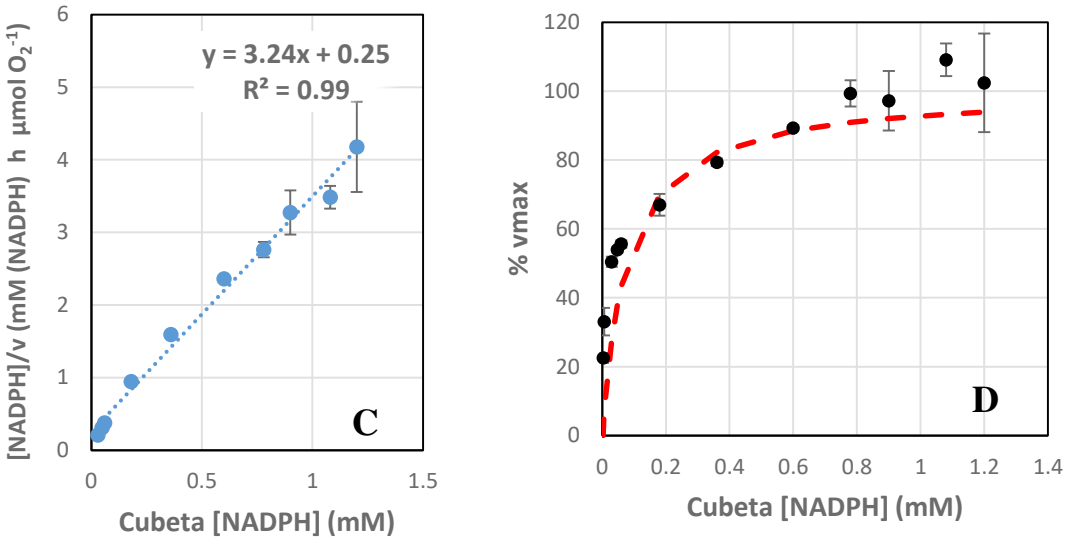


Figure 3.1. Enzyme-kinetics graphical analysis Effect of substrates, [NADH] and [NADPH], on the rate of reduction of INT in *A. aurita* **A:** Hanes-Woolf linearization ([S]/v) versus [S]) of the Michaelis-Menten equation described in B. **B:** Michaelis-Menten curve (rectangular hyperbola) describing the relation between percentage activity and [NADH]. Black points: the results of the study. Red-dashed line: Line of the Michaelis-Menten equation (Eqn. 3.2) based on the corresponding V_{max} and K_m generated from the Hanes-Woolf linearization in A (Eqn. 3.1), K_m=0.064 mM NADH, V_{max}= 0.801 µmol O_2 h^{-1}. **C:** Hanes-Woolf linearization of the Michaelis-Menten equation described in D. **D:** Michaelis-Menten curve describing the relation between percentage of activity and [NADPH]. Black points: the results of the study. Red line: Line of the Michaelis-Menten equation (Eqn. 3.2) based on the corresponding V_{max} and K_m generated from the Hanes-Woolf linearization in C (Eqn. 3.1), K_m=0.078 mM NADPH, V_{max}= 0.308 µmol O_2 h^{-1}. Standard deviation error bars, n=3.

The optimum pH according to Fig. 3.2 was around 8.5 and the effect of temperature could be corrected by using an Ea of 38.83±1.8 kJ mol^{-1} (Fig. 3.3). In addition, the assay maintained the linearity in the dilution test (Fig. 3.4) allowing correction using dilution factors. The dilution test also allowed the ratios between biomass and homogenate volume to be established.

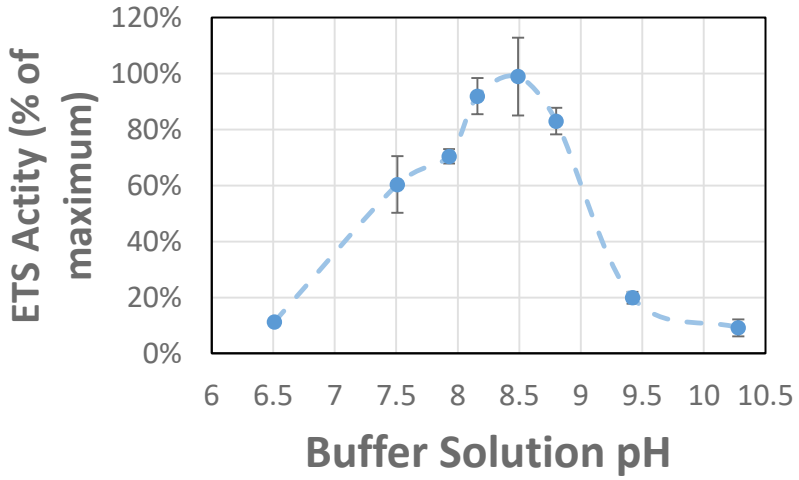


Figure 3.2. Effect of pH on the rate of ETS enzyme activity in *A. aurita*. Results standardized by the maximum activity. n=3

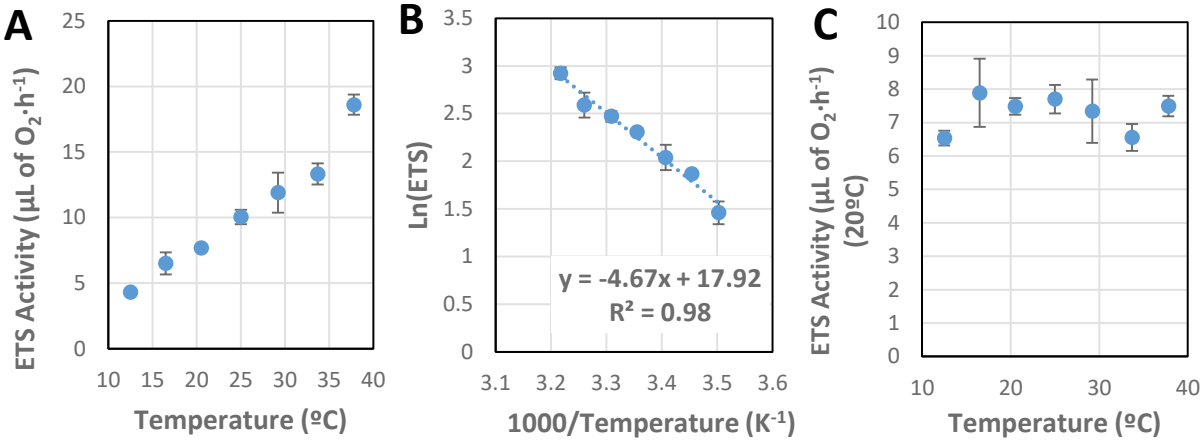


Figure 3.3. Relationship between temperature and ETS enzyme activity in *A. aurita*. **A:** Effect of temperature on the enzyme activity (the reaction rate). **B:** Linear regression of the Arrhenius plot based on the Arrhenius equation (Eqn 3.3) for the calculation of the activation energy, Ea, using the slope. Ea = -slope*Rg =-(-4.67)*(8.314) = 38.83 kJ mol^{-1}. **C:** Use of Ea determined in B and the Arrhenius equation to correct activities at the same temperature (20ºC) using Eqn 3.4. n=3 and the error bars are standard deviation.

$$ETS_T = ETS_{T0} \cdot e^{\frac{Ea \cdot \left(\frac{1}{T0} - \frac{1}{T}\right)}{Rg}} \qquad (3.4)$$

Where ETS_T is the corrected ETS activity at the desired temperature, ETS_{T0} is the original ETS activity at the reference temperature. Ea and Rg are as above. T is the temperature to which you want to convert, T0 is the original reference temperature.

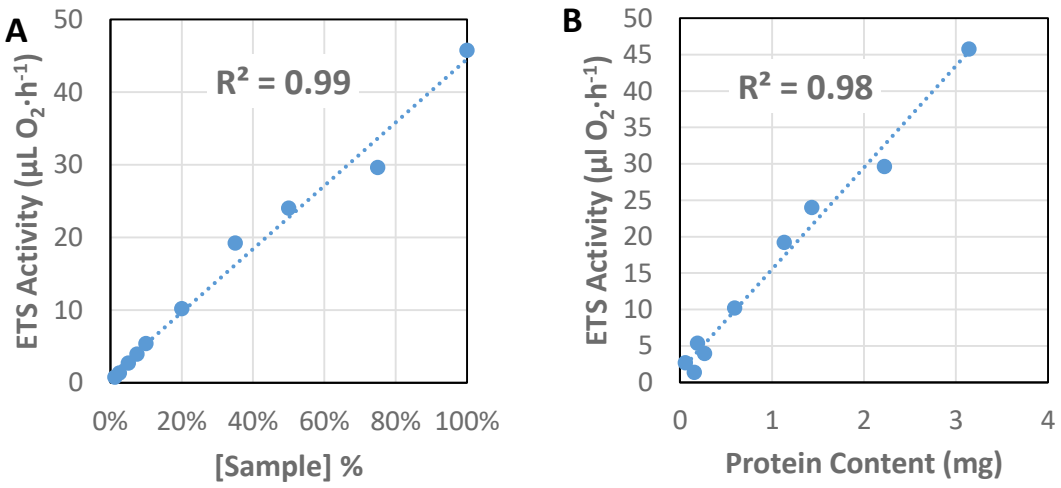


Figure 3.4. Relationship between activity and enzyme concentration from a sample from *A. aurita* **A:** Enzyme activity at different enzyme concentrations. 100% is equivalent to a concentration of 1 individual of approximately 350mg per 1 ml of buffer. **B:** ETS activity of the dilutions against their protein content. Protein, here, is an index of biomass as well as the enzyme concentration.

It is worth mentioning some of the similarities and differences with the previous assay conditions. The concentration of NADH and pH are the same as those proposed by Owens & King (1975) after similar tests. However, they recommended a much lower concentration of NADPH (0.25mM). This is not very serious since, at least in heterotrophs, because the activity obtained with NADPH is considerably less than that obtained with NADH. So, the difference between the activity with both substrates and the activity with only NADH is low (Savenkoff et al., 1995). In general, the Michaelis-Menten curve of NADPH could not be described well by a rectangular hyperbola so it is possible that the behaviour of the NADPH-INT oxidoreductase and NADPH is more complex in *A. aurelia*. With respect to the activation energy that was determined by Owens & King (1975), namely 54.4 - 66.9 kJ mol^{-1}, I found it to be lower (38.83±1.8 kJ mol^{-1}) which is closer to that of Sizer (1942) for NADH dehydrogenase (41.48kJ mol^{-1}). This may have implications on the thermal corrections made previously and on the relationships with Ea in different organisms. In summary, these were the conditions used in this work and those that I recommend for future investigations with jellyfish. However, above all, it is recommended each new investigator carry out his/her own tests when working with different organisms or in a different laboratory. This recommendation extends to the absorption coefficients or extinction coefficients of the INT which, while that of Owens & King was 15.9 mM^{-1} cm^{-1} and have been used by other authors, our extinction coefficients ranged from 12.8 to 13.8 mM^{-1} cm^{-1}.

3.4. CONCLUSIONS

Based on these tests the optimum conditions for the ETS kinetic assay in jellyfish based on *A. aurita* were determined. We recommend the use of a phospahte buffered solution at a pH of 8.5 for the homogenate and reagents so the reaction takes place at 8.5. The substrate solution should have a concentration of 1.7mM of NADH and 1.7mM of NADPH so the reaction takes place at a concentration of 1mM.

The resulting activation energy of this reaction in jellyfish based on the temperature test was 38.83 kJ mol^{-1}. The enzymatic reaction is linearly related with enzyme concentration. We encourage testing the extinction coefficient of the INT solution.

The measurements presented in chapters 4, 5 and 6 were carried out based on these conditions. Together with the tests carried out in chapter 2 and the experiments described above, it was possible to apply techniques to study respiration at a macroscopic level and at an enzymatic level. In the case of measurements on jellyfish, the results were used to estimate carbon demand and energy release in the form of ATP associated with respiratory processes.

REFERENCES

Arrhenius, S., 1889. Über die Reaktionsgeschwindigkeit bei der Inversion von Rohrzucker durch Säuren. Zeitschrift für physikalische Chemie De Gruyter Oldenbourg 4: 226–248.

Bisswanger, H., 2014. Enzyme assays. Perspectives in Science Elsevier 1: 41–55, http://dx.doi.org/10.1016/j.pisc.2014.02.005.

Brooks, H. B., S. Geeganage, S. D. Kahl, C. Montrose, S. Sittampalam, M. C. Smith, & J. R. Weidner, 2004. Basics of Enzymatic Assays for HTS. Assay Guidance Manual 1–10, http://www.ncbi.nlm.nih.gov/pubmed/22553875.

Christensen, J. P., & T. T. Packard, 1977. Sediment metabolism from the northwest African upwelling system. Deep Sea Research Elsevier 24: 331–343.

Eyring, H., 1935. The activated complex in chemical reactions. The Journal of Chemical Physics American Institute of Physics 3: 107–115.

Finlay, B. J., A. Span, & C. Ochsenbein-Gattlen, 1983. Influence of physiological state on indices of respiration rate in protozoa. Comparative biochemistry and physiology 74A: 211–219.

Gómez, M., S. Torres, & S. Hernzández-León, 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. South African Journal of Marine Science 17: 15–20.

Haldane, J. B. S., & K. G. Stern, 1932. Allgemeine Chemie der Enzyme. T. Steinkopff.

Hanes, C. S., 1932. Studies on plant amylases. Biochemical Journal 26: 1406–1421.

Hofstee, B. H. J., 1952. On the evaluation of the constants Vm and Km in enzyme reactions. Science JSTOR 116: 329–331.

Iguchi, N., H. Iwatani, K. Sugimoto, S. Kitajima, N. Honda, & O. Katoh, 2017. Biomass, body elemental composition, and carbon requirement of *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) in the southwestern Japan Sea. Plankton and Benthos Research 12: 104–114.

King, F. D., & T. T. Packard, 1975. Respiration and the activity of the respiratory electron transport system in marine zooplankton. Limnology and Oceanography 20: 849–854.

Laidler, K. J., 1984. The development of the arrhenius equation. Journal of Chemical Education 61: 494–498.

Lineweaver, H., & D. Burk, 1934. The Determination of Enzyme Dissociation Constants. Journal of the American Chemical Society 56: 658–666.

Low, P. S., J. L. Bada, & G. N. Somero, 1973. Temperature adaptation of enzymes: roles of the free energy, the enthalpy, and the entropy of activation. Proceedings of the National Academy of Sciences National Acad Sciences 70: 430–432.

Michaelis, L., & M. L. Menten, 1913. The kinetics of the inversion effect. Biochem. Z 49: 333–369.

Osma, N., I. Fernández-Urruzola, M. Gómez, S. Montesdeoca-Esponda, & T. T. Packard, 2016a. Predicting in vivo oxygen consumption rate from ETS activity and bisubstrate enzyme kinetics in cultured marine zooplankton. Marine Biology 163: 146.

Osma, N., F. Maldonado, I. Fernández-Urruzola, T. T. Packard, & M. Gómez, 2016b. Variability of respiration and pyridine nucleotides concentration in oceanic zooplankton. Journal of Plankton Research Oxford University Press 38: 537–550.

Owens, T. G., & F. D. King, 1975. The measurement of respiratory electron-transport-system activity in marine zooplankton. Marine Biology 30: 27–36.

Packard, T. T., 1971. The Measurement of Respiratory Electron-transport Activity in Marine Phytoplankton. Journal of Marine Research 29: 235–244.

Packard, T. T., & J. P. Christensen, 2004. Respiration and vertical carbon flux in the Gulf of Maine water column. Journal of Marine Research 62: 93–115.

Packard, T. T., M. L. Healy, & F. A. Richards, 1971. Vertical Distribution of the Activity of the Respiratory Electron Transport System in Marine Plankton. Limnology and Oceanography 16: 60–70.

Purcell, J. E., V. Fuentes, D. Atienza, U. Tilves, D. Astorga, M. Kawahara, & G. C. Hays, 2010. Use of respiration rates of scyphozoan jellyfish to estimate their effects on the food web. Hydrobiologia 645: 135–152.

Robinson, P. K., 2015. Enzymes: principles and biotechnological applications. Essays in Biochemistry 59: 1–41.

Savenkoff, C., T. T. Packard, M. Rodier, M. Gérino, D. Lefèvre, & M. Denis, 1995. Relative contribution of dehydrogenases to overall respiratory ETS activity in some marine organisms. Journal of Plankton Research 17: 1593–1604.

Segel, I. H., 1976. Biochemical Calculations, John and Sons. Inc. New York .

Thuesen, E. V., & J. J. Childress, 1994. Oxygen consumption rates and metabolic enzyme activities of oceanic California Medusae in relation to body size and habitat depth. Biological Bulletin 187: 84–98.

CHAPTER 4

RESPIRATORY METABOLISM AND BIOCHEMICAL COMPOSITION IN THE MEDUSAE, *Aurelia aurita* AND *Pelagia noctiluca*

ABSTRACT

We investigated jellyfish respiration and biochemical composition in two scyphozoan species, *Aurelia aurita* and *Pelagia noctiluca*. Respiratory activity was determined using enzymatic analysis of the respiratory electron transport system (ETS). This ETS activity was stoichiometrically converted to potential respiration (Φ), in oxygen units, and compared to aerobic respiration (*R*) determined by optodes and to wet biomass (WM). Regression analysis between *R* and both Φ and WM, showed strong power function correlation [R (µl O_2 h^{-1}) = 0.78 Φ (µl O_2 h^{-1}) $^{0.90}$ (r^2 = 0.97)]. [R (µl O_2 h^{-1}) = 0.23 WM (g) $^{0.93}$ (r^2 = 0.97)]. The biochemical composition was studied through the measurement of protein, lipid and carbohydrate content. These analyses revealed that WM specific lipid content is higher in smaller life stages and decreases as the size increases while protein content remained stable. The content of these biochemical components was used to calculate the composition based energy content in both species. This parameter was not very constant due to the changes in lipids content. It was also compared with bomb calorimetry studies of jellyfish tissue.

Respiratory carbon demand (*RCD*) and minimum mass-specific-carbon-requirements (*MCR*) were determined, separately, using *R* and Φ. These were used to calculate jellyfish-prey-consumption impact, as well as to calculate the global cnidarian respiratory carbon oxidation rate (2.27 – 3.15 Gt C y^{-1}). Heterotrophic energy transformation (*HET*), was also derived from the respiratory measurements. The mitochondrial, mass-specific potential *HET* was 10.03±5.31 and 11.05±4.88 J d^{-1} g WM^{-1} for *A. aurita* and *P. noctiluca*, respectively. These values are within the *HET* range calculated from *R* results from literature for *A. aurita* and *P. noctiluca.* These are nearly 1000-fold greater than the sun's mass-specific-energy-production rate (0.017 J d^{-1} g^{-1}), but at a world wide scale cnidarian HET is only equivalent to 2-3% of the solar energy fixed by primary production. More *HET* calculations from respiration would promote energy circulation modelling in ecosystems that would advance understanding energy-flux dynamics that have been traditionally deduced from biomass and elemental circulation modelling.

4.1. INTRODUCTION

Here, we attempt to improve and provide tools to research the role of jellyfish in oceanic carbon and energy transformations through the determination of respiratory carbon demand (*RCD*) and heterotrophic energy transformation (*HET*) in two scyphozoan jellyfish, *Aurelia aurita* (Linnaeus, 1758) and *Pelagia noctiluca* (Forsskål, 1775). Living systems, from individuals to ecosystems, are controlled by two main transformations. One has cyclical behavior, biomass, while the other one has directional behavior, energy (Odum, 1968). Respiration provides information about the basal energy and carbon requirements needed by an organism to resist the force of entropy, the tendency to decompose to the elements and heat. All cells resist this decomposition by converting energy in food to ATP and heat. However, respiration also quantifies the flow of both carbon and energy fixed by primary producers to the different trophic levels (Karl, 2014). Therefore, studying the respiration of different components of Earth's ecosystems helps understand the requirements, roles and relevance of each of these components. Gelatinous zooplankton (jellyfish) are an abundant component of oceanic ecosystems and have been understudied compared to the crustacean component (Raskoff et al., 2003; Haddock, 2004; Henschke et al., 2016).

New research has recently focused on the biology and ecology of gelatinous zooplankton in recognition of their changing roles in ocean ecosystems. Scyphozoans are important members of this zooplankton group. Research on their trophic dynamics has evidenced their consumption of fish eggs, fish larvae, and other fish life-stages; their consumption of multiple zooplankton species; and other members of pelagic ecosystems (Purcell, 1997, 2003; Purcell & Arai, 2001; Hansson et al., 2005; Boero, 2013; Choy et al., 2017; Chiaverano et al., 2018). Pauly et al. 2009 described them as the main competitors of zooplanktivorous fish. Hence, they are both predator and competitor of many commercially important fish species. There have been substantial efforts to quantify and assess the impact of this consumption (Larson, 1987a; Morand et al., 1987; Schneider, 1989; Purcell, 1997; Uye & Shimauchi, 2005; Ishii & Tanaka, 2006; Han et al., 2012; Iguchi et al., 2017; Nagata & Morandini, 2018). Other studies have taken into account the dense aggregations of some jellyfish and have predicted widespread and severe ecological changes (Malej, 1989; Mills, 1995; Schneider & Behrends, 1998; Hansson et al., 2005; Ishii & Tanaka, 2006; Condon et al., 2011; Boero, 2013; Iguchi et al., 2017; Schiariti et al., 2018). Quantifying prey-consumption rates of these organisms will help assess their effect on the recovery of populations in areas where jellyfish are proliferating (Purcell et al., 2007; Richardson et al., 2009; Boero, 2013). The impact of anthropogenic stressors on the rise of jellyfish blooms is complicated and has been brought into question (Pitt et al., 2018) although given the reality of worldwide ocean dumping, over pollution, over-fishing, and colossal increases in coastal construction (Stoett, 2019) the impact is likely large. Especially since some scyphozoan species fall in the category of "weed species" that can proliferate in our highly disturbed future marine ecosystems (Doubleday & Connell, 2018).

Here, to assess their impacts on coastal and pelagic ecosystems, we investigated metabolism rates in *A. aurita* and *P. noctiluca*. We estimated prey-consumption rates in these organisms through analysis of their metabolism (Larson, 1987a; Purcell et al., 2010). Respiratory metabolism is directly related to the carbon utilization of an organism (Ishii & Tanaka, 2006). In mitochondria, the organic carbon of different food sources is oxidized to produce energy in the form of ATP (adenosine triphosphate), through a series of enzyme-mediated reactions that end with the reduction of O_2 to H_2O and the production of CO_2. This vital process of respiration never stops. Furthermore, it

provides an estimation of an organism's minimum metabolic carbon requirement. We determined respiration (*R*) via the physiological oxygen consumption over time, measured using optodes during incubation. We measured potential respiration (Φ) via the enzymatic activity of the respiratory electron transport system (ETS). Using this enzymatic technique provides a way to assess the metabolism of large individuals whose respiration cannot easily be measured directly using incubations (Purcell et al., 2010; Iguchi et al., 2017).

Food consumption associated with respiration was determined indirectly by employing diet compositions and assimilation values from the literature. Heterotrophic energy transformation (*HET*), previously described as Heterotrophic Energy Production, can be calculated from respiration as the amount of energy from respiratory metabolism stored in the form of ATP in both eukaryotes and prokaryotes (Packard et al., 2015). Here, *HET* was determined in jellyfish for the first time. We used it, both to describe the energy circulation through the ecosystem, as suggested by Karl (2014), and to describe mitochondrial energetic potentials in jellyfish as inspired by Lane (2015).

4.2. MATERIALS AND METHODS

4.2.1. Specimen collection

The jellyfish used here were provided by Loro Parque Foundation's aquarium in Tenerife (Canary Islands, Spain). The *Aurelia aurita* culture traced its origin to a stock-culture from the Madrid aquarium (Spain). *Pelagia noctiluca* specimens were captured during a jellyfish bloom that occurred in the Canary Islands in 2012. Generations of these two species have been cultivated at Loro Parque since then. Their culturing conditions were: filtered sea water at 18-20ºC, 33 PSU (‰) and 8.2 pH. *A. aurita* was fed thrice daily with *Artemia* nauplii and *P. noctiluca* was fed both *A. aurita* portions and *Artemia* nauplii. *A. aurita* had, been fed *Artemia* nauplii, *ad libitum,* right before the experiment while *P. noctiluca* had not been fed right before the experiment so we cannot be sure of their nutritional state.

4.2.2. Oxygen consumption by physiological respiration (*R*)

First, jellyfish R was determined using oxygen sensitive optodes to monitor oxygen (O_2) consumption (Fibox-4 by Presens (Regensburg, Germany) (Lilley & Lombard, 2015)). The organisms were transferred carefully, in seawater, to containers ready for incubation and O_2 monitoring. [Caution! Because jellyfish are so fragile and easily injured, great care must be taken during manipulation to avoid harming and stressing them.] The containers had an optode spot already installed inside. The container size depended on the organism's size and were selected to maintain a volume (CV)-to-wet mass (WM) ratio (Purcell et al., 2010) above 100 mL g^{-1}. Such containers were 5-6 mL glass tubes, 60 mL glass bottles, 350 mL glass jars, 600mL glass jars; and 1 L glass jars. The CV-to-WM ratios were closer to 30 mL g^{-1} for the biggest *A. aurita* sample and most *P. noctiluca* medusa stages. The incubations were made in darkness at 20°C. Periodically, before measuring, the containers were inverted several times to mix the seawater and to prevent stagnation. After mixing, the dissolved O_2 concentration ($[O_2]$) was measured every 20 min approximately during 5-6 h. The R was calculated as the change in $[O_2]$ during the incubation time ($\Delta[O_2]/\Delta t$) i.e. the slope of the O_2-versus-time curve. An identical-size vessel, filled only with filtered seawater, was used as a control.

4.2.3. Sample storage

Once the R measurements were completed, the organisms were stored at -80°C to prevent enzyme degradation until enzymatic analysis (Ahmed et al., 1976). The ephyrae were carefully pipetted, into labeled 2 mL microvials. We recommend the use of fire-polished, Pasteur glass pipettes for the transfer of ephyrae. For the other-sized organisms, excess seawater was allowed to drain from their bodies for a few seconds before storing them in labeled, appropriately-sized plastic-bags. The storing resembled the procedure that used liquid nitrogen, described by Thuesen & Childress (1994) for jellyfish samples. Their article describes storing samples at -80°C without loss of enzyme activity for at least 6 months. The samples here, used for respiratory enzyme analysis, were weighed directly after being removed from the -80°C freezer.

4.2.4. ETS analysis and potential respiration (Φ)

The ETS assay used here, was performed kinetically (an absorbance time-course) as described by Packard and Christensen (2004). The chemistry was based on Packard et al. (1974) as improved by Owens & King (1975). The *A. aurita* and *P. noctiluca* ephyrae were homogenized using an ultrasonic probe (Cole Parmer) with a Vibracell VCX 130 ultrasonic processor (Sonics, (Newtown, CT, USA)) in 0.1M phosphate buffer (0.1M Na_2HPO_4, 0.1 mM KH_2PO_4, 75 µM $MgSO_4 \cdot 7H_2O$, polyvinylpyrrolidone (1.5 $mg \cdot mL^{-1}$), TRITON X-100 ($2mL \cdot L^{-1}$) (Acros Organics, Geel, Belgium (#21568-2500))) at 8.5 pH. The *P. noctiluca* medusa samples were homogenized with a motor-driven teflon-glass tissue-grinder at 4000 rpm for 2 min, in 0.1M phosphate buffer. A glass microfibre filter (GF/F Circle, 25 mm) was inserted into the tissue grinder to serve as an abrasive (Packard, 1971). The filter was reduced to its microfibers within a minute. In the case of the biggest specimens, they were first blended, in a mechanical liquefier, with a known volume of deionized water (Puranity TU3), sufficient to macerate the sample (Packard, 1971; King et al., 1978). Then, a known aliquot of that blended homogenate was ground up in buffer with a GF/F filter in the teflon-glass tissue-grinder as described above. The maximum final volume-to-mass ratio, for *A. aurita,* was kept close to 10 mL homogenate per g sample and, for *P. noctiluca,* close to 15 mL homogenate per g sample. These homogenization steps were always performed at 0-4°C in an ice baths to retard enzyme degradation (Packard, 1971). The crude homogenate was then centrifuged at 4000 rpm for 10 min at 0-4°C using a refrigerated microcentrifuge. The resulting supernatant contained the enzymes for analysis. The enzyme analysis consisted of mixing, in a cuvette, 0.1 mL of the supernatant, 0.3 mL of the buffered substrates (NADH (1.7mM) and NADPH (1.7mM)) and 0.1 mL of INT solution (2 mg INT tetrazolium salt, 3-(4-Iodophenyl)-2-(4-nitrophenyl)-5-phenyl-2H-tetrazol-3-ium chloride (INT) (Acros Organics, Geel, Belgium) per mL deionized H_2O). The reaction in the cuvette was monitored in the Cary100 UV-Vis Spectrophotometer (Agilent Technologies (Santa Clara, CA, USA)) for 8 min at 490nm as INT was reduced to a dark-red formazan. From the evolution of this absorbance the rate of formazan production can be determined with the extinction coefficient of that batch of INT (13.76 mM^{-1} cm^{-1}). The potential respiration (Φ) associated with this enzymatic system, was calculated knowing that 4 moles of electrons transferred down the ETS would reduce 2 moles of INT and normally reduce, at cytochrome oxidase, one mole of O_2. This means that an ETS activity of 4 µmol e^- h^{-1} is stoichiometrically equivalent to 1 µmol O_2 h^{-1} of Φ (Packard, 1985). Blanks without homogenate were needed to control for any non-enzymatic INT reduction (Maldonado et al., 2012). Residual crude homogenate was stored at -20°C for biochemical determinations.

4.2.5. Biomass & composition

The frozen jellyfish samples were weighed before homogenization and ETS analysis. Protein was measured in the spare ETS homogenates that had been stored at -20°C. The crude homogenates of *P. noctiluca* medusa had to be diluted by half before analysis. The protein analysis was based on the Biuret reaction as described by Lowry et al. (1951) and modified by Rutter 1967. This technique employed Folin-reagent (Sigma-Aldrich, Madrid, Spain (F9252)). The protein method was modified here (Martínez et al., 2020) by the addition of Dodecyl sulphate sodium salt (SDS) (Acros Organics, Geel, Belgium (223145000)) as suggested by Markwell et al. (1978). Lipid content was also measured in these homogenates following the methodology described by Bligh and Dyer (1959) using a chloroform based extraction and determined based on the "index of total lipids" defined by Knight et al. (1972). The homogenates did not require dilution for the lipid analysis. Carbohydrate content was also measured using the phenol and H_2SO_4 based reaction proposed by Dubois et al. (1956). However, in most *A. aurita* samples and in the smaller *P. noctiluca* samples the homogenates were too diluted and the values fell below the limits of detection.

The content in proteins, lipids and, when available, carbohydrates were used to calculate composition based energy contents and energy densities (Doyle et al., 2007) like in Clarke et al. (1992) with the average enthalpies of combustion 23.9, 39.5 and 17.5 KJ g^{-1} for proteins, lipids and carbohydrates, respectively.

4.2.6. Carbon demand calculations from respiration

As a fundamental property of respiration, CO_2 is produced from the oxidation of organic matter. Accordingly, from R and Φ we can calculate the associated carbon demand (CD) using a conversion factors (*F*) proposed by Larson (1987a) and used by many others, including Iguchi et al. (2017); and Ikeda et al. (2017). The daily carbon demand, associated with respiration (*RCD*), in mg C d^{-1} g WM^{-1}, can be calculated from the respiration, *R*, in µl O_2 h^{-1} g WM^{-1}, by equation (4.1) based on Uye & Shimauchi (2005):

$$RCD = \frac{R \cdot RQ \cdot F \cdot 24}{10^3 \cdot AE} \qquad (4.1)$$

Where: *RQ* is the unitless ratio of CO_2-produced to O_2-consummed (0.85) (Schneider, 1989); *F* is the C:CO_2 conversion factor, 0.536 µg C (µl CO_2)$^{-1}$; *AE* is the assimilation efficiency, 0.8 (described by Schneider (1989) as the percentage of the carbon consumed that is assimilated); 24h per day; and dividing by 10^3 converts micrograms to milligrams. From this calculation of *RCD*, in units of mg C d^{-1} g WM^{-1}, we can calculate the mass-specific, minimum carbon-requirements (g C g C^{-1} d^{-1}) (*MCR* in % d^{-1}), associated with respiration, as inspired by Ishii & Tanaka (2006), equation (4.2):

$$MCR = \frac{RCD}{WDC \cdot DCC \cdot 10^3} \cdot 100 \qquad (4.2)$$

Here, *WDC* (g dry mass/g WM) is a wet to dry mass conversion factor of 0.036 (3.6%) for *A. aurita* (Lucas, 1994; Uye & Shimauchi, 2005) and 0.054 (5.4%) for *P. noctiluca* (average from (Morand et al., 1987; Malej et al., 1993)). The *DCC* (g carbon content/g dry mass) is a dry mass to carbon content conversion factor of 0.039 (3.9%) for *A. aurita* (Ishii & Tanaka, 2006; Purcell et al., 2010) and 10% for *P. noctiluca* (average from (Morand et al., 1987; Malej et al., 1993)); and dividing by 10^3 converts milligrams to grams.

4.2.7. Heterotrophic Energy Transformation (*HET*) from respiration

Respiration rates can be used to calculate the associated energetics (ATP production) and hence energy transformation dynamics for different organisms. This respiratory ATP generation (*HET*) is an important mechanism in all organisms and the main way energy is released from organic matter in heterotrophic organisms (Lane, 2006; Packard et al., 2015). Ochoa (1943) showed how 3 ATP molecules are produce for every oxygen atom (O) used in respiration. This number is called the P/O ratio, where "P" refers to phosphate. Now, after 65 years of research, the P/O ratio is thought to be closer to 2.5 (Ferguson, 2010; Moran et al., 2012). It is governed by the protonmotive force as first described by Peter Mitchell (Mitchell, 1961, 2011). Likely, it varies slightly from organism to organism, depending on the biochemical efficiency of their ATP synthase. Nevertheless, from respiration, we can calculate *HET* in any plankton sample, in any oceanic region, and even in our jellyfish samples. *HET* is calculated from *R* using equation (4.3):

$$HET\ (\mathrm{J}\ d^{-1}\ (\mathrm{g\ WM})^{-1}) = R\ \ (\mu\mathrm{mol\ O_2\ h^{-1}\ (g\ WM)^{-1}}) \cdot\ 2\ \cdot\ 2.5\ \cdot\ 0.048 \cdot 24 \qquad (4.3)$$

Where 2 is the number of electron pairs that participate in reduction of one mole of O_2 to 2 moles of H_2O; 2.5 is the modified P/O ratio, the ATP produced by the flow of one electron pair; 0.048 is the free energy (ΔG, in J (µmol ATP)$^{-1}$) asociated with ATP, (Alberty & Goldberg, 1992; Ferguson, 2010; Moran et al., 2012; Packard et al., 2015). The final constant, 24, converts *HET* to daily production. To calculate potential *HET*, one can use Φ, calculated from ETS activity, instead of *R*, measured physiologically.

4.3. RESULTS

4.3.1. Respiration (R) & ETS analysis (Φ)

The specimens used here ranged in size from 4 mg ephyra to 50 g medusa, for *Aurelia aurita,* and 10 mg ephyra to 70 g medusa, for *Pelagia noctiluca*. The average WM-specific *R* in *A. aurita* was 31.82±20.52 µl O_2 h^{-1} g WM^{-1} and 17.07±6.49 µl O_2 h^{-1} (g WM)$^{-1}$ in *P. noctiluca*. The average WM-specific Φ in *A. aurita* was 39.01±20.66 µl of O_2 h^{-1} g WM^{-1} and 42.96±18.97 µl of O_2 h^{-1} (g WM)$^{-1}$ in *P. noctiluca*. These results fall slightly on the higher end of the ranges for medusa compiled in Bailey et al. 1995.
Figs. 4.1, 4.2 & 4.3 show the relationship between the physiological measurements and the enzymatic measurements, as well as, the relationship between the physiological measurements and the wet biomass (WM).

The linear and log-linear regression analysis show a good relation between R and Φ from the ETS analysis, equivalent to that between *R* and WM (Figs. 4.1, 4.2, 4.3). Since the ETS activity is responsible for *R*, the difference between the results for *A. aurita* and *P. noctiluca* is likely due to their different feeding states. While *A. aurita* was recently fed *ad libitum, P. noctiluc*a samples were not fed in this way right before the respiration measurements. This could explain their different *R*:Φ ratios (95%±60%, 43%±17% respectively). The equations between *R* and WM found here (Figs. 4.1A, 4.2A, 4.3A), have both the exponents and the coefficients within the range observed in the literature when converted to the same units. The exponent is generally close to 1 in cnidarians and ctenophores (Uye & Shimauchi, 2005; Møller & Riisgård, 2007; Shimauchi & Uye, 2007; Purcell et al., 2010; Gambill & Peck, 2014; Lilley & Lombard, 2015).

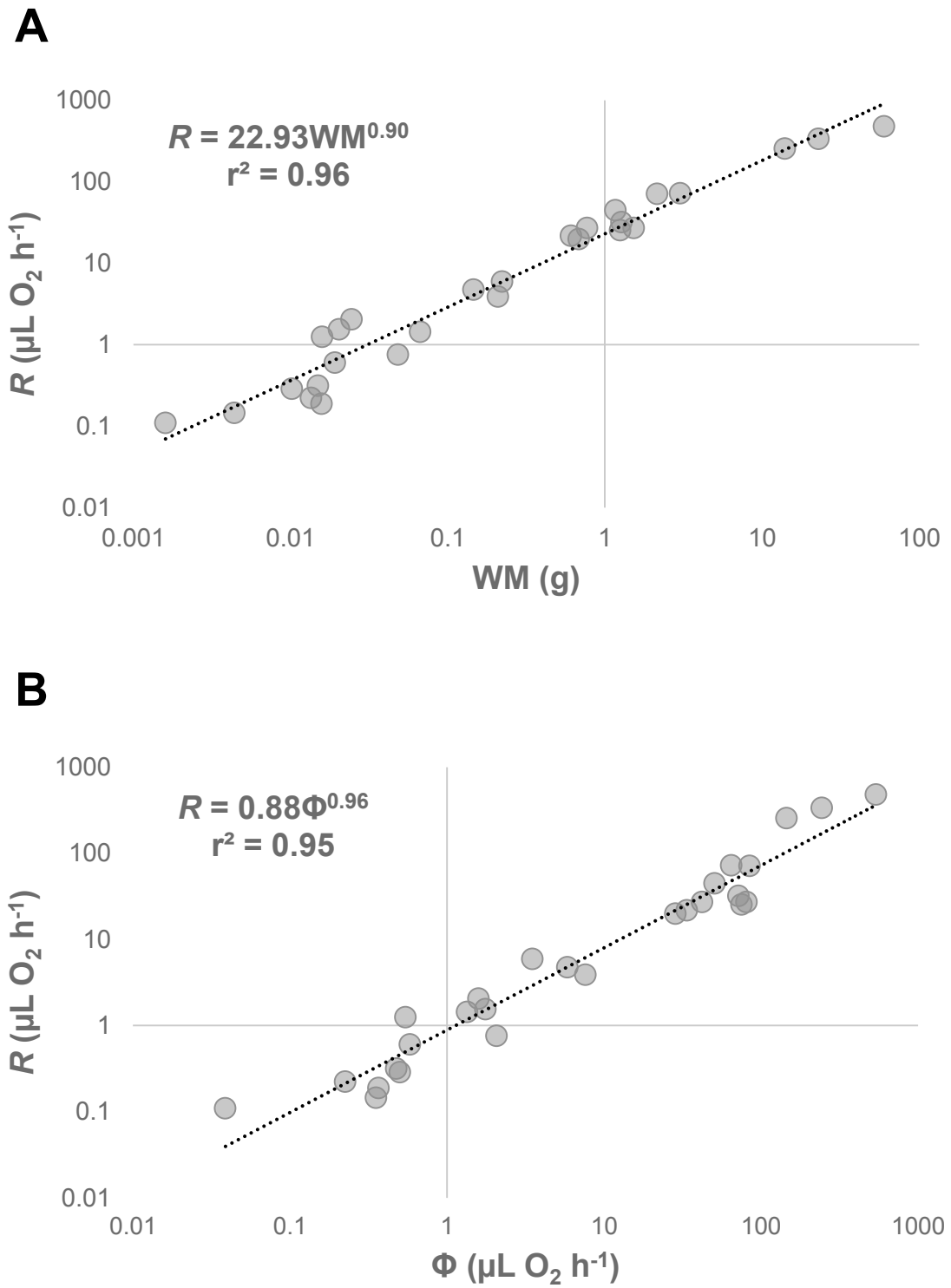


Figure 4.1. Physiological respiration (*R*) in *Aurelia aurita* (n=24 p<0.001). Regression analysis: **(A)** with wet mass (WM); **(B)** with potential respiration (Φ). Both plots' axes follow logarithmic scales. The power functions and coefficient of determinations (r^2) are shown. The corresponding linear functions were **(A)** $R = 8.93\ WM + 16.64$, $r^2 = 0.89$; **(B)** $R = 0.98\ \Phi - 1.18$, $r^2 = 0.91$.

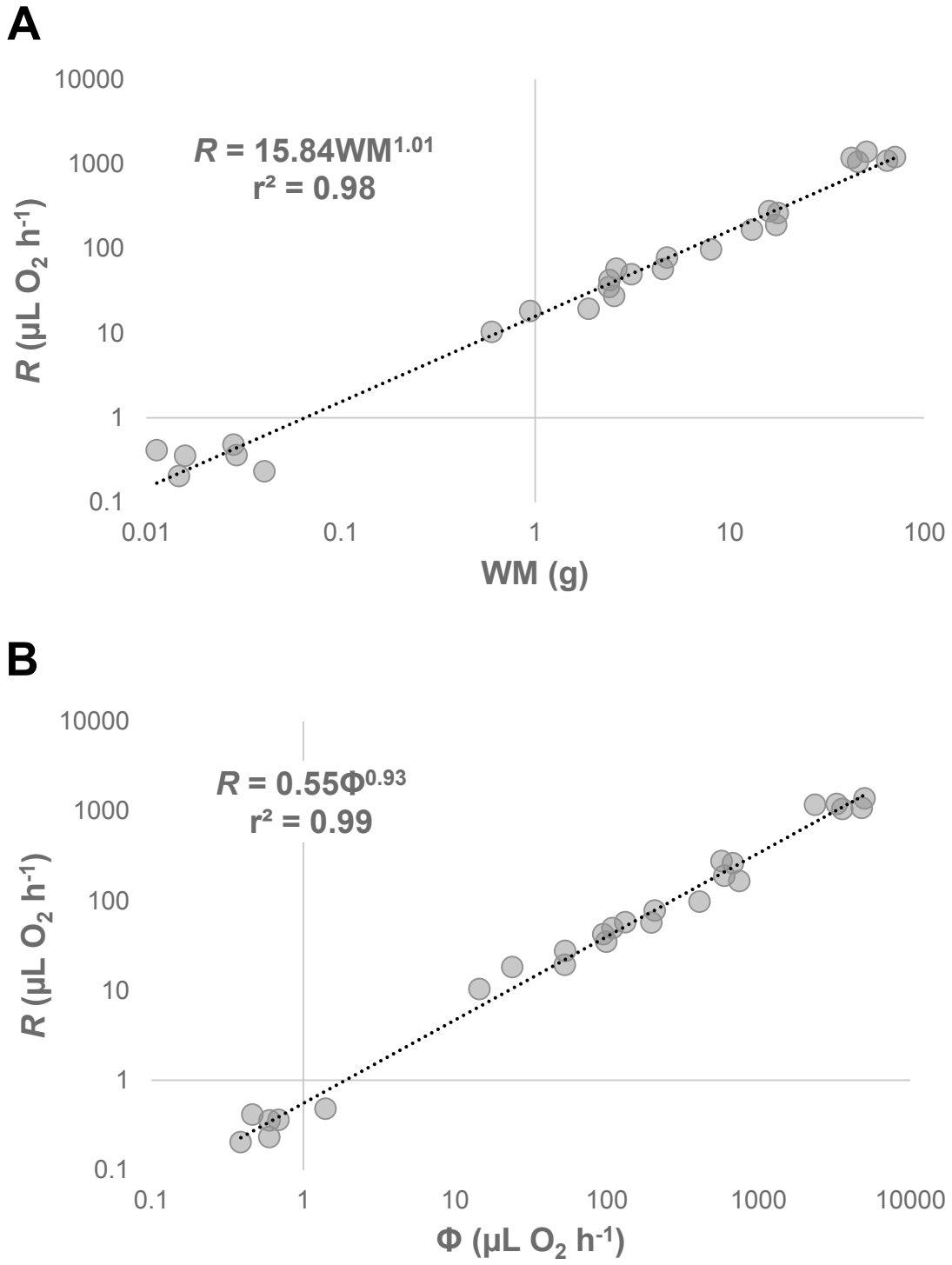


Figure 4.2. Physiological respiration (*R*) in *Pelagia noctiluca* (n=25 p<0.001). Regression analysis: (a) with wet mass (WM); (b) with potential respiration (Φ). Both plots' axes follow logarithmic scales. The power functions and coefficient of determinations (r^2) are shown. The corresponding linear functions were (A) R = 20.47 WM - 12.93, r^2 = 0.92; (B) R = 0.29 Φ + 26.87, r^2 = 0.92.

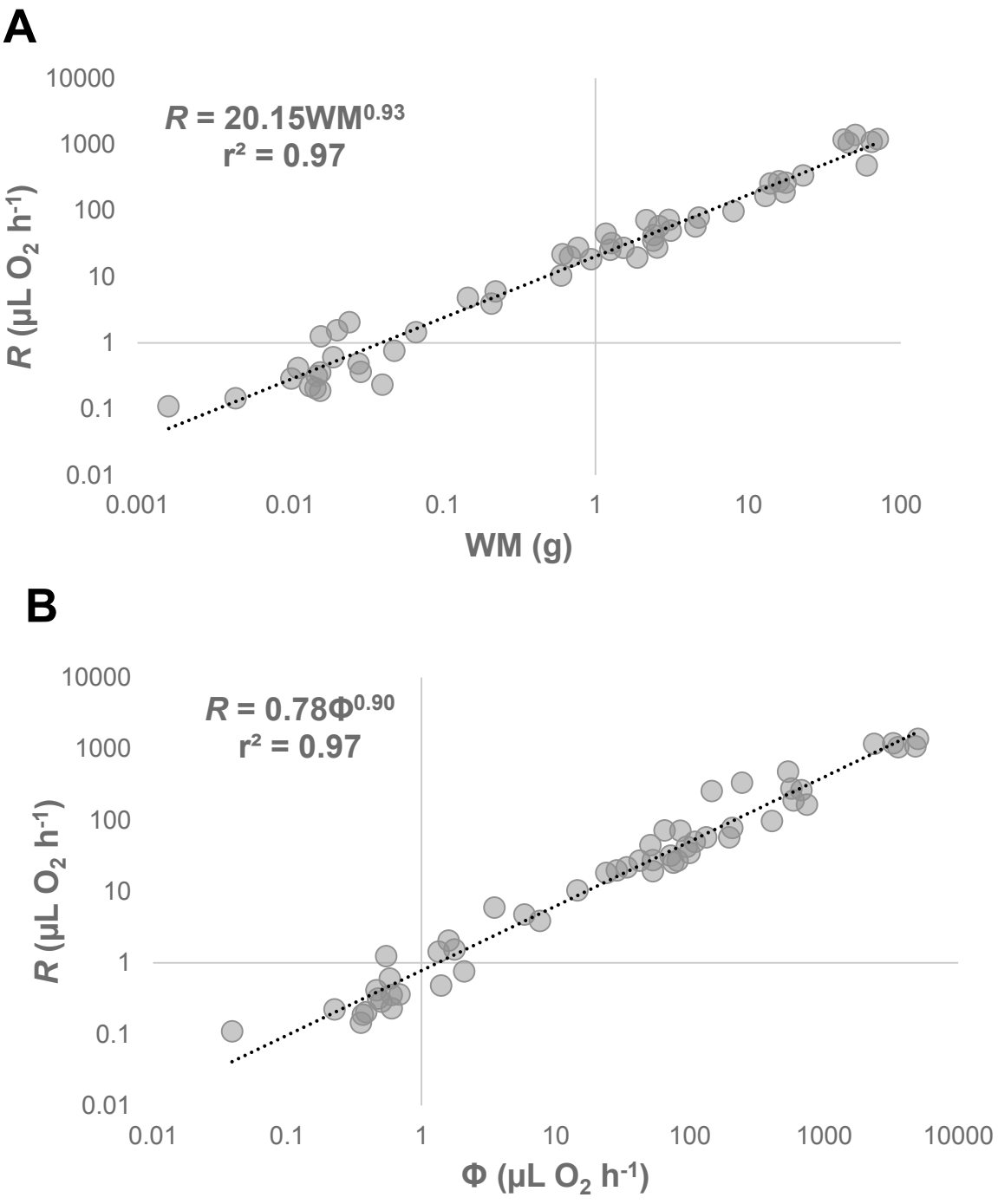


Figure 4.3. Physiological respiration (*R*) in both scyphozoans (n=49 p<0.001). Regression analysis: (a) with wet mass (WM); (b) with potential respiration (Φ). Both plots' axes follow logarithmic scales. The power functions and coefficient of determinations (r^2) are shown. The corresponding linear functions were (A) $R = 17.93WM + 1.18$, $r^2 = 0.86$; (B) $R = 0.29\ \Phi + 31.40$, $r^2 = 0.91$.

4.3.2. Biomass & composition

The log-linear regression analysis of mg of protein (*P*) and DM (converted from WM using 3.42% (Lucas, 1994)) in *A. aurita* (Log *P*(mg) = 1.60 + 1.04 Log DM (g), r^2=0.97, n=21) showed a similar relationship to the one found by Lucas (1994). They also used the method of Lowry (Log *P*(mg) = 1.75 + 1.13 Log DM (g), r^2= 0.95, n= 103). In the case of lipids the resulting loglinear function with DM was (Log *L*(mg) = 0.37 + 0.25 Log DM (g), r^2=0.57, n=22). This did not match, as well as protein did, the results of Lucas (1994) (Log L(mg) = 1.19 + 0.77 Log DM (g), r^2= 0.83, n= 57). However the lipids results from the smaller size fractions are absent in Lucas (1994).

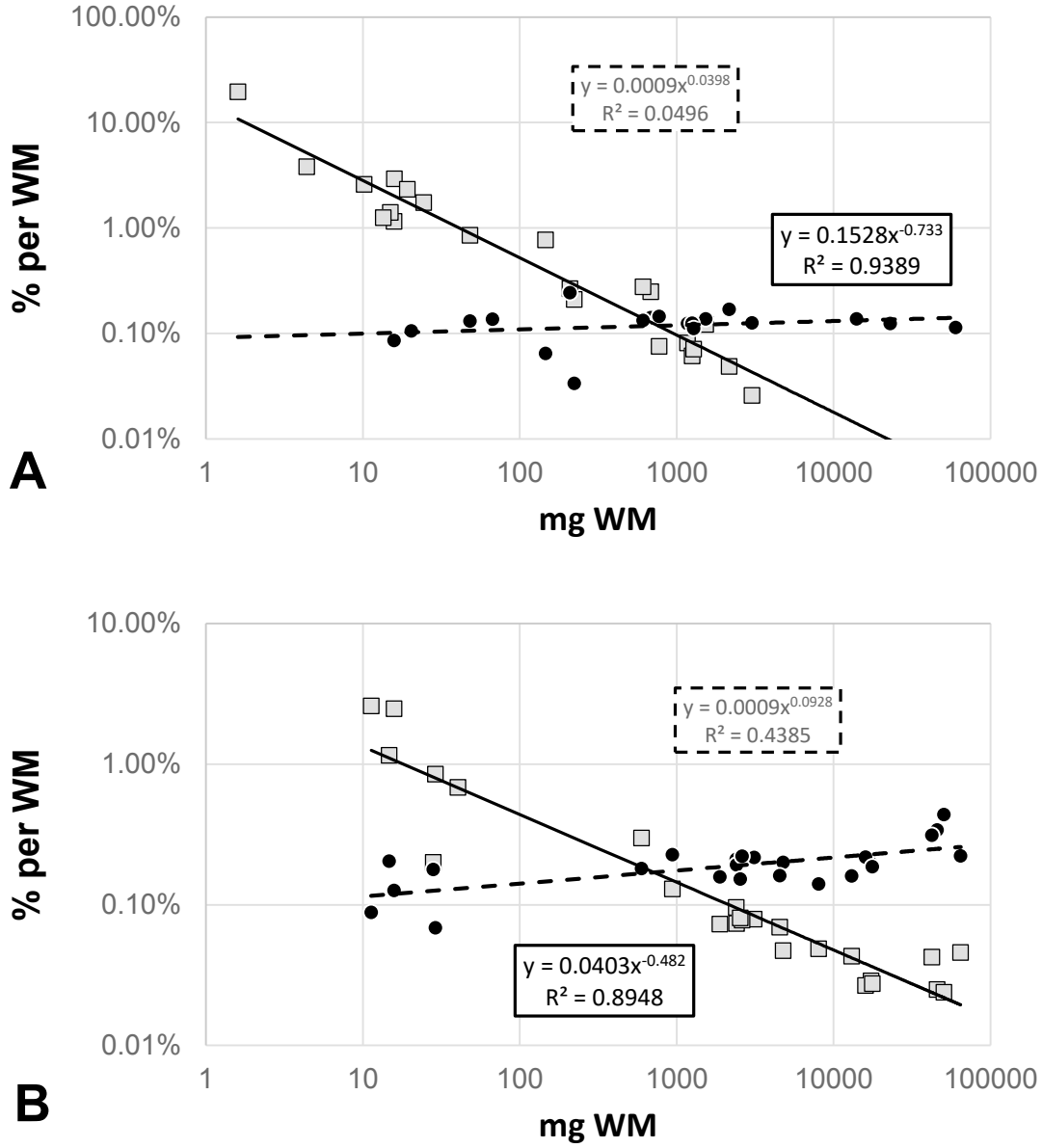


Figure 4.4. Protein (black circles) and lipid (gray squares) composition of *Aurelia aurita* (A) and *Pelagia noctiluca* (B) in the different sized samples.

The average protein content per WM in *A. aurita* was 0.13±0.04% while in *P. noctiluca* it was 0.19±0.08%. This last value is lower than the measurements in *P. noctiluca* by Malej et al. (1993) using the Bradford protein method (0.94±0.22%, n=10). Meanwhile lipids show some interesting new findings since lipid content was not as constant as protein. Lipid content was higher in ephyrae than in medusae. *A. aurita* lipid content showed a decreasing potential trend with size. Smaller samples had an average lipid content between 1 and 4% and bigger samples between 1 and 0.05% with the smallest sample resulting in a 19.55% (Fig. 4.4A). *P. noctiluca* showed a similar decreasing potential trend with smaller samples having a lipid content per WM between 0.2 and 3% and bigger samples between 0.3 and 0.02% (Fig. 4.4). These values in smaller samples are much higher than those reported in Lucas (1994) and Malej et al. (1993) which reported compositions of larger samples using Bligh & Dyer (1959) and Barnes & Blackstock (1973), respectively. This high lipids in early smaller samples also caused lipid content to be higher than protein during early stages and switching in larger samples. Due to this changing composition the resulting composition based energy density calculated like in Clarke et al. (1992) also decreased without being constant. Nevertheless, the resulting average energy densities were 0.68±1.56 and 0.19±0.27 KJ g WM^{-1} in *A. aurita* and *P. noctiluca,* respectively.

4.3.3. Carbon demand from respiration

Our respiration measurements and calculations facilitated the determination of respiratory carbon requirements and mass-specific minimum carbon-requirements. In Table 4.1, these values are compared to similar results from the literature.

Knowing the carbon composition of prey and our calculated *RCD*, we estimated the prey quantities necessary to support a jellyfish's respiratory metabolism (Table 4.2). For example, Artemia nauplii's carbon weight (Makridis & Vadstein, 1999; Pereira et al., 2014) is approximately 0.86 µg C $individual^{-1,}$ from which we calculated a potential consumption rate of 291.65 – 948.37 artemia $nauplii \cdot d^{-1} \cdot g\ WM^{-1}$ for *A. aurita*, based on the *RCD* estimated from potential respiration (Table 4.1).

The main prey of *A. aurita* are copepods (genus: Acartia, Oithona,…), Balanid nauplii, fish eggs, fish larvae, cladocerans and other larvae (Båmstedt, 1990; Omori et al., 1995; Hansson et al., 2005; Uye & Shimauchi, 2005; Pereira et al., 2014) and the main prey of *P. noctiluca* are copepods, euphausiaceans, larvaceans, hydrozoans, fish larvaes, fish eggs, mollusk larvaes, chaetognaths, pteropods and cladocerans (Larson, 1987a; Malej, 1989; Malej & Malej, 2004; Sabatés et al., 2010; Canepa et al., 2014; Purcell et al., 2014; Tilves et al., 2016; Milisenda et al., 2018). Table 4.2 shows the potential consumption rates based on potential *RCD*, if certain organisms are preyed upon exclusively by this study's species. The carbon contents of these prey were obtained from the literature. These numbers facilitate estimations of jellyfish trophic impacts, from the approximate number of individuals and their average mass. Based on the dietary composition of *P. noctiluca* as reported by Sabatés et al. 2010 (49-72% for copepods, 6-12% for fish larvae), we approximated the consumption impact of these individuals when present in the surrounding waters over areas impacted by these jellyfish blooms. The consumption values can be used to study the impact jellyfish have on fish larvae and zooplankton populations of a certain area (Malej, 1989; Bailey et al., 1995; Tilves et al., 2016; Iguchi et al., 2017; Nagata & Morandini, 2018).

Table 4.1. Ranges of respiratory carbon demand (*RCD*) and weight-specific carbon minimum-requirements (*MCR*), calculated for *A. aurita* and *P. noctiluca*, from *R*, Φ, and similar parameters in the literature.

	RCD (mg C · d^{-1} · g WM^{-1})	*MCR* (%·d^{-1})	Reference
A. aurita	0.109 – 1.139	7.77 – 81.15	**This study (*R*)**
	0.124 – 1.181	8.81 – 84.10	**This study (Φ)**
	0.038 – 0.114	3 – 8	**Larson (1987b)**
	0.035	2.47	**Schneider (1989)**
	0.07 – 0.56	5 - 40	**Båmstedt (1990)**
	0.14 – 3.68	10 - 262	**Båmstedt et al. (1994)**
	0.01 – 0.08	0.58 – 5.56	**Ishii & Tanaka (2001)**
	0.06 – 0.305	4.7 – 23.4	**Uye & Shimauchi (2005)**
	0.012 – 0.284	0.8 – 20.3	**Ishii & Tanaka (2006)**
	0.053 – 0.072	3.77 – 5.10	**Han et al. (2012)**
	0.057 – 0.168	4.09 – 11.88	**Iguchi et al. (2017)**
P. noctiluca	0.078 – 0.499	1.46 – 9.33	**This study (*R*)**
	0.208 – 1.355	3.79 – 25.33	**This study (Φ)**
	0.920	16.21	**Davenport & Trueman (1985)**
	0.116 – 3.003	1 – 56.14	**Malej (1989)***
	0.047 – 0.775	0.82 – 13.66	**Malej et al. (1993)**
	0.001 – 0.112	0.01 – 1.98	**Lilley & Lombard (2015)**

*Temperature between 19 and 23ºC

Table 4.2. Potential consumption rates per species derived from potential *RCD* and carbon contents of zooplankters. The data was drawn from the literature. Based on Bailey et al. (1995).

Prey Examples		Average Carbon (µg Ind^{-1})	Ref. Carbon Content	Potential Daily Prey Consumption (Ind d^{-1} g WM^{-1})	
				Aurelia aurita	*Pelagia noctiluca*
Copepods	***Oithona davisae***	0.2	**Uye & Shimauchi (2005)**	1254 - 4078	1640.0 – 4232.8
Copepods	***Acartia Tonsa***	4	**Omori (1978)**	62.7 - 204	82 – 211.64
Balanid nauplii	**-**	1	**Uye & Shimauchi (2005)**	251 - 816	328 – 846.56
Euphausiacea	**-**	1240	**Lindley (1999)**	0.2 – 0.66	0.26 – 0.68
Euphausiacea	***Tessarabrachion oculatus***	6820	**Omori (1969)**	0.04 – 0.12	0.05 – 0.12
Cladocerans	***Bosmina sp.***	1.03	**Walve & Larsson (1999)**	243.75 – 792.61	318.76 – 822.71
Cladocerans	***Evadne sp.***	0.72	**Walve & Larsson (1999)**	347.16 – 1128.9	453.98 – 1171.7
Fish eggs	***Sardina Pilchardus***	30	**Pereira et al. (2014)**	8.36 – 27.19	10.93 – 28.22
Fish eggs	***Gadus Morhua***	43	**Jørgensen (1985)**	5.83 – 18.97	7.63 – 19.69
Fish Larvae	***Sardina Pilchardus***	50	**Pereira et al. (2014)**	5.02 – 16.31	6.56 – 16.93
Fish Larvae	***Gadus Morhua***	100	**Jørgensen (1985)**	2.51 – 8.16	3.28 – 8.47

In this work we use as example the impact on the trophic systems in the water of the Canary archipelago where jellyfish play a relevant role as both predator and prey (Couce-Montero et al., 2015) and *P. noctiluca* periodically blooms (Rodríguez et al., 2015). Based on the studies of zooplankton abundances measured in the waters around Gran Canaria (Table 4.3) we can estimate the density of *P. noctiluca* necessary to have a destructive effect in the same waters (Table 4.3).

Table 4.3. *P. noctiluca* biomass concentration ([PN]) necessary to potentially consume a certain prey concentration ([Prey]) daily. The calculations are based on prey concentrations calculations in the surrounding waters of Gran Canaria based on the literature and shelf prey percentages of Sabatés et al. (2010) (%prey, 8% for fish larvae, 72% for zooplankton). [PN] = [Prey]/(*RCD*(Φ) * (%prey) (For [Prey] in Ind m^{-3} units, potential-daily-prey-consumption can be used instead of *RCD*). The PN units are g WM m^{-3}.

	[Prey]		Reference	[PN]
Zooplankton	1087	Ind m^{-3}	**Gómez & Hernández-León (1997)**	7.13 – 18.41
	610.94	Ind m^{-3}	**Mingorance et al. (2004)**	4.01 - 10.35
	2.46	mg C m^{-3}	**Hernández-León et al. (2007)**	4.04 - 10.42
	0.60	Ind m^{-3}	**Landeira et al. (2009)**	0.004 - 0.010
	5.50	mg C m^{-3}	**Hernández-León et al. (2010)**	9.02 - 23.27
	4.98	mg C m^{-3}	**Moyano & Hernández-León (2011)**	8.16 - 21.07
	11.01	mg C m^{-3}	**Schmoker et al. (2012)**	18.06 - 46.62
	6.44	Ind m^{-3}	**Landeira & Lozano-Soldevilla (2018)**	0.04 - 0.11
Fish Larvae	0.34	Ind m^{-3}	**Bécognée et al. (2006)**	0.02 - 0.05
	0.33	Ind m^{-3}	**Moyano et al. (2009)**	0.02 - 0.05
	0.72	Ind m^{-3}	**Moyano & Hernández-León (2011)**	0.04 - 0.11

4.3.4. Heterotrophic energy transformation (*HET*) from respiration

As in the case of *RCD* in Table 4.1, the respiration results from the literature can be used to calculate *HET* based on equation 3. The results of these calculations are shown in Table 4.4. In our study, we calculate the average *HET* and average potential *HET* in *A. aurita* to be 8.18±5.28 and 10.03±5.31 J d^{-1} g WM^{-1} and in *P. noctiluca* to be 4.39±1.67 and 11.05±4.88 J d^{-1} g WM^{-1}. If *HET* were measured in other organisms, we may, in the future, be able to assess and compare their energy transforming capabilities and study energy circulation (Karl, 2014). This may also be useful to address Margalef's hypothesis regarding complexity and energy expenditure (Margalef, 1963). Inspired by a calculation of energy release in mitochondria by Lane (2015), we compare the *HET* by scyphozoan mitochondria with the mass-specific energy-release rate of the sun. The sun releases 0.017 J d^{-1} g^{-1} (Williams, 2018). From Table 4.4, the resulting average *HET* was approximately 6 J d^{-1} g $WM^{-1}$and the average potential *HET* was approximately 10 J d^{-1} g WM^{-1} of both species together. Consequently, the scyphozoan WM specific heterotrophic energy transformation is more than 500-times, greater than the sun's energy release rate! (If we used jellyfish dry-mass in the calculation, the difference would be more than a thousand-fold greater).

Table 4.4. Ranges of wet-mass-specific, *HET* calculated for *A. aurita* and *P. noctiluca*, from *R*, Φ, and *HET* calculated from respiration measurements in the literature.

	HET **(J d^{-1} g WM^{-1})**	**Reference**
A. aurita	2.05 - 21.43	**This study (*R*)**
	2.33 - 22.21	**This study (Φ)**
	0.71 - 2.14	**Larson (1987b)**
	0.66	**Schneider (1989)**
	1.32 - 10.53	**Båmstedt (1990)**
	2.63 - 69.22	**Båmstedt et al. (1994)**
	0.19 - 1.50	**Ishii & Tanaka (2001)**
	1.13 - 5.74	**Uye & Shimauchi (2005)**
	0.23 - 5.34	**Ishii & Tanaka (2006)**
	0.99 – 1.35	**Han et al. (2012)**
	1.07 - 3.16	**Iguchi et al. (2017)**
P. noctiluca	1.47 - 9.39	**This study (*R*)**
	3.91 - 25.49	**This study (Φ)**
	17.31	**Davenport & Trueman (1985)**
	3.89 - 100.89	**Malej (1989)**
	0.88 - 14.58	**Malej et al. (1993)**
	0.02 - 2.11	**Lilley & Lombard (2015)**

4.4. DISCUSSION

4.4.1. Measuring *R* and Φ

The differences between *Pelagia noctiluca* and *Aurelia aurita* have several possible explanations (Figs. 4.1, 4.2). As mentioned in the results section, feeding may affect *R*:Φ ratios as observed by Hernández-León & Gómez (1996), Packard et al. (1996), Herrera et al. (2011) and Osma et al. (2016) where well-fed organisms have higher respiration and the ratio decreased in organisms as they were denied food. This trend was based on a comparison with an expected basal *R*:Φ ratio of 0.5 (50%, Cleland (1967)). Also, the different CV:WM ratios could also explain part of the difference between the results of the two species since smaller incubation volumes may repress respiratory metabolism Purcell et al. (2010), hence lower *R*. These enzymatic measurements are useful to

promote our understanding of the respiratory metabolism of these jellyfish. At least, they are as useful as biomass measurements (Figs. 4.1, 4.2, and 4.3) which are traditionally used to study metabolic processes (Brown et al., 2004). However, biomass, alone, does not convey information about the physiological state of an organism, but combined with enzymatic measurements, it can (Osma et al., 2016).

Iguchi et al. (2017) reported ETS activities in 10 *A. aurita* medusae (Table 4.5). They used this information to estimate *R* in the giant jellyfish *Nemopilema nomurai*. They saw great potential in the application of the ETS assay, but were cautious about their results, advocating further studies in their final paragraphs. The results here for the 6 medusae closest in size to medusae in their study (Table 4.5) showed a considerable difference. The difference between studies may have several explanations. We measured R with optodes and they used the Winkler technique which only measured the initial and final O_2 concentrations from the incubations. It is an endpoint-type of analysis. Furthermore, their long (24 h) incubations may have led to O_2-limited respiration (Gnaiger, 2001; Purcell et al., 2010).

Table 4.5. Comparison between ETS results (mean ± s.d.) on *A. aurita* medusae from Iguchi et al. (2017) and from our study. Note that in the literature. The ratio, *R*/Φ, is often referred to as *R*/ETS (Filella et al., 2018).

	Iguchi et al. (2017)	**This Study**
n	10	6
Size range (g WM)	0.7 - 1.9	0.7 - 1.5
***R* (µl $O_2 \cdot h^{-1} \cdot$ g WM^{-1})**	5.9 ± 1.4	28.6 ± 8
Φ (µl $O_2 \cdot h^{-1} \cdot$ g WM^{-1})	7.1 ± 2.6	52.1 ± 6.8
***R*/Φ**	0.9 ± 0.3	0.57 ± 0.2

For example, if the jellyfish were incubated in 250 mL with seawater (35 salinity, 20°C) assuming 1 atm pressure, the initial O_2 would be approximately 5.4 mL L^{-1}. This means that there would have been 1.3 mL O_2 in the 250-mL container. Given the average respiration rate obtained in our study, 28.6 µl O_2 h^{-1} g WM^{-1}, a jellyfish, of 1 g, would have consumed 0.7 mL of O_2 after 24 h, more than 50% of the original amount. Also, if the jellyfish, in the experiments of Iguchi et al. (2017), were not swimming properly during the incubation, i.e., if no "active swimming" was observed for 24 h, they would have consumed less O_2 than they normally would (Purcell et al. 2010).

In addition, there are other factors in the study of Iguchi et al. (2017), apart from the deterioration mentioned in their article, that could have led to a loss in enzymatic activity. They describe freezing the homogenates, instead of the samples, with liquid nitrogen (-196°C) and then storing them at -80°C. In our experience, this leads to much loss in enzyme activity within 24 h (Ahmed et al., 1976). Furthermore, they also used the substrate concentrations of Owens & King (1975) which we have found could slightly underestimate ETS activity in scyphozoans. They also used the extinction coefficient of INT, 15.9 mM^{-1} cm^{-1}, from Packard & Williams (1981) rather than determining the INT-formazan extinction coefficient for their own solutions. Most research teams do the same, but we have found that this practice can lead to underestimations of ETS. In summary, Iguchi et al. (2017) inadvertently, may have underestimated both respiration and ETS activity. To resolve this type of discrepancy in the literature, an international

intercalibration workshop or program needs to be organized for ETS analysis. In the late 1980s this was done for DOC analysis. More recently, in 2014, SCOR working-group 144 held a workshop in Vancouver, BC to compare measurements of DNA, RNA, and protein (Torres-Beltrán et al., 2019). A similar workshop is needed for ETS analysis.

4.4.2. Comparison of *RCD* and *HET* with the Literature

In the case of *A. aurita*, in general, the results from this study are higher than most other similar estimations (Table 4.1). This could correspond once again to the feeding conditions. For example, the jellyfish in our experiments were fed before the respiratory measurements, while Uye & Shimauchi (2005) collected the medusae from the ocean and left them for a 2h acclimation and then proceeded to measure their respiration. The short unknown "starvation" period could have allowed the jellyfish to maintain their relatively low metabolism compared to the fed conditions of our study. In other cases, as in Larson (1987b), the use of antibiotics to decrease the associated bacterial respiration without knowing the impact of the antibiotics on the jellyfish's metabolism could be responsible of these differences. The reason for the discrepancy is a quandary.

Båmstedt (1990) and Båmstedt et al. (1994) (Table 4.1) reported exceptional "carbon specific daily rations" of up to 40%, in high prey abundance conditions. Båmstedt et al. (1994), working with natural zooplankton samples, obtained "carbon specific daily rations" greater than 100% for *A. aurita* ephyrae and greater than 250% for medusae when the food source was composed of high density large mesozooplankters (>1000μm). Hence, the *MCR* asociated with respiration was shown to be higher after copious food supply and, under adequate conditions, the carbon ingestion exceeded the organisms' own carbon mass. This implied that metabolism can adapt to encounters of prey aggregations by increasing consumption rates (Omori et al., 1995). This is also reflected in the closeness between *R* and Φ in the case of *A. aurita*.

From our *A. aurita* ephyrae (E) *RCD* results, we calculated the average *RCD* and potential *RCD* per individual (2.88±1.09 and 4.47±2.36 μg C ind^{-1} d^{-1}) and compared it to the daily carbon feeding rate in ephyrae of *Aurelia coerulea* (6.3 μg C ind^{-1} d^{-1} as determined from ciliate clearance rates (Kamiyama, 2018)). Assuming close biological similarity between the two species of *Aurelia*, this comparison suggests that our *RCD* calculations constitute 46 to 71% of the total carbon requirements of *Aurelia* ephyra. There are fewer results of *P. noctiluca* respiration with which to compare, but this study's measurements seem to fall in range with other observations (Table 4.1). However, there is a lot of dispersion arguing that more studies are necessary. Since *HET* is calculated from respiration too, it also was in range with values calculated from the literature (Table 4.4) and the same points discussed for *RCD* could explain some of variability in *HET* calculations.

4.4.3. Estimations based on the *RCD* and *HET* calculated

In 2012 there was an unprecedented bloom of *P. noctiluca* along the coast of Gran Canaria associated with circulation shifts in the Mediterranean Sea. On one northern beach, only, there were reports of a total of 13 tonnes of *P. noctiluca* carcasses being cleaned from the sands reaching 3 tonnes in 3 days (Deidun, 2012; Rodríguez et al., 2015). Based on the values shown in Table 4.5, this bloom (smack) density could have had a big impact on the local zooplankton and fish larvae (Table 4.3). The impact would have been particularly noticeable on the northern area because zooplankton communities there are less abundant (Landeira et al., 2009) and because it is where *P.*

noctiluca first arrived according to regional circulation (Rodríguez et al., 2015). After this event of 2012, monitoring programs such as Red Promar employed the concept of citizen science to document the occurrence of *P. noctiluca*. In 2014 this group reported sightings through out the north and east coast of the island near areas studied for fish larvae populations (Moyano et al., 2009). Accordingly, one can assume that these areas were also impacted during the 2012, 2014 and 2019 events. The impacts of these jellyfish smacks consuming prey can be more devastating if their timing coincides with the highest abundance of fish larvae (Moyano et al., 2009; Tilves et al., 2016) (Table 4.3). A chance encounter of jellyfish with high prey density would clearly increase consumption rates (Omori & Hamner, 1982; Malej, 1989). Since prey are not distributed homogenously in the water column and since they migrate nocturnally (Landeira et al., 2009; Tilves et al., 2016), the encounter and its impact could be more severe. Malej (1989) noted that *P. noctiluca* interactions within an ecosystem differed greatly between dispersed and aggregated distributions. They observed scenarios where the zooplankton stock would be eliminated in less than 2 days and scenarios where it would take 100 days. Hence *P. noctiluca* aggregations could overwhelm regional populations.

On a much larger scale, Lucas et al. (2014) estimated that the total gelatinous zooplankton biomass, in the upper 200m of the world ocean, was 38.3 Tg C (1 Tg = 10^{12} g). Of this amount, 92% were cnidarians (35.24 Tg C). Based on the average physiological *MCR* (17.67±10.82% g C d^{-1} g C^{-1}) and potential *MCR* (24.48±12.48% g C d^{-1} g C^{-1}) of the two species of this study (Table 4.1) and the estimated biomass of planktonic cnidarian in the upper 200m (Lucas et al., 2014), we can estimate that the cnidarian respiratory carbon consumption range in the upper 200m of the world ocean is between 6.23 - 8.71 Tg C d^{-1} (2.27 – 3.15 Gt C y^{-1}) (1 Gt = 10^{15}g). This value will correspond to approximately 21.9 – 30.3% of the upper 200m global mesozooplankton respiratory carbon in the world ocean (10.4 Gt C y^{-1} (28.49 Tg C d^{-1})), according to the review of Hernández-León & Ikeda (2005). This would imply an impact of 3.8-5.3% on the sum of the global primary production and allocthonous organic matter input (59.2 Gt C y^{-1}) (Del Giorgio & Duarte, 2002; Hernández-León & Ikeda, 2005; Steinberg & Landry, 2017). If the calculations were made with the sum of the global primary production and allocthonous organic matter input, according to Behrenfeld & Falkowski (1997) and Behrenfeld et al. (2005) (43.5 - 67 Gt C y^{-1}), the percentage impact would be similar.

Karl (2014) encourages a better understanding of energy dynamics in our ecosystems. To help achieve this objective, the *HET* was calculated (Table 4.4). Packard et al. (2015) proposed *HET* as a helpful tool to assess respiration-related energy flow in aquatic ecosystems. Using the average *HET* and potential *HET* of the two species (0.139 – 0.192 KJ h^{-1} g C^{-1}, we calculated from *HET* (Table 4.4), biomass, and the biomass of cnidarians (35.24 Tg C) in the upper 200m of the world ocean (Lucas et al., 2014), we estimate the *HET* associated with cnidarians in the upper 200 m of the world ocean to range from 0.117 to 0.162 EJ d^{-1} (1 EJ = 10^{18} J). This energy, released through the oxidation of organic matter, is equivalent to 2 – 3% of the energy fixed from the sun during photosynthetic primary production according to the energy calculations of Dewar et al. (2006) (62.7 TW (5.42 EJ d^{-1})) (Behrenfeld & Falkowski, 1997). If the energy stored by photosynthesis in the form of glucose (Dewar et al., 2006; Barber, 2009) is equivalent to 2813 KJ mol^{-1} and the energy stored in form of ATP during the respiratory process of glucose is 1900 KJ mol^{-1}, then, there is an energy expenditure 913 KJ mol^{-1}; the efficiency of the process is close to 68% as described by Barber (2009). As a result, the loss, due to consumption of intermediary organisms in the food-web up to the cnidarian predator,

will condition the efficiency of this energy transfer and increase the percentage of energy released before the cnidarian *HET*.

HET can also be compared to the solar energy that reaches ocean surface using the values of Trenberth et al. (2009) (161 TW m^{-2}) and the global ocean surface area used in Lucas et al. (2014) (361,900,000 km^2). The resulting estimation of the global solar energy that reaches earth is approximately 5034 EJ d^{-1}. Based on these values for solar energy input and primary production (Dewar et al., 2006; Trenberth et al., 2009), the percentage of solar energy fixed by the ocean is 0.108% which coincides with the estimations of 0.1% by Barber (2009). The percentage of this initial solar energy reaching the ocean's surface, that is then used in respiration by the described cnidarian population, is approximately 0.0023 – 0.0032%.

Energy-wise, jellyfish are an interesting member of marine ecosystems because they have a low energy density but a high digestion speed, which makes it a useful alternative prey to several opportunistic predators (Arai et al., 2003; Hays et al., 2018). Arai (1997) published the approximate average values of the gross energy density of *A. aurita* and *P. noctiluca* tissue as 2.95 and 3.6 KJ g DM^{-1}, respectively (Doyle et al., 2007). The values for *P. noctiluca* agree with Milisenda et al. 2014 (gonad tissue,11.51 KJ g DM^{-1}; somatic tissue, 2.19 KJ g DM^{-1}). When the values from Arai (1997) are normalized by WM (106.2 and 192.6 J g WM^{-1}), using the conversions cited earlier we can compare it with our average composition based energy densities (676±1556 and 193±273 J g WM^{-1} in *A. aurita* and *P. noctiluca*, respectively). The energy content in the literature differs widely with the composition based energy density here presented. This could be due to the large energy density in smaller sized samples with higher lipid concentration (Fig. 4.4). The ranges are very wide, hence the values in the literature fall inside the results obtained here. We can normalize *HET* by energy density i.e. energy specific *HET*. In *A. aurita*, energy specific *HET* and potential energy specific HET ranged from 0.2 to 15.8% J d^{-1} J^{-1} and from 0.1 to 28.6% J d^{-1} J^{-1}, respectively. In *P. noctiluca* the ranges were 0.5 to 7.7% J d^{-1} J^{-1} and 1 to 26.7% J d^{-1} J^{-1}. Since energy density was higher in smaller samples the energy specific HET increased with size in both species. While in smaller samples respiration required around 1% of their energy content daily, in the largest sample this number was closer to 15 or 20% daily. Energy specific *HET* and energy specific potential *HET* using the energy density measured by Arai (1997) results in 7.71 and 9.45% d^{-1} in *A. aurita* and 2.28 and 5.74% d^{-1} in *P. noctiluca*. So, to satisfy their respiratory metabolic demands these organisms transformed, daily, less than 10% of their bodies' worth in energy. This type of energy analysis, as well as the other calculations described in this section may allow new perspectives on the contributions of different groups of organisms to the energy flow and balances of the biosphere.

4.5. CONCLUSIONS

The regression analysis of the results of this study show good linear and log-linear relationships between WM and *R* and between Φ and *R*. The resulting log-linear regression analysis, for the combined data of *Aurelia aurita* and *Pelagia noctiluca*, of *R* with WM and Φ resulted in the equations: $R = 20.15\ WM^{0.93}$ (r^2=0.97) and $R = 0.78\ \Phi^{0.90}$ (r^2=0.97). Determination of Φ through ETS analysis is a useful tool to study the respiratory physiology of jellyfish. These respiratory parameters can advance understanding of jellyfish impacts on ecosystems through calculations of *RCD* and *HET*.

The average potential *RCD*s determined in this study were 0.53±0.28 and 0.59±0.26 mg C d^{-1} g WM^{-1} for *A. aurita* and *P. noctiluca* respectively.
The results of this investigation revealed the prey-comsumption impact of *P. noctiluca* blooms in the waters of Gran Canaria. They showed that blooms of 20 g WM m^{-3} could severely affect zooplankton and fish larvae populations.

Based on this study's results and literature estimations of global jellyfish populations these medusae may account for nearly a third of the mesozooplankton's respiratory carbon oxidation in the upper 200m. This is 3.8 to 5.3% of the carbon produced in primary production. The *HET* is equivalent to between 2 and 3% of the energy fixed by primary production.

The potential *HET* associated with the mitochondria of *A. aurita* was 10.03±5.31 and of *P. noctiluca* 11.05±4.88 J d^{-1} g WM^{-1}. This is nearly 1000 times greater than the mass-specific-energy-production rate of the sun!

Simultaneously, studying the composition it was observed that the lipid content was higher in smaller sizes and decrease with size. This affected the energy content of the samples and has implications on the ecology and biochemistry of the jellyfish as they age.

REFERENCES

Ahmed, S. I., R. A. Kenner, & F. D. King, 1976. Preservation of enzymic activity in marine plankton by low-temperature freezing. Marine Chemistry 4: 133–139.

Alberty, R. A., & R. N. Goldberg, 1992. Standard Thermodynamic Formation Properties for the Adenosine 5′-Triphosphate Series. Biochemistry 31: 10610–10615.

Arai, M. N., 1997. A funcional biology of Scyphozoa.—Chapman S Hall. London, Weinheim.

Arai, M. N., D. W. Welch, A. L. Dunsmuir, M. C. Jacobs, & A. R. Ladouceur, 2003. Digestion of pelagic Ctenophora and Cnidaria by fish. Canadian Journal of Fisheries and Aquatic Sciences 60: 825–829.

Bailey, T. G., M. J. Youngbluth, & G. P. Owen, 1995. Chemical composition and metabolic rates of gelatinous zooplankton from midwater and benthic boundary layer environments off Cape Hatteras, North Carolina, USA. Marine Ecology Progress Series 122: 121–134.

Båmstedt, U., 1990. Trophodynamics of the scyphomedusae *Aurelia aurita*. Predation rate in relation to abundance , size and type of prey organism. Journal of Plankton Research 12: 215–229.

Båmstedt, U., M. B. Martinussen, & S. Matsakis, 1994. Trophodynamics of the two scyphozoan jellyfishes, *Aurelia aurita* and *Cyanea capillata*, in western Norway. ICES Journal of Marine Science 51: 369–382.

Barber, J., 2009. Photosynthetic energy conversion: Natural and artificial. Chemical Society Reviews 38: 185–196.

Barnes, H., & J. Blackstock, 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilun method for ‘total’lipids. Journal of Experimental Marine Biology and Ecology Elsevier 12: 103–118.

Bécognée, P., C. Almeida, A. Barrera, A. Hernández-Guerra, & S. Hernández-León, 2006. Annual cycle of clupeiform larvae around Gran Canaria Island, Canary Islands. Fisheries Oceanography 15: 293–300.

Behrenfeld, M. J., E. Boss, D. A. Siegel, & D. M. Shea, 2005. Carbon-based ocean productivity and phytoplankton physiology from space. Global Biogeochemical Cycles 19:.

Behrenfeld, M. J., & P. G. Falkowski, 1997. Photosynthetic rates derived from satellite-based chlorophyll concentration. Limnology and Oceanography 42: 1–20.

Bligh, E. G., & W. J. Dyer, 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology NRC Research Press 37: 911–917.

Boero, F., 2013. Review of jellyfish blooms in the Mediterranean and Black Sea. Studies and Reviews. General Fisheries Commission for the Mediterranean. Rome, http://www.vliz.be/en/imis?refid=226251.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, & G. B. West, 2004. Toward a metabolic theory of ecology. Ecology Wiley Online Library 85: 1771–1789.

Canepa, A., V. Fuentes, A. Sabatés, S. Piriano, F. Boero, & J. M. Gili, 2014. *Pelagia noctiluca* in the Mediterranean In Pitt, K. A., & C. H. Lucas (eds), Jellyfish Blooms. Springer Science, Dordrecht: 1–304.

Chiaverano, L. M., K. L. Robinson, J. Tam, J. J. Ruzicka, J. Quiñones, K. T. Aleksa, F. J. Hernandez, R. D. Brodeur, R. Leaf, S. Uye, M. B. Decker, M. Acha, H. W. Mianzan, & W. M. Graham, 2018. Evaluating the role of large jellyfish and forage fishes as energy pathways, and their interplay with fisheries, in the Northern Humboldt Current System. Progress in Oceanography 164: 28–36.

Choy, C. A., S. H. D. Haddock, & B. H. Robison, 2017. Deep pelagic food web structure as revealed by in situ feeding observations. Proceedings. Biological sciences 284:.

Clarke, A., L. J. Holmes, & D. J. Gore, 1992. Proximate and elemental composition of gelatinous zooplankton from the Southern Ocean. Journal of Experimental Marine Biology and Ecology 155: 55–68.

Cleland, W. W., 1967. Enzyme kinetics. Annual review of biochemistry Annual Reviews 4139 El Camino Way, PO Box 10139, Palo Alto, CA 94303-0139, USA 36: 77–112.

Condon, R. H., D. K. Steinberg, P. A. del Giorgio, T. C. Bouvier, D. A. Bronk, W. M. Graham, & H. W. Ducklow, 2011. Jellyfish blooms result in a major microbial respiratory sink of carbon in marine systems. Proceedings of the National Academy of Sciences 108: 10225–10230.

Couce-Montero, L., V. Christensen, & J. J. Castro, 2015. Effects of small-scale and recreational fisheries on the Gran Canaria ecosystem. Ecological Modelling Elsevier B.V. 312: 61–76, http://dx.doi.org/10.1016/j.ecolmodel.2015.05.021.

Davenport, J., & E. R. Trueman, 1985. Oxygen uptake and buoyancy in zooplanktonic organisms from the tropical eastern atlantic. Comparative Biochemistry and Physiology Part A: Physiology 81A: 857–863.

Deidun, A., 2012. Lessons from Chadwick Lakes. Allied Newspapers Ltd.

Del Giorgio, P. A., & C. M. Duarte, 2002. Respiration in the open ocean. Nature 420: 379–384, www.nature.com/nature.

Dewar, W. K., R. J. Bingham, R. L. Iverson, D. P. Nowacek, L. C. St. Laurent, & P. H. Wiebe, 2006. Does the marine biosphere mix the ocean?. Journal of Marine Research 64: 541–561.

Doubleday, Z. A., & S. D. Connell, 2018. Weedy futures: can we benefit from the species that thrive in the marine Anthropocene?. Frontiers in Ecology and the Environment 16: 599–604.

Doyle, T. K., J. D. R. Houghton, R. McDevitt, J. Davenport, & G. C. Hays, 2007. The energy density of jellyfish: Estimates from bomb-calorimetry and proximate-composition. Journal of Experimental Marine Biology and Ecology 343: 239–252.

Dubois, M., K. Gilles, J. K. Hamilton, P. A. Rebers, & F. Smith, 1956. A colorimetric method for the determination of sugars. Nature 168: 167.

Ferguson, S. J., 2010. ATP synthase: From sequence to ring size to the P/O ratio. Proceedings of the National Academy of Sciences 107: 16755–16756.

Filella, A., I. Baños, M. F. Montero, N. Hernández-Hernández, A. Rodríguez-Santos, A. Ludwig, U. Riebesell, & J. Arístegui, 2018. Plankton community respiration and ETS activity under variable CO2 and nutrient fertilization during a mesocosm study in the subtropical North Atlantic. Frontiers in Marine Science 5: 1–11.

Gambill, M., & M. A. Peck, 2014. Respiration rates of the polyps of four jellyfish species: Potential thermal triggers and limits. Journal of Experimental Marine Biology and Ecology 459: 17–22.

Gnaiger, E., 2001. Bioenergetics at low oxygen: Dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. Respiration Physiology 128: 277–297.

Gomez, M., & S. Hernandez-Leon, 1997. Estudio de la comunidad mesozooplantónica en relación a un efecto de isla en aguas de Gran Canaria. VIERAEA 26: 11–21.

Haddock, S. H. D., 2004. A golden age of gelata: past and future research on planktonic ctenophores and cnidarians. Hydrobiologia 530: 549−556.

Han, C., J. Chae, J. Jin, & W. Yoon, 2012. Estimation of the minimum food requirement using the respiration rate of medusa of *Aurelia aurita* in Sihwa Lake. Ocean Science Journal 47: 155–160.

Hansson, L. J., O. Moeslund, T. Kiørboe, & U. Riisgård, 2005. Clearance rates of jellyfish and their potential predation impact on zooplankton and fish larvae in a neritic ecosystem (Limfjorden, Denmark). Marine Ecology Progress Series 304: 117–131.

Hays, G. C., T. K. Doyle, & J. D. R. Houghton, 2018. A Paradigm Shift in the Trophic Importance of Jellyfish?. Trends in Ecology and Evolution 33: 874–884.

Henschke, N., J. D. Everett, A. J. Richardson, & I. M. Suthers, 2016. Rethinking the Role of Salps in the Ocean. Trends in Ecology and Evolution Elsevier Ltd 31: 720–733, http://dx.doi.org/10.1016/j.tree.2016.06.007.

Hernández-León, S., G. Franchy, M. Moyano, I. Menéndez, C. Schmoker, & S. Putzeys, 2010. Carbon sequestration and zooplankton lunar cycles: Could we be missing a major component of the biological pump?. Limnology and Oceanography 55: 2503–2512.

Hernández-León, S., & M. Gómez, 1996. Factors affecting the respiration/ETS ratio in marine zooplankton. Journal of Plankton Research 18: 239–255.

Hernández-León, S., M. Gómez, & J. Arístegui, 2007. Mesozooplankton in the Canary Current System: The coastal-ocean transition zone. Progress in Oceanography 74: 397–421.

Hernández-León, S., & T. Ikeda, 2005. A global assessment of mesozooplankton respiration in the ocean. Journal of Plankton Research 27: 153–158.

Herrera, A., M. Gómez, L. Molina, F. Otero, & T. Packard, 2011. Rearing techniques and nutritional quality of two mysids from Gran Canaria (Spain). Aquaculture Research 42: 677–683.

Iguchi, N., H. Iwatani, K. Sugimoto, S. Kitajima, N. Honda, & O. Katoh, 2017. Biomass, body elemental composition, and carbon requirement of *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) in the southwestern Japan Sea. Plankton and Benthos Research 12: 104–114.

Ikeda, H., C. Mizota, & S. I. Uye, 2017. Bioenergetic characterization in *Aurelia aurita* (Cnidaria: Scyphozoa) polyps and application to natural polyp populations. Marine Ecology Progress Series 568: 87–100.

Ishii, H., & F. Tanaka, 2001. Food and feeding of *Aurelia aurita* in Tokyo Bay with an analysis of stomach contents and a measurement of digestion times. Hydrobiologia 451: 311–320.

Ishii, H., & F. Tanaka, 2006. Respiration rates and metabolic demands of *Aurelia aurita* in Tokyo Bay with special reference to large medusae. Plankton Benthos Research 1: 64–67, https://www.jstage.jst.go.jp/article/pbr/1/1/1_1_64/_pdf.

Jørgensen, L., 1985. Carbon and Nitrogen Utilization in Developing Eggs and Larvae of Cod. Fisheries Research 3: 337–342.

Kamiyama, T., 2018. Planktonic ciliates as food for the scyphozoan *Aurelia coerulea*: feeding and growth responses of ephyra and metephyra stages. Journal of Oceanography Springer Japan 74: 53–63.

Karl, D. M., 2014. Solar energy capture and transformation in the sea. Elementa: Science of the Anthropocene 2:.

King, F. D., A. H. Devol, & T. T. Packard, 1978. Plankton metabolic activity in the eastern tropical North Pacific. Deep-Sea Res 25: 689–704.

Knight, J. A., S. Anderson, & J. M. Rawle, 1972. Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. Clinical Chemistry 18: 199–202.

Landeira, J. M., & F. Lozano-Soldevilla, 2018. Seasonality of planktonic crustacean decapod larvae in the subtropical waters of Gran Canaria Island, NE Atlantic ; Estacionalidad de las larvas de crustáceos decápodos en las aguas subtropicales de la isla de Gran Canaria, Atlántico NE. Scientia Marina 82: 119–134, https://doi.org/10.3989/scimar.04683.08A.

Landeira, J. M., F. Lozano-soLdevilla, S. Hernández-León, & E. D. Barton, 2009. Horizontal distribution of

invertebrate larvae around the oceanic island of Gran Canaria: the effect of mesoscale variability. Scientia Marina 73: 214–8358.

Lane, N., 2006. Power, sex, suicide: mitochondria and the meaning of life. Oxford University Press.

Lane, N., 2015. The vital question: energy, evolution, and the origins of complex life. WW Norton & Company.

Larson, R. J., 1987a. Respiration and Carbon Turnover Rates of Medusae from the NE Pacific. Comparative Biochemistry and Physiology Part A: Physiology 87: 93–100, https://www.sciencedirect.com/science/article/pii/0300962987904300.

Larson, R. J., 1987b. A Note on the Feeding, Growth, and Reproduction of the Epipelagic Scyphomedusa *Pelagia noctiluca* (Forskal). Biological Oceanography 4:.

Lilley, M. K. S., & F. Lombard, 2015. Respiration of fragile planktonic zooplankton: Extending the possibilities with a single method. Journal of Experimental Marine Biology and Ecology 471: 226–231.

Lindley, J. A., 1999. Dry weight carbon and nitrogen content of some euphausiids from the north Atlantic Ocean and the Celtic Sea. Journal of Plankton Research 21: 2053–2066.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, & R. J. Randall, 1951. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry 193: 265–275.

Lucas, C. H., 1994. Biochemical composition of *Aurelia aurita* in relation to age and sexual maturity. Journal of Experimental Marine Biology and Ecology 183: 179–192.

Lucas, C. H., D. O. B. Jones, C. J. Hollyhead, R. H. Condon, C. M. Duarte, W. M. Graham, K. L. Robinson, K. A. Pitt, M. Schildhauer, & J. Regetz, 2014. Gelatinous zooplankton biomass in the global oceans: Geographic variation and environmental drivers. Global Ecology and Biogeography 23: 701–714.

Makridis, P., & O. Vadstein, 1999. Food size selectivity of *Artemia franciscana* at three developmental stages. Journal of Plankton Research 21: 2191–2201.

Maldonado, F., T. T. Packard, & M. Gómez, 2012. Understanding tetrazolium reduction and the importance of substrates in measuring respiratory electron transport activity. Journal of Experimental Marine Biology and Ecology 434–435: 110–118.

Malej, A., 1989. Behaviour and trophic ecology of the jellyfish *Pelagia noctiluca* (Forsskhl, 1775) malej 1989. Journal of Experimental Marine Biology and Ecology 126: 259–270, https://www.researchgate.net/publication/229086652_Behaviour_and_trophic_ecology_of_the_jellyfish_Pelagia_noctiluca_Forssk.

Malej, A., J. Faganeli, & J. Pezdič, 1993. Stable isotope and biochemical fractionation in the marine pelagic food chain: the jellyfish *Pelagia noctiluca* and net zooplankton. Marine Biology: International Journal on Life in Oceans and Coastal Waters 116: 565–570.

Malej, A., & A. Malej, 2004. Invasion of the Jellyfish *Pelagia noctiluca* in the Northern Adriatic: a non-success story In Dumont, H. J., T. A. Shiganova, & U. Niermann (eds), Aquatic Invasions in the Black, Caspian, and Mediterranean Seas. Dordrecht: 273–285.

Margalef, R., 1963. On Certain Unifying Principles in Ecology. The American Naturalist XCVII: 357–374.

Markwell, M. A. K., S. M. Haas, L. L. Bieber, & N. E. Tolbert, 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Analytical Biochemistry 87: 206–210.

Martínez, I., A. Herrera, M. Tames-espinosa, D. R. Bondyale-juez, V. Romero-kutzner, T. T. Packard, & M. Gómez, 2020. Protein in marine plankton: A comparison of spectrophotometric methods. Journal of Experimental Marine Biology and Ecology Elsevier 526: 151357.

Milisenda, G., S. Rosa, V. L. Fuentes, F. Boero, L. Guglielmo, J. E. Purcell, & S. Piraino, 2014. Jellyfish as prey: Frequency of predation and selective foraging of *Boops boops* (vertebrata, actinopterygii) on the mauve stinger *Pelagia noctiluca* (cnidaria, scyphozoa). PLoS ONE 9: 1–10.

Milisenda, G., S. Rossi, S. Vizzini, V. L. Fuentes, J. E. Purcell, U. Tilves, & S. Piraino, 2018. Seasonal variability of diet and trophic level of the gelatinous predator *Pelagia noctiluca* (Scyphozoa). Scientific Reports 8: 12140.

Mills, C. E., 1995. Medusae, siphonophores, and ctenophores as planktivorous predators in changing global ecosystems Introduction Observations from blue-water diving and. ICES J. mar. Sci 52: 575–581.

Mingorance, M. C., F. Lozano-Soldevilla, J. A. Garcia-Braun, J. M. Landeira, J. M. Espinosa, & J. I. Gómez, 2004. Estudio de la distribución vertical de la comunidad mesozooplanctónica en aguas de la isla de Tenerife (Islas Canarias). Revista de la Academia Canaria de Ciencias 114: 99–114.

Mitchell, P., 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 189: 291–292.

Mitchell, P., 2011. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biochimica et Biophysica Acta - Bioenergetics Elsevier B.V. 1807: 1507–1538, http://dx.doi.org/10.1016/j.bbabio.2011.09.018.

Møller, L. F., & H. U. Riisgård, 2007. Respiration in the scyphozoan jellyfish *Aurelia aurita* and two hydromedusae (*Sarsia tubulosa* and *Aequorea vitrina*): Effect of size, temperature and growth. Marine Ecology Progress Series 330: 149–154.

Moran, L. A., H. R. Horton, K. G. Scrimgeour, & M. D. Perry, 2012. Principles of biochemistry. Pearson Boston.

Morand, P., C. Carré, & D. C. Biggs, 1987. Feeding and metabolism of the jellyfish *Pelagia noctiluca* (scyphomedusae, semaeostomae). Journal of Plankton Research 9: 651–665.

Moyano, M., & S. Hernández-León, 2011. Intra- and interannual variability in the larval fish assemblage off Gran Canaria (Canary Islands) over 2005-2007. Marine Biology 158: 257–273.

Moyano, M., J. M. Rodríguez, & S. Hernández-León, 2009. Larval fish abundance and distribution during the late winter bloom off Gran Canaria Island, Canary Islands. Fisheries Oceanography 18: 51–61.

Nagata, R. M., & A. C. Morandini, 2018. Diet, prey selection, and individual feeding rates of the jellyfish *Lychnorhiza lucerna* (Scyphozoa, Rhizostomeae). Marine Biology Springer Berlin Heidelberg 165: 1–17.

Ochoa, S., 1943. Efficiency of aerobic phosphorylation in cell-free heart extracts. Journal of Biological Chemistry 151: 493–505.

Odum, E. P., 1968. Energy flow: A historical review. Am. Zoologist 8: 11–18.

Omori, M., 1969. Weight and chemical composition of some important oceanic zooplankton in the North Pacific Ocean. Marine Biology 3: 4–10.

Omori, M., 1978. Some Factors Affecting on Dry Weight, Organic Weight and Concentrations of Carbon and Nitrogen in Freshly Prepared and in Preserved Zooplankton. Internationale Revue der gesamten Hydrobiologie und Hydrographie 63: 261–269.

Omori, M., & W. M. Hamner, 1982. Patchy distribution of zooplankton: Behavior, population assessment and sampling problems. Marine Biology 72: 193–200.

Omori, M., H. Ishii, & A. Fujinaga, 1995. Life history strategy of *Aurelia aurita* (Cnidaria, Scyphomedusae) and its impact on the zooplankton community of Tokyo Bay. ICES Journal of Marine Science 52: 597–603.

Osma, N., I. Fernández-Urruzola, M. Gómez, S. Montesdeoca-Esponda, & T. T. Packard, 2016. Predicting in vivo oxygen consumption rate from ETS activity and bisubstrate enzyme kinetics in cultured marine zooplankton. Marine Biology 163: 146.

Owens, T. G., & F. D. King, 1975. The measurement of respiratory electron-transport-system activity in marine zooplankton. Marine Biology 30: 27–36.

Packard, T., E. Berdalet, D. Blasco, S. O. Roy, L. St-Amand, B. Lagacé, K. Lee, & J. P. Gagné, 1996. CO2 production predicted from isocitrate dehydrogenase activity and bisubstrate enzyme kinetics in the marine bacterium *Pseudomonas nautica*. Aquatic Microbial Ecology 11: 11–19.

Packard, T. T., 1971. The Measurement of Respiratory Electron-transport Activity tn Marine Phytoplankton. Journal of Marine Research 29: 235–244.

Packard, T. T., 1985. Measurement of electron transport activity of marine microplankton . In Williams, P. J., & H. W. Jannasch (eds), Advances in Aquatic Microbiology. Academic Press, New York: 207–261.

Packard, T. T., D. Harmon, & J. Boucher, 1974. Respiratory electron transport activity in plankton from upwelled waters. Tethys 6: 213–222.

Packard, T. T., N. Osma, I. Fernández-Urruzola, L. A. Codispoti, J. P. Christensen, & M. Gómez, 2015. Peruvian upwelling plankton respiration: Calculations of carbon flux, nutrient retention efficiency, and heterotrophic energy production. Biogeosciences 12: 2641–2654.

Packard, T. T., & P. J. Williams, 1981. Rates of respiratory oxygen consumption and electron transport in surface seawater from the northwest Atlantic. Oceanologica Acta 4: 351–358.

Pauly, D., W. Graham, S. Libralato, L. Morissette, & M. L. Deng Palomares, 2009. Jellyfish in ecosystems, online databases, and ecosystem models. Hydrobiologia 616: 67–85.

Pereira, R., M. A. Teodósio, & S. Garrido, 2014. An experimental study of *Aurelia aurita* feeding behaviour: Inference of the potential predation impact on a temperate estuarine nursery area. Estuarine, Coastal and Shelf Science 146: 102–110.

Pitt, K. A., C. H. Lucas, R. H. Condon, C. M. Duarte, & B. Stewart-Koster, 2018. Claims That Anthropogenic Stressors Facilitate Jellyfish Blooms Have Been Amplified Beyond the Available Evidence: A Systematic Review. Frontiers in Marine Science 5: 1–11.

Purcell, J. E., 1997. Pelagic cnidarians and ctenophores as predators : Selective predation, feeding rates, and effects on prey populations. Annales de l'Institut océanographique 73: 125–137, http://cat.inist.fr/?aModele=afficheN&cpsidt=2441173.

Purcell, J. E., 2003. Predation on zooplankton by large jellyfish, *Aurelia labiata*, *Cyanea capillata* and *Aequorea aequorea*, in Prince William Sound, Alaska. Marine Ecology Progress Series 246: 137–152, https://www.int-res.com/abstracts/meps/v246/p137-152/.

Purcell, J. E., & M. N. Arai, 2001. Interactions of pelagic cnidarians and ctenophores with fish: a review. Hydrobiologia 451: 27–44.

Purcell, J. E., V. Fuentes, D. Atienza, U. Tilves, D. Astorga, M. Kawahara, & G. C. Hays, 2010. Use of respiration rates of scyphozoan jellyfish to estimate their effects on the food web. Hydrobiologia 645: 135–152.

Purcell, J. E., U. Tilves, V. L. Fuentes, G. Milisenda, A. Olariaga, & A. Sabatés, 2014. Digestion times and predation potentials of *Pelagia noctiluca* eating fish larvae and copepods in the NW Mediterranean Sea. Marine Ecology Progress Series 510: 201–213.

Purcell, J. E., S. I. Uye, & W. T. Lo, 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: A review. Marine Ecology Progress Series 350: 153–174.

Raskoff, K., F. Sommer, W. Hamner, & K. Cross,

2003. Collection and Culture Techniques for Gelatinous Zooplankton. The Biological Bulletin 204: 68–80, http://www.biolbull.org/cgi/content/abstract/204/1/68%5Cnpapers2://publication/uuid/13C25FD6-CECC-46B7-9395-58F050AD1722.

Richardson, A. J., A. Bakun, G. C. Hays, & M. J. Gibbons, 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends in Ecology and Evolution 24: 312–322.

Rodríguez, G., G. Clarindo, & L. McKnight, 2015. Jellyfish outbreaks in coastal city beaches from a management perspective. Coastal Cities and their Sustainable Future 1: 277–288.

Rutter, W. J., 1967. Protein determinations in embryos In Wilt, F. H., & N. K. Wessels (eds), Methods in developmental biology. New York: 671–683.

Sabatés, A., F. Pagès, D. Atienza, V. Fuentes, J. E. Purcell, & J. M. Gili, 2010. Planktonic cnidarian distribution and feeding of *Pelagia noctiluca* in the NW Mediterranean Sea. Hydrobiologia 645: 153–165.

Schiariti, A., M. S. Dutto, A. Carrara Morandini, R. M. Nagata, D. Y. Pereyra, F. A. Puente Tapia, L. Díaz Briz, & G. Genzano, 2018. An Overview of the Medusozoa from the Southwestern Atlantic In Hoffmeyer, M. S., M. E. Sabatini, F. P. Brandini, D. L. Calliari, & N. H. Santinelli (eds), Plankton Ecology of the Southwestern Atlantic. Springer Nature 2018, Gewerbestrasse, Switzerland: 413–449.

Schmoker, C., J. Arístegui, & S. Hernández-León, 2012. Planktonic biomass variability during a late winter bloom in the subtropical waters off the Canary Islands. Journal of Marine Systems Elsevier B.V. 95: 24–31, http://dx.doi.org/10.1016/j.jmarsys.2012.01.008.

Schneider, G., 1989. Estimation of food demands of Aurelia aurita medusae populations in the kiel bight/western baltic. Ophelia 31: 17–27.

Schneider, G., & G. Behrends, 1998. Top-down control in a neritic plankton system by *Aurelia aurita* medusae — a summary. Ophelia 48: 71–82.

Shimauchi, H., & S. I. Uye, 2007. Excretion and respiration rates of the scyphomedusa *Aurelia aurita* from the Inland Sea of Japan. Journal of Oceanography 63: 27–34.

Steinberg, D. K., & M. R. Landry, 2017. Zooplankton and the Ocean Carbon Cycle. Annual Review of Marine Science 9: 413–444, http://www.annualreviews.org/doi/10.1146/annurev-marine-010814-015924.

Stoett, P. J., 2019. Global ecopolitics: Crisis, governance, and justice. University of Toronto Press.

Thuesen, E. V., & J. J. Childress, 1994. Oxygen consumption rates and metabolic enzyme activities of oceanic California Medusae in relation to body size and habitat depth. Biological Bulletin 187: 84–98.

Tilves, U., J. E. Purcell, V. L. Fuentes, A. Torrents, M. Pascual, V. Raya, J. M. Gili, & A. Sabatés, 2016. Natural diet and predation impacts of *Pelagia noctiluca* on fish eggs and larvae in the NW Mediterranean. Journal of Plankton Research 38: 1243–1254.

Torres-Beltrán, M., A. Mueller, M. Scofield, M. G. Pachiadaki, C. Taylor, K. Tyshchenko, C. Michiels, P. Lam, O. Ulloa, K. Jürgens, J.-H. Hyun, V. P. Edgcomb, S. A. Crowe, & S. J. Hallam, 2019. Sampling and Processing Methods Impact Microbial Community Structure and Potential Activity in a Seasonally Anoxic Fjord: Saanich Inlet, British Columbia. Frontiers in Marine Science 6: 1–16, https://www.frontiersin.org/article/10.3389/fmars.2019.00132/full.

Trenberth, K. E., J. T. Fasullo, & J. Kiehl, 2009. Earth's Global Energy Budget. Bulletin of the American Meteorological Society 90: 311–323, http://journals.ametsoc.org/doi/abs/10.1175/2008BAMS2634.1.

Uye, S., & H. Shimauchi, 2005. Population biomass, feeding, respiration and growth rates, and carbon budget of the scyphomedusa *Aurelia aurita* in the Inland Sea of Japan. Journal of Plankton Research 27: 237–248.

Walve, J., & U. Larsson, 1999. Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. Journal of Plankton Research 21: 2309–2321.

Williams, D. R., 2018. Sun Fact Sheet. Nasa Official. , https://nssdc.gsfc.nasa.gov/planetary/factsheet/sunfact.html.

CHAPTER 5

OCEAN SAILORS, *Physalia physalis* AND *Velella velella*: ECOPHYSIOLOGICAL REFLECTIONS OF THEIR BIOCHEMICAL COMPOSITION AND RESPIRATORY METABOLISM

ABSTRACT

In this study we shed light on the role as prey and predator of two important representatives of the widely spread pleustonic ecosystem, *Physalia physalis* and *Velella velella*. We verify the implications their wind driven drifting nature has on their metabolisms and test if their composition matches that of other consumed gelatinous species. To investigate their nutritional value as prey we analysed the biochemical composition (wet and dry biomass, Protein (P), Lipid (L), Carbohydrate (K)) of the two hydrozoans and calculated the corresponding energy content (Ec). To study the relation of their physiology with their passive propulsion and to estimate their impact as predators we estimated carbon demand associated with their respiratory metabolism (physiological respiration (R) and potential respiration (Φ)). The water content in these organisms was lower than other gelatinous zooplankton probably due to the presence of their chitin structures. The biochemical composition per dry mass fell inside the range for gelatinous zooplankton in the literature with slightly higher protein content and Ec. The Ec per wet mass (WM) was in general higher than other gelatinous species excluding arctic specimens. The respiratory metabolism per protein content of both hydrozoans was in general lower than in pulsating jellyfish. The R:Φ was low compared to previous studies on zooplankton. The respiratory carbon demand was lower than the carbon demand calculated demand from prey ingestion studies. There was a relation between the respiration and their drifting, fairly inactive, lifestyle compared to other more active members of the gelatinous zooplankton. The P, L and K content of these pleustonic hydrozoans resembles that of other predated gelatinous zooplankton but in general have lower water content. We propose that these results supported both hypotheses. These results are the first published respiration measurements for both species. Further research is needed to test alternative explanations to these observations.

5.1. INTRODUCTION

Physalia physalis (Linnaeus, 1758) (Portuguese man-o-war) is a well-known cystonect siphonophore, a hydrozoan, which lives in the air-sea water interface thanks to its specialized buoyant sail-like crest, the pneumatophore. Probably its pleustonic nature, living at the very surface of the ocean, and its world-wide distribution has made it more accessible and led it to be the first formally described siphonophore (Mapstone, 2014). However, to cultivate it in the laboratory, specialized aquarium design is required that has retarded its study more so than the study of other cnidarians (Pierce, 2006). Biologically, each individual is composed of a colony of zooids, separate but dependant animals, each providing specific functions to the colony (Mackie et al., 1988; Munro et al., 2019). Similarly, *Velella velella* (Linnaeus, 1758) is another pelagic colonial hydrozoan, of the order Anthoathecata, suborder Capitata, circulating in air-sea interface driven by the wind on its chitin sail-like structure (Bieri, 1977; Schuchert, 2010). Both jellyfish rely on wind for their propulsion through the ocean.

In addition, both species provide regulating, supporting and cultural services for ecological and societal benefits associated with jellyfish, according to the nomenclature of Doyle et al. (2014). More attention is now being paid to these organisms as smacks of them are increasingly invading beaches and harbours reducing their touristic value (Purcell et al., 2015; Araya et al., 2016; Betti et al., 2019) and, in the case of *P. physalis,* endangering tourists with their potent toxin (Prieto et al., 2015). In addition, these smacks impact local ecology due to *P. physalis'* predation on fish and fish larvae, and *V. velella*'s predation on zooplankton and fish eggs (Bieri, 1966; Purcell, 1984; Purcell et al., 2015; Zeman et al., 2018). Addressing the impact of these and other gelatinous zooplankton is a priority in order to understand their competition with and predation on fish (Pauly et al., 2009). In particular, studying the physiological requirements of these organisms can help quantify the prey necessary to sustain them, as well as, illuminate the relationships between lifestyle and metabolism. Respiration (R), involving incubations, measures the carbon and oxygen (O_2) consumption by the organisms. However, with gelatinous zooplankton, these incubations may encounter logistical issues (Purcell et al., 2010), especially if the jellyfish under study are floating colonial ones. So, to overcome the problems of measuring respiration directly, we tested the use of an enzymatic technique by measuring the activity of the respiratory electron transport system (ETS), the potential respiration (Φ), that drives physiological respiration (Chance & Williams, 1955a, 1955b, 1955c; Osma et al., 2016; Gnaiger et al., 2019). Having both R and Φ should allow us to assess how much of its respiratory potential capacity the organism is using.

During respiration, organic carbon is oxidized to CO_2 to release energy in the form heat or stored in the form of adenosine triphosphate (ATP). From the respiratory rate we can determine the energy (heterotrophic energy transformation previously named heterotrophic energy production (Packard et al., 2015)) and respiratory carbon demand (Uye & Shimauchi, 2005) associated with this O_2 consumption. Since these pleustonic predators can form big blooms that swarm through small areas (Fleming et al., 2013; Prieto et al., 2015; Ferrer & Pastor, 2017; Pires et al., 2018; Ghilardi, 2019), quantifying the carbon demand of these organisms will also facilitate assessment of the potential impact these blooms preying on fish larvae and zooplankton. We also wanted to study the relation between the respiratory metabolism and the locomotion of gelatinous zooplankton. When thinking of jellyfish moving through the ocean we envision an umbrella-shaped jelly, like *Aurelia aurita*, calmly propelling itself along by pulsations to destinations unknown. It's propulsion efficiency is very high (Gemmell et al., 2014, 2020;

Hoover & Miller, 2015), yet for its cousins, *Physalia physalis and Velella velella,* who exploit the wind for their locomotion, their propulsion efficiency should be even more efficient. We hypothesized that metabolic indices such biomass-specific physiological respiration (R), potential respiration (Φ) and derived parameters such as HEP (heterotrophic energy production) or the R:Φ ratio should be lower in *P. physalis and V. velella* than in pulsating hydrozoans or scyphozoans. Here, taking advantage of chance-beaching of smacks of *P. physalis and V. velella* on the north coast of Gran Canaria Island, we were able to, both, examine this hypothesis and quantify the predation of these pleustonic hydrozoans by calculating their basal carbon demand from respiration measurements.

On the other hand, apart from their predatory nature, these hydrozoans are prey for a wide variety of organisms. Many such as turtles, nudibranchs, cirripeds, crabs, birds, cephalopods, sharks, fish (especially sunfish) have been described eating these jellyfish (Bieri, 1966; Jenkins, 1983; Arai, 1988, 2005; Frick et al., 2009; Griffin et al., 2019; Thiebot & McInnes, 2020).

According to Hays et al. (2018) the trophic importance of jellyfish is experiencing a paradigm shift. Based on the review by Thiebot & McInnes (2020), energy content, in some cases, is insufficient to explain predation. This begs further research on capture practice, opportunism and self-medication using jellyfish. Furthermore, along with these new perspectives, both articles agree that further studies on the dietary "value" of gelatinous prey are needed. *P. physalis* and *V. velella* are actively hunted by several gelativore predators, we hypothesized that the biochemical and energy content per wet mass (WM) of these two species was at least equal to that of other gelatinous zooplankton because they experience a considerable amount of predation. We suspect the biochemical and energy content per WM may be higher because these species have chitinous structures absent other more fragile gelatinous zooplankton. To understand the nutritional value of *P. physalis* and *V. velella,* protein, lipid, carbohydrate content (biochemical composition) and their associated energy value were determined analytically. The intent was to provide insight into the role of these pleustonic creatures, not only as predators, but also as prey, i.e., insight on integral members of mostly overlooked food webs. Besides, it may inform on the feeding strategies of gelativore predators, in general.

In summary, in this work we provided the first biochemical analysis on protein, lipid, carbohydrate composition, energy content, as well as the first measurements of respiration in *P. physalis* and *V. velella* both using incubations and enzymatic techniques. The respiration results were then used to determine heterotrophic energy transformation (HET) and respiratory carbon demand (RCD) and compared with carbon ingestion calculated from ingestion rates in the literature. These parameters help shed light on the physiological respiratory activity of these organisms, which we hypothesized would be low due to their wind propelled locomotion; and their trophic importance as prey, which we hypothesized would be higher per wet mass than other gelatinous organism with higher water content.

5.2. MATERIALS AND METHODS

5.2.1. Specimen collection, sample storage, and biomass determination

Both *V. velella* and *P. physalis* specimens were collected in the north-eastern beaches of Gran Canaria (Canary Island, Spain). These organisms arrived naturally, in large numbers (smacks), in January and February 2017. They were carefully collected by gloved-hands while being beached by the tide and then transported live in containers half full of seawater. Once in the laboratory, some samples were stored as described in the sample storage section and other were maintained alive in approximately 40 L (40 cm depth) tanks with filtered sea water that was changed daily. Organisms in tanks had their pneumatophore periodically moistened and rinsed using pipettes and spray bottles, and also manually moved away from the tank glass edges. After measuring physiological respiration (R), as described in the following subsection, sample wet mass (WM) was determined. To measure WM, samples were blotted dry using a paper towel by putting plastic netting between the sample and the paper to prevent the paper sticking to the sample. Then the samples were stored in labelled plastic bags at -80ºC. Subsequently, extra samples were stored, following this procedure. Another group of samples (*P. physalis*, n=60, WM range: 0.27 - 15.31 g; *V. velella*, n=24, WM range: 0.02 - 0.60 g) were used to determine the percentage of dry mass (DM). These organisms were dried at 60ºC in aluminium foil and weighted periodically until the mass stopped changing significantly (24 - 60 h). The final measure was recorded as the DM.

5.2.2. Physiological respiration (R) (O_2 consumption), ETS analysis and potential respiration (Φ)

R was determined by monitoring O_2 consumption by the living samples during an incubation. The decrease in the O_2 dissolved in the filtered sea water inside the sealed incubation chambers was measured using O_2 optodes (Fibox-4 by Presens (Regensburg, Germany); Lilley & Lombard (2015)). The organisms were place carefully in the incubation chambers. Here, the volume of the chambers depended on the size of the organism. Either BOD bottles (~60 mL) or glass jars (~600 mL) sealed with Parafilm© under its lid (Purcell et al., 2010) were used. The floating nature of these organisms posed a challenge when deciding how the incubation would be performed. In the end, R was measured with the organism completely submerged since the tests with the incubation chambers half full gave lower results (Fig. 5.1). Blank seawater incubations, without organisms were performed to correct for background O_2 variations. All incubations took place in the dark at 18ºC during 2-3 h. The seawater was mixed slightly before O_2 measurements by magnetic stirring or by gently shaking the jar. These problems associated with incubating pleustonic organisms to determine respiration were avoided by using the enzymatic technique used next. The ETS analysis was based on Owens & King (1975) assay, modified for small reaction volumes and kinetic analysis according to Packard & Christensen (2004). To liberate the mitochondrial enzymes, the jellyfish tissue was homogenized in 0.1 M phosphate buffer (0.1 M Na_2HPO_4, 0.1 mM KH_2PO_4, 75 µM $MgSO_4 \cdot 7H_2O$, polyvinylpyrrolidone (PVP-40) (1.5 mg mL^{-1}), TRITON X-100 (2 mL L^{-1})) at 8.5 pH. The homogenization was performed with a motor-driven Teflon-glass tissue-grinder at 4000 rpm for 2-3 min using a glass microfibre filter (GF/C Circle, 25 mm) as an abrasive (Packard, 1971). Then a known aliquot of that homogenate was diluted to achieve a measureable concentration. The final volume-to-mass ratio after the dilution

was kept close to 30 mL homogenate per g sample never exceeding 60 mL homogenate per g sample. These homogenization steps were always performed at 0 - 4°C in an ice bath. The crude homogenate was then centrifuged at 4000 rpm for 10 min at 0 - 4°C. The resulting supernatant contained the enzymes for analysis. The enzyme analysis consisted of mixing, in a cuvette, 0.1 mL of the supernatant, 0.3 mL of the buffered substrates (NADH (1.7 mM) and NADPH (1.7 mM)) and 0.1 mL of INT solution (2 mg INT per mL deionized H_2O). Then the cuvette was quickly placed in the Cary100 UV-Vis Spectrophotometer and the reaction was monitored for 8 min at 490 nm as INT was reduced to a dark-red formazan. This batch of INT had an extinction coefficient of 12.8 mM^{-1} cm^{-1}. This extinction coefficient is necessary to calculate the concentration of formazan from the measured absorbance. The rate of formazan production is stoichiometrically related to the rate of O_2 reduction by those enzymes i.e. the potential respiration (Φ) associated with this enzymatic system (Packard, 1985). Blanks without homogenate and blanks without substrates were needed to control for any non-enzymatic and non-respiratory INT reduction (Maldonado et al., 2012). Spare crude homogenate was stored at -20°C for biochemical determinations.

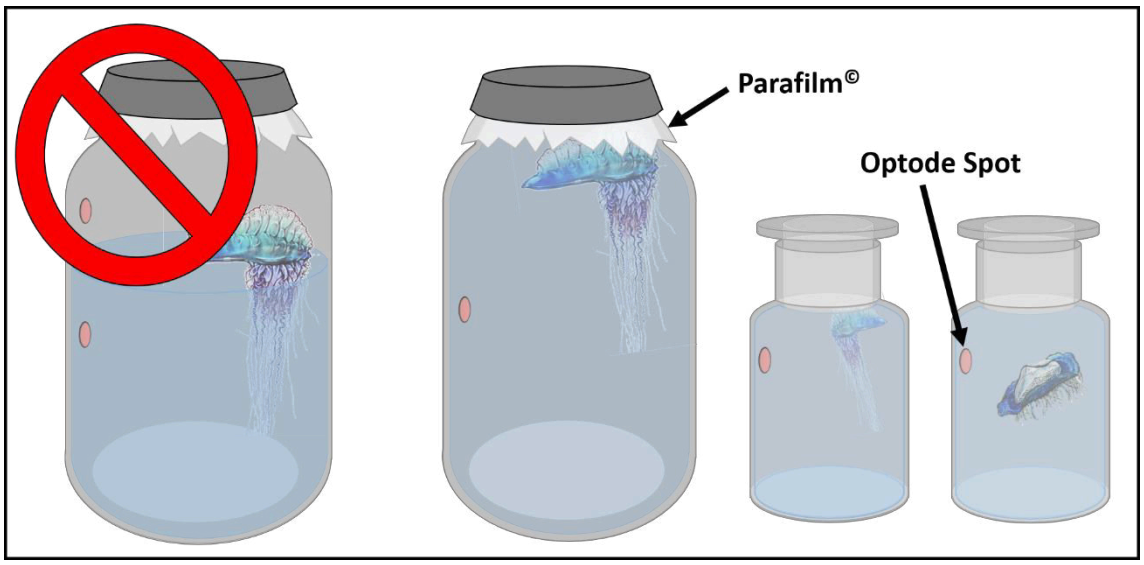


Figure. 5.1. Examples of the physiological respiration incubations of the hydrozoans in this study. Optodes spots installed inside. Left: Discarded half-filled incubation for *P. physalis*. Right: Completely full incubations of *P. physalis* and *V. velella* in 600 mL Jars or 60 mL BOD bottles depending on the size of the organism.

5.2.3. Carbon demand calculations and heterotrophic energy transformation (*HET*) from respiration

The daily carbon demand, associated with respiration (RCD), in mg C d^{-1} (g WM)$^{-1}$, can be calculated from the respiration, R, in µL O_2 h^{-1} (g WM)$^{-1}$, by eqn 5.1 (Uye & Shimauchi, 2005):

$$RCD = R \cdot RQ \cdot F \cdot 24 \cdot (10^3 \cdot AE)^{-1} \qquad (5.1)$$

where: RQ is the unitless ratio of CO_2-produced to O_2-consummed (0.85); F is the C:CO_2 conversion 0.536 µg C (µL CO_2)$^{-1}$; AE is the assimilation efficiency described by Schneider (1989) as the percentage of the carbon consumed that is assimilated, in this

case, 0.9 according to the findings of (Purcell & Kremer, 1983); 24 converts hours to days; and dividing by 10^3 converts micrograms to milligrams. The same respiration rates were used to calculate the heterotrophic energy transformation (HET) in units of J d^{-1} (g WM)$^{-1}$, the associated energetics of the adenosine triphosphate (ATP) production (Packard et al., 2015; Azzaro et al., 2019). It is calculated from R, in units of μmol O_2 h^{-1} (g WM)$^{-1}$, using eqn (5.2):

$$HET = R \cdot 2 \cdot 2.5 \cdot 0.048 \cdot 24 \qquad (5.2)$$

where 2 is the number of electron pairs that participate in reduction of one mole of O_2 to 2 moles of H_2O; 24 converts hours to a day; 2.5 is the modified P/O ratio, the ATP produced by the flow of one electron pair to atomic oxygen; and 0.048 is the energy asociated with ATP, ΔG in J (μmol ATP)$^{-1}$. The constants and ratios are explained in Alberty & Goldberg, 1992; Ferguson, 2010; Moran et al., 2012; Packard et al., 2015. The original concept can be found in Ochoa (1943). To calculate potential HET (p-HET) from ETS activity, one uses Φ instead of R.

5.2.4. Biochemical composition analysis: protein, lipid, carbohydrate & energy content determinations

The protein content was measured in the samples homogenized for the ETS assay and diluted with buffer, when necessary, to reach a ratio of 120 mL of homogenate per g of sample. The analysis was based on the Biuret reaction as described by Lowry et al. (1951) and modified by Rutter (1967). It was modified here by the addition of Dodecyl sulphate sodium salt (SDS) as suggested by Markwell et al. (1978) (Martínez et al., 2020). In brief, it consists of the reduction of phosphomolybdotungstate to heteropolymolybdenum, a spectrophotometric analysis, and a calculation based on a bovine serum albumin (BSA) standard-curve. The samples may require further dilution using the phosphate homogenization buffer to achieve measureable protein concentrations.

The lipid content was measured in samples homogenized as previously describe without any further dilution. The content was determined based on the "index of total lipids" as defined by Knight et al. (1972) (Marsh & Weinstein, 1966; Barnes & Blackstock, 1973; De Coen & Janssen, 1997). We followed the methodology described by Bligh & Dyer (1959) for the extraction of lipids using 250 μL of sample, 500 μL of chloroform, 500 μL of methanol and 200 μL of bi-distilled water; mixing well after every addition. This mixture was centrifuged in vials for 10 min at 2600 g and 4°C. 100 μL of the resulting bottom phase were mixed with 500 μL of H_2SO_4 (95%) and then charred in a muffle furnace at 200°C for 15 min. The charred sample solutions were cooled for 5 min and 40 μL were mixed with 1 mL of phospho-vanillin reagent. The mixture was then incubated in darkness at 37°C for 15 min. After 5 min of cooling the solutions' absorbance were measured at 525 nm in the spectrophotometer. A standard curve ranged from 0 and 4.8 mg mL^{-1} of olive oil dissolved in chloroform was used for calculations.

The carbohydrate content was measured in samples homogenized as described previously without any further dilution. The content was studied using the method proposed in Dubois et al. (1956) where 150 μL of the homogenate were mixed with 150 μL of phenol solution (5%) and 750 μL of sulfuric acid (95%). The solution was left for 10 min and then mixed with a vortex mixer and incubated in darkness for 10 min at 30°C. After cooling for 5 min the absorbance of the solution at 485 nm was measured spectrophotometrically. A standard curve of glucose dissolved in homogenization buffer ranging between 0 and 1.5 mg mL^{-1} was used.

Energy content, based on average enthalpies of combustion: 23.9, 39.5 and 17.5 kJ g^{-1} for protein, lipid and carbohydrate, respectively, was determined according to Clarke et al. (1992). The result of the sum of each component multiplied by its respective enthalpy of combustion provided an estimation of the energy associated with the biochemical composition of the organism.

5.3. RESULTS

5.3.1. Biomass: *WM*, *DM*, and biochemical composition.

The biochemical composition of *P. physalis* and *V. velella* has been measured for the first time and is summarized in Table 5.1. It provides ratios, factors for inter-conversion between WM, DM and biochemical content for these hydrozoans. The water content for *P. physalis* and *V. velella* was 85.24±4.08% and 90.68±1.06%, respectively. The WM ranges for the determination of DM/WM were 0.27-15.3g for *P. physalis* (n=60) and 0.02-0.60g for *V. velella* (n=24). For protein (P), the WM ranges were 0.08-10.60g for *P. physalis* (n=47) and 0.18-1.07g for *V. velella* (n=11). For lipid (L) and carbohydrate (K), the WM ranges were 0.55-2.18g for *P. physalis* (n=6) and 0.18-0.35 for *V. velella* (n=6). The colonies of the two species have different WM ranges; siphonophore colonies of *P. physalis* can reach heavier masses as they grow. WM-specific results appear to be higher in *P. physalis,* perhaps because it had a lower water content. However, the biochemical composition is similar when normalized by DM instead; with the exception of lipids which seem to be slightly higher in *P. physalis*. These new measurements in *P. physalis* and *V. velella* show the typical relative composition where carbohydrates are the least concentrated component and protein the most concentrated one. This composition is common amongst jellyfish (Lucas, 2009). Only in Khong et al. (2016) was the carbohydrate higher in their three edible jellyfish because carbohydrates were not measured but calculated from the other components. In our case, protein content was an order of magnitude higher than the other components and lipid was around double the carbohydrate content (Table 5.1).

Table 5.1. Biochemical composition for *P. physalis* and *V. velella*, in terms of means and standard deviations (SD) of dry mass (DM), protein (P), lipids (L) and carbohydrate (K), normalized by wet mass (WM) and * by estimated dry mass (DM).

	Physalia physalis		***Velella velella***	
	Mean	**SD**	**Mean**	**SD**
DM/WM (%)	14.76	4.08	9.32	1.06
P/WM (%)	3.17	1.24	2.39	0.76
L/WM (%)	0.62	0.19	0.24	0.15
K/WM (%)	0.3	0.05	0.17	0.08
P/DM (%)*	21.5	9.53	25.66	8.15
L/DM (%)*	4.23	1.3	2.52	1.65
K/DM (%)*	2.01	0.34	1.79	0.86

5.3.2. Respiratory metabolism

P. physalis and *V. velella* incubations were complicated by the buoyancy of these jellyfish. After observing a lower O_2 consumption signal in half-filled jars, we opted for submerging the jellyfish completely during the incubation (Fig. 5.1). R and Φ normalized by WM, estimated DM and P are displayed in Table 5.2. The respiratory results are similar in both colonial organisms. We used both physiological and enzymological respiratory results to calculate the R:Φ ratio. This, in theory, is the percentage of the potential respiratory activity that is being used for respiration by the sample or individual organism; it is also a stress index (St-Amand et al., 1999). Regarding *V. velella and P. physalis*, both have a low R:Φ ratio (*V. velella*: 0.09 ± 0.04, *P. physalis*: 0.11 ± 0.08) indicating that only about 10% of the respiratory capacity is being used by the jellyfish. The rest, 90%, is held in reserve. In other zooplankton, according to Hernández-León & Gómez (1996), the R:Φ ratio is closer to the value of 0.50, the ratio that would be expected for optimum enzyme control according to Cleland (1967). When the carbon demand (RCD) associated with the metabolism (R and Φ) was calculated the physiological and the potential RCD was determined in mg C, d^{-1} (g WM)$^{-1}$: 0.72 ± 0.38 & 7.83 ± 3.59 for *P. physalis* and 0.68 ± 0.33 & 7.89 ± 2.55 for *V. velella*.

Table 5.2. Respiratory metabolism as means and standard deviations (SD). Both R and Φ are normalized by wet mass (WM), by estimated dry mass (DM), and by protein (P). The sample WM range was 0.174 - 5.81 g (n=11) for *P. physalis* and 0.193 - 1.07 g (n=5) for *V. velella*.

	Physalia physalis		***Velella velella***	
	Mean	**SD**	**Mean**	**SD**
R (µL O_2 h^{-1} (g WM)$^{-1}$)	59.48	31.53	56.62	27.43
R (µL O_2 h^{-1} (mg DM)$^{-1}$)	0.40	0.21	0.61	0.29
R (µL O_2 h^{-1} (mg P)$^{-1}$)	1.81	0.73	2.94	1.12
Φ (µL O_2 h^{-1} (g WM)$^{-1}$)	644.69	295.44	649.66	210.02
Φ (µL O_2 h^{-1} (mg DM)$^{-1}$)	4.37	2.00	6.97	2.25
Φ (µL O_2 h^{-1} (mg P)$^{-1}$)	19.40	6.23	36.19	13.51
R/Φ	0.11	0.08	0.09	0.04

5.3.3. Energy content (Ec) and heterotrophic energy transformation (HET)

According to Table 5.3, the average WM-specific Ec, based on the biochemical composition, in *P. physalis* (0.98±0.35 kJ (g WM)$^{-1}$) was slightly above that in *V. velella* (0.67±0.24 kJ (g WM)$^{-1}$). However, when normalized by dry mass instead, the Ec was similar in both hydrozoan colonies (6.63±2.67 kJ (g DM)$^{-1}$ for *P. physalis* and 7.16±2.55 kJ (g DM)$^{-1}$) for *V. velella*, Table 5.3). The average WM-specific HET (15.29±8.11 and 14.56±7.05 J d^{-1} (g WM)$^{-1}$) and potential HET (165.78±75.97 and 167.06±54.01 J d^{-1} (g WM)$^{-1}$) were similar between both colonies, which was expected since R and Φ were also

similar (Table 5.2). These parameters facilitate analysis based on energy instead of biomass and provide information energy flux through an ecosystem that elemental and mass analysis cannot (Karl, 2014). Here, we divided HET by Ec (Table 5.3) to obtain another metric, the energy-content-specific heterotrophic energy transformation, i.e., the energy released during respiration normalized by the energy available in the biochemical composition. It is a form of energy transformation efficiency (HET/Ec) where a low number signifies high efficiency and a high number, the reverse. In Table 5.3, we calculate HET/Ec and find that both *P. physalis* and *V. velella* use, in respiration, an equivalent of approximately 2% of their energy content and a maximum of around 20%. The inverse of the Ec-specific HEP and potential HEP (Ec/HEP) showed that it takes between 65 and 6 days for *P. physalis* to use, through respiration, its own energy content and between 46 and 4 days in the case of *V. velella*.

Table 5.3. Wet mass (WM) and dry mass (DM)-specific energy content (Ec) determined from biochemical composition and WM-specific heterotrophic energy transformation (HET) and potential HET (p-HET) derived from physiological respiration (R) and potential respiration (Φ).

	Physalia physalis		***Velella velella***	
	Mean	**SD**	**Mean**	**SD**
Ec (kJ (g WM)$^{-1}$)	0.98	0.35	0.67	0.24
Ec (kJ (g DM)$^{-1}$)	6.63	2.67	7.16	2.55
HET (R) (J d^{-1} (g WM)$^{-1}$)	15.29	8.11	14.56	7.05
p-HET (Φ) (J d^{-1} (g WM)$^{-1}$)	165.78	75.97	167.06	54.01
HET (R)/Ec (% d^{-1})	1.56%	0.83%	2.18%	1.06%
p-HET (Φ)/Ec (% d^{-1})	16.94%	7.76%	25.03%	8.09%

5.4. DISCUSSION

5.4.1. Biomass & biochemical composition

Both *V. velella* and *P. physalis* possess resistant chitin structures essential for their floatability and survivability in the pleuston (Munro et al., 2019). These structures may increase their DM:WM ratio compared to other members of their respective orders. The average water content per WM in these samples (Table 5.1) are the lowest percentages of water-content found in the medusae, ctenophores and thaliaceans compiled by Bailey et al. (1995), Ikeda (2014) and Lucas et al. (2011). The water-content in those similarly soft-bodied organisms ranged between 95 and 97.6% (92.82 exceptionally in pyrosomes). *P. physalis* was even closer to crustacean and fish water-content which ranged between 63.8 and 88.9% (Bailey et al., 1995). Similarly, when compared with other DM:WM ratios, that included diverse gelatinous zooplankton, the DM:WM ratio of the polychaete *Tomopteris* was, at 15.49%, the closest to our hydrozoans (Clarke et al., 1992). The other organisms in Clarke's study, which included a siphonophore and an

athecate hydroid, ranged between 4 and 5%. Normalizing by WM or DM gave different interpretations so both results were provided. Hence, a gelativore predator preying upon *P. physalis* or *V. velella* will ingest less water per gram ingested compared to other gelatinous prey of the same wet mass.

To avoid the difference in water-content, the following comparisons are DM-specific. We compared the protein (P), lipid (L) and carbohydrate (K) content with similar observations in the literature. In general, the P/DM in *P. physalis* and *V. velella* at 21.5 ± 9.53% and 25.66 ± 8.15% was higher than in most other hydrozoans and Scyphozoans (Table 5.1, Fig. 5.2), but lower than *Aglantha digitale* (56.5%, 21.6 - 22.1%), *Hybocodon prolifer* (23 - 31%), gonad tissue of *Aurelia aurita* (2.07 - 28.56%), gonad and oral arm tissue of *Cyanea capillata* (22.6 – 34.7%) and some edible jellyfish species (19.95 - 53.87%) (Ikeda, 1972; Percy & Fife, 1981; Lucas, 1994; Doyle et al., 2007; Khong et al., 2016). These differences could be explained by the presence of protein based venoms in the case of *P. physalis*, (Tamkun & Hessinger, 1981), extracellular fluids (Lane et al., 1965), protein based blue pigments (Zagalsky & Herring, 1977) and chitin-protein complexes associated with the pneumatophore (Gainey, 1972; Roberts, 1992). These could increase the protein concentration in our jellyfish. Details on the amino acid composition of the protein content of *P. physalis* can be found in Gross et al. 1958 and Lane et al. (1965). The latter described the amino acid concentration in the extracellular fluid of *P. physalis* as 200 to 500 times higher than those in *A. aurita*.

Detailed descriptions of lipid classes in *P. physalis* can be found in Stillway (1976) and Joseph (1979) and in both *P. physalis* and *V. velella* in Lopes et al. (2016). The lipids described in those articles are responsible for the concentrations observed here (Table 5.1). The arachidonic acid (20:4ω6), eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3), have been proposed to be essential fatty acids that may explain jellyfish demand by some predators (Arai et al., 1989; Stenvers et al., 2020). All have been described in relative high percent of lipid composition in both *P. physalis* and *V. velella* (Stillway, 1976; Lopes et al., 2016). It should be mentioned that there have been mixed results in the detection of arachidonic acid in *P. physalis*. The average concentration of lipids appears to be slightly lower in *V. velella* than in *P. physalis*. This could be due to their diets (Lopes et al., 2016). Accordingly, the predation on fish larvae by *P. physalis* and copepods by *V. velella* affect their lipid composition so it could also affect lipid concentration. But the difference between the two could also be caused by the lipids associated to the nematocyst capsule and toxin of *P. physalis* (Stillway, 1974, 1976; Joseph, 1979).

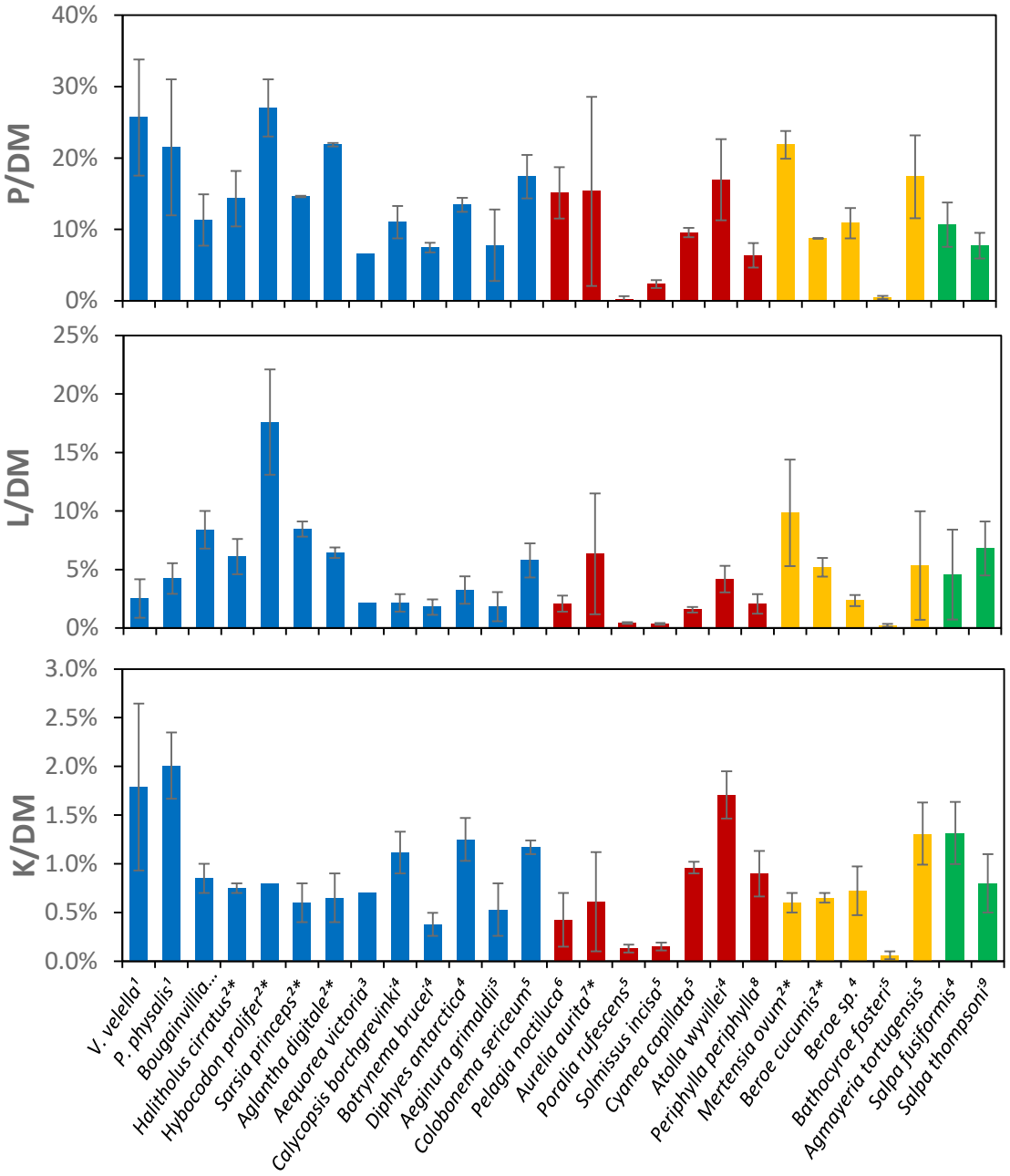


Figure 5.2. Compilation of gelatinous zooplankton biochemical composition measurements (mean±SD) from the literature with special attention to hydrozoan results. All values are normalized per dry mass (DM). Colours indicate the following gelatinous groups: Blue (Hydrozoan: Anthoathecata, Siphonophorae, Trachymedusae, Lepthothecata), red (Scyphozoan: Semaestomeae, Coronatae), yellow (Ctenophore), green (Thaliacean). Only articles containing protein (P), lipid (L) and carbohydrate (K) data were included. [1]This study's results, [2]Percy & Fife (1981) (Arctic species), [3]Arai et al. (1989), [4]Clarke et al. (1992), [5]Bailey et al. (1995), [6]Malej et al. (1993), [7]Lucas (1994), [8]Lucas (2009), [9]Dubischar et al. (2006), *Instead of mean±SD, these values represent the range (midpoint ± half the range).

Both lipid results (4.23±1.3% and 2.52±1.65%, Table 5.1) fall in range with observations in the literature of other hydrozoans and Scyphozoans (Fig. 5.2) (Clarke et al., 1992; Malej et al., 1993; Lucas, 1994; Bailey et al., 1995) and are above the lipid content of the edible jellyfish in Khong et al. (2016). However, as shown in Percy & Fife (1981), Larson

& Harbison (1989) and Nelson et al. (2000), *P. physalis* and *V. velella* have lower *L/DM* than Arctic gelatinous species (7.7 - 31%). Larson & Harbison (1989) suggested that difference in lipid content was caused by diet since Arctic prey are high in lipid during certain times of the year. The lipid content in *V. velella* can be affected by the lipids produced by the symbiotic dinoflagellates and by the exposure to light during its pleustonic life (Mortillaro et al., 2009).

The carbohydrate content in the species analysed in this study (2.01±0.34% and 1.79±0.86%, Table 5.1) was within, or slightly above, the range found in the literature for hydrozoans and scyphozoans (0.13 – 1.7%) (Fig. 5.2) (Larson & Harbison, 1989; Clarke et al., 1992; Malej et al., 1993; Lucas, 1994; Bailey et al., 1995; Doyle et al., 2007; Lucas, 2009). Even including crustaceans and fish (Bailey et al., 1995), the K/DM of the two hydrozoans of this study were some of the highest results except for the edible jellyfish species in Khong et al. (2016), probably because, instead of measuring, they calculated K based on the other components. Carbohydrates are in general the lowest of the three biochemical components. The composition of this carbohydrate content has been analysed by Gross et al. (1958), paying particular attention to *P. physalis*' float, where it showed a high hexose concentration but similar to other invertebrates in that study. Chitin structures present in both species studied may be affecting the measurement of carbohydrate in their tissue.

The biochemical analysis show that these two hydrozoans have a comparable, if not higher, content of protein, lipid and carbohydrate per DM but the content of this biochemical components per WM is much higher than most other gelatinous zooplankton with the exception of lipids in certain arctic species. As we hypothesised, *P. physalis* and *V. velella* would provide predators of more organic content and less water than other gelatinous zooplankton prey of the same WM.

5.4.2. Respiratory metabolism

Due to logistical problems associated with incubation, we encourage research on enzyme-based alternatives. Another reason for improving enzyme-based techniques is that they are useful when working with fragile gelatinous zooplankton which are often seriously damaged during sampling, hindering live measurements (Purcell et al., 2010).

Wet mass (WM)-specific results for *V. velella* and *P. physalis* (Table 5.2) appear to be greater than in scyphozoans used in similar respiratory analyses, specially the Φ (*A. aurita*: 31.82 ± 20.52 μL O_2 h^{-1} (g WM)$^{-1}$, 39.01 ± 20.66 μL O_2 h^{-1} (g WM)$^{-1}$; *Pelagia noctiluca*: 17.07 ± 6.49 μL O_2 h^{-1} (g WM)$^{-1}$, 42.96 ± 18.97 μL O_2 h^{-1} (g WM)$^{-1}$; R and Φ respectively; Chapter 4). The water content in this study's two hydrozoans was lower; so when normalized by dry mass the physiological respiration results (Table 5.2) were in range with the previous scyphozoan results (0.93 ± 0.60 μL O_2 h^{-1} (mg DM)$^{-1}$, 0.32 ± 0.12 μL O_2 h^{-1} (mg DM)$^{-1}$, *A. aurita* and *P. noctiluca* respectively). Nevertheless, Φ is much higher in our hydrozoans, in most cases. Protein-specific Φ in *A. aurita* and *P. noctiluca* were 32.95 ± 19.72 and 24.43 ± 11.33 μL O_2 h^{-1} (mg P)$^{-1}$, slightly higher than Φ in *P. physalis* but still under the Φ of *V. velella* (Table 5.2). *V. velella*'s high Φ could be partially explained by interference of the photosynthetic enzymatic machinery of the endosymbiotic algae (Bondyale-Juez et al., 2017, Chapter 2), but still, the reason for the high Φ in these two hydrozoans is still a conundrum that challenges our hypothesis.

We compared our R measurements with other values of R in the literature in both scyphozoans and hydrozoans (Fig. 5.3).

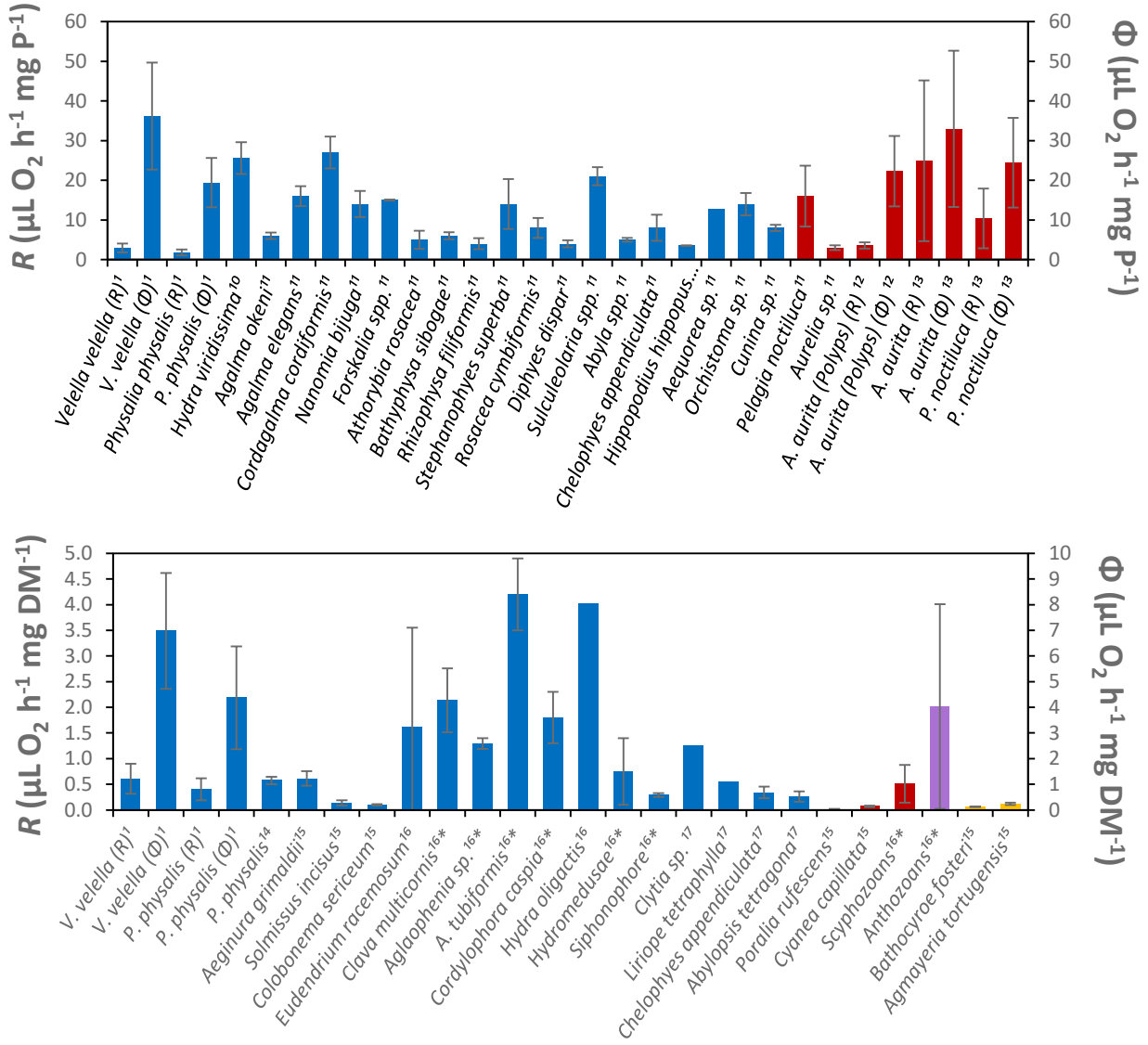


Figure 5.3. Compilation of gelatinous zooplankton respiration measurements from the literature with special attention on the hydrozoan respiratory measurements. Note the primary ordinate on the left is for physiological respiration (*R*) and the secondary ordinate on the right is for potential respiration (Φ). If the source of the data measured both R and Φ the species has a (R) or (Φ) to indicate so, otherwise measurements are R. Colours indicate the following gelatinous groups: Blue (Hydrozoan: Anthoathecata, Siphonophorae, Trachymedusae, Lepthothecata, Limnomedusae), orange (Scyphozoan: Semaestomeae, Coronatae), purple (Anthozoan), yellow (Ctenophore). Top: average respiratory values normalized by mg of protein (P). Bottom: average respiratory values normalized by mg of dry mass (DM). [1]This study's results, [10]Pardy & Dieckmann (1975), [11]Biggs (1977b), [12]Purcell et al. (2019), [13]Bondyale et al. unpublished, [14]Larimer & Ashby (1962), [15]Bailey et al. (1995), [16]Barangé et al. (1989), [17]Nival et al. (1972) *Instead of mean±SD, these values represent the range (midpoint ± half the range).

No other study of R in *V. velella* was found. *R* measurements in *P. physalis* tissue were reported in Larimer & Ashby (1962). Their results ranged from 75 – 106 µL O_2 h^{-1} (g WM)$^{-1}$ at 25°C, slightly above, but close to our results (59.48 ± 31.53 µL O_2 h^{-1} (g WM)$^{-1}$,

Table 5.2). *WM*-specific results were generally higher due to the lower water content compared to other gelatinous zooplankton, so the following comparisons will be DM- or protein (P)-specific. Compared to the study from Bailey et al. (1995) the results here (0.40 ± 0.21 and 0.61 ± 0.29 μL O_2 h^{-1} (mg DM)$^{-1}$) are higher than in most gelatinous organisms (0.01 - 0.14 μL O_2 h^{-1} (mg DM)$^{-1}$) except the Narcomedusa, *Aeginura grimaldii* (0.61 ± 0.14 μL O_2 h^{-1} (mg DM)$^{-1}$) and *in situ* incubations of other hydrozoan and scyphozoan species that were close (0.21 - 0.31 μL O_2 h^{-1} (mg DM)$^{-1}$). However, considering that these measurements by Bailey et al. (1995) were at temperatures between 4 and 7°C it is reasonable to conclude that the *R* results here presented (0.40 ± 0.21 and 0.61 ± 0.29 μL O_2 h^{-1} (mg DM)$^{-1}$, Table 5.2) are not very different.

On the contrary, our protein-specific results (1.81±0.73 and 2.94±1.12 μL O_2 h^{-1} (mg P)$^{-1}$), Table 5.2) are below similar results from a variety hydrozoan species including several siphonophores (4.25 - 38.57 μL O_2 h^{-1} (mg P)$^{-1}$), as measured by Biggs (1977b). However, these measurements by Biggs (1977b) were performed at 26±3°C so once again it is reasonable to assume that our R results are low but in range. Detailed comparison between the siphonophore species present in Biggs (1977b) shows that individuals of the suborder Cystonectae, *Bathyphysa sibogae* and *Rhizophysa filiformis*, had some of the lowest respiratory values. Both jellyfish are from the same suborder as *P. physalis* and neither pulsate. This is consistent with our hypothesis that using wind (or currents) for movement through the ocean permits life at low levels of respiration and that can be detected by a low R:Φ ratio. Meanwhile, genus from the suborder Physonectae like *Forskalia, Agalma* or *Cordagalma* and suborder Calycophorae *Sulculeolaria* or *Stephanophyes* had the highest respiratory values and pulsate frequently (Bone & Trueman, 1982; Costello et al., 2015). However, there were several exceptions like results of species of the genus *Diphyes* and *Abyla* which had low respiration even though they pulsate. This is not consistent with our hypothesis. Similarly, our respiration results are lower than those compiled in Barangé et al. (1989) for sessile hydrozoans. That is hard to reconcile. In addition, Nival et al. (1972) presented very similar respiration rates in pulsating hydrozoans at 15°C.

We hypothesised that these surface dwellers should have a low R:Φ, due to their drifting nature. Few studies have measured both R and Φ in cnidarian. Measurements of the pulsating planktonic medusae, *Pelagia noctiluca* and *Aurelia aurita* gave R:Φ ratios of 0.43±0.17 and 0.95±0.6, respectively (Chapter 4). Iguchi et al. (2017) observed a high R:Φ ratio of 0.9±0.3 in *A. aurita*. Low R:Φ (0.19 ± 0.10) was found in sessile polyps of *A. aurita* (Purcell et al., 2019). The R:Φ in this study (0.11±0.08 and 0.09±0.04 in *P. physalis* and *V. velella* respectively) resembled the low ratio of *A. aurita* polyps. Since both hydrozoans in this study are wind-propelled polyp colonies perhaps there could be a low ratio amongst sessile life forms that do not need ATP for locomotion. In the case of the free-floating, wind-driven hydrozoans in this study, a low metabolism may allow them to survive prolonged periods without prey consumption as they sail along at the surface of pelagic oligotrophic waters (Biggs, 1977b). In the case of *V. velella* the presence of endosymbiotic photosynthetic dinoflagelates in *V. velella* (Blank & Trench, 1986) may allow it to survive without prey and live with even a lower R:Φ ratio (Bondyale-Juez et al., 2017). Low R:Φ ratios may support our hypothesis from a respiratory stand, but we realize our measurements need confirmation and replication (Schmidt, 2009) to strengthen it. For example, the jellyfish sampled were close to being stranded and it is possible that in their previous days near the shore these organisms had not been feeding as much as they do in the open ocean. Lane et al. (1965) suggested that *P. physalis* could not provide reliable physiological data after being maintained for more than a few hours due to problems with interaction with aquariums. In our experiments,

no organism that clouded the water or deflated abnormally was used. Also gases from the floats from both organisms could be increasing the O_2 concentration during incubation. Munro et al. (2019) describes both gas release in young *Physalia* and the unlikely release of gas naturally in mature colonies. Carter (1931) suggested there is an exchange between pneumatophore of *Physalia*, *Velella* and *Porpita* species with the exterior. However, Larimer & Ashby (1962) checked that carbon monoxide (CO) was the gas mainly secreted, while O_2 and N_2 diffused inward. Carter (1931) even speculated on the possibility of pneumatophore having a respiratory function which could also reduce the consumption of the O_2 dissolved in the water if they are able to obtain it from the gases in their pneumatophore. Pickwell (1966) also speculated with the idea of gas exchange between the pneumatophore and the exterior with production of bubbles in the case of vertical migrating siphonophores which is probably not the case of *P. physalis*. No abnormal deflation, production of bubbles or sudden increase in O_2 was observed. Nevertheless, these issues should be considered. They also encourage research on enzymatic alternatives. So, we tentatively consider low R:Φ ratios to support our hypothesis, but we realize that future research addressing alternative explanations for the low ratios is needed.

5.4.3. Comparing respiratory carbon demand (*RCD*) with carbon ingestion estimations

Purcell (1984) identified fish larvae as the main prey of *P. physalis*. She found that *P. physalis,* with floats between 1 and 20 cm, captured fish larvae between 2 and 20 mm in length. It was estimated that about 120 fish larvae were consumed daily by each individual. Based on Harris et al. (1986), the carbon content (g C) per fish larvae can be estimated using the equation, $C=0.44 \cdot 10^{-3} \cdot L^{3.272}$ (where C is carbon in grams and L is the length, in cm, for each larvae) for *Gadus chalcogramma* from the Gadidae family, one of the families sampled by Purcell (1984). We then calculated the range of carbon content based on the fish length range in Purcell (1984) (0.002 to 4.25 mg C) and determine the associated carbon demand based on the amounts of fish consumed (about 120 individuals). Hence, according to Purcell (1984), the carbon demand per individual, associated with those predation rates, ranged between 0.27 and 510.04 mg C d^{-1} ind^{-1}. In the *P. physalis* colonies, here, the RCD per colony and potential RCD per colony, in mg C d^{-1} ind^{-1}, ranged from 0.19 - 3.51 and 0.90 - 50.49, respectively. If we consider that our study's siphonophores' float length ranged from 2.39 to 6.77 cm, it is reasonable that our results are closer to the lower values in Purcell (1984). Our results are consistent with predation observations, keeping also in mind that the amount of the ingested C destined for respiration will be lower than the total C ingested. Consequently, RCD would be the minimum C requirements. For example, Purcell & Kremer (1983) found that the siphonophore, *Sphaeronectes gracilis,* at low food levels, ingested 5.5 times more C than necessary to meet metabolic demands and even 9 times more at high food levels. This observation agrees with the low metabolic strategy hypothesised, but begs the question, "what happens to the rest of the assimilated carbon in these colonial pelagic hydrozoans?" Is it invested in growth, reproduction or the generation of specific organic substances?

Based on the carbon content measured by Beers (1966) on *P. physalis* (31.4% per *DM*) the carbon-specific R and Φ would be 1.56% d^{-1} and 16.90% d^{-1}. Therefore, according to these results *P. physalis* would need to consume less than 2% of its own carbon mass daily to meet its physiological respiratory requirements with a maximum of around 17% in high activity conditions. This fits into the range found in other gelatinous zooplankton

(0.6 – 18.28 % C d^{-1}) as observed by Bailey et al. (1995) . The inverse of the carbon-specific RCD shows that it takes between 65 and 6 days to use its own carbon content for respiratory processes. This result is very similar to the inverse of the Ec-specific HET of *P. physalis* described in the Results Section (64 – 6 days, Table 5.3).

In the case of *V. velella,* there has been some discussion regarding its main prey. According to the most recent findings (Bieri, 1966; Purcell et al., 2012, 2015; Zeman et al., 2018), its main prey are cladocerans, euphausid larvae, copepods and fish eggs. For example, in Zeman et al. (2018) *V. velella* guts contained 43% cladocerans, 19% copepods (6% cyclopoida, 13% calanoid), 28% fish eggs and 5% invertebrate eggs. In Purcell et al. (2015), the prey found in *V. velella*'s gastrozoids were mainly calanoid copepods and euphausid larvae (calycopsis and furcilia). Converting the ingestion rates (nº of prey consumed colony^{-1} d^{-1}) that appear in these articles to carbon mass, we can compare the values obtained from ingestion studies with our metabolic results. The approximate carbon content for the different types of prey (µg C ind^{-1}) obtained from the literature are: Walve & Larsson (1999) for the calanoid copepod (1.55) and the cladoceran (0.72); Pereira et al. (2014) for the fish eggs (30); Lindley (1999) and Guglielmo et al. (2015) for the euphausid larvae (10 for calycopsis, 984 for furcilia). From the ingestion rates in Purcell et al. (2015) we obtained the range 4.10 – 13.09 mg C ind^{-1} d^{-1} for 94.9% of the prey ingested and from Zeman et al. (2018) we obtained the range 0.02 – 7.62 mg C ind^{-1} d^{-1} for 84% of the prey ingested. Our results show that the RCD per colony ranged from 0.21 – 0.35 mg C ind^{-1} d^{-1} and potential RCD per colony ranged from 1.4 – 6.2 mg C ind^{-1} d^{-1}. It must also be considered that *V. velella* colonies used for our research had a sail length between 1.16 and 3.39 cm. Sail lengths from Purcell et al. (2015) ranged between 1.7 - 5.2cm and from Zeman et al. (2018), between 1.9 - 3.3cm. Our results are in range, but in general, lower than previous ingestion studies. These observations are similar to those made with the *P. physalis* comparison. The ingestion may be higher to fulfil the demand for other biological processes other than respiration, such as *V. velella*'s 0.5 mm daily growth (in length) describe by Bieri (1977). Low carbon use during metabolism is in agreement with our hypothesis of the low metabolic strategy of these pleuston wanderers.

In both cases, it is also possible that nearshore samples may not exhibit the same healthy metabolism that open ocean samples would. Regardless, these respiration measurements bring us closer to understanding trophic exchanges and physiological strategies taking place in the pleustonic ecosystem.

5.4.4. HET and Energy content (E_C)

The WM-specific HET results for *P. physalis* (15.29 ± 8.11 J d^{-1} (g WM)$^{-1}$) and *V. velella* (14.56 ± 7.05 J d^{-1} (g WM)$^{-1}$) (Table 5.3) were higher than in *A. aurita* (8.18±5.28 J d^{-1} (g WM)$^{-1}$) and *P. noctiluca* (4.39±1.67 J d^{-1} (g WM)$^{-1}$) (Chapter 4). This could be explained by the lower water content in the hydrozoans of this study. When HET was normalized by Ec (1.56 ± 0.83% d^{-1} and 2.18 ± 1.06% d^{-1}, Table 5.3) the resulting HET/Ec was higher in *A. aurita* and *P. noctiluca* (7.71 ± 4.97% d^{-1} and 2.28±0.87% d^{-1}, respectively) but not by much. However, since Φ was so much higher, in this study's hydrozoans, the resulting p-HET (165.78 ± 75.97 J d^{-1} (g WM)$^{-1}$ and 167.06 ± 54.01 J d^{-1} (g WM)$^{-1}$) and p-HET/Ec (16.94 ± 7.76% d^{-1} and 25.03 ± 8.09% d^{-1}) were higher than in the previously mentioned scyphozoans (Table 5.3). HET is a fairly new parameter and further study is needed to understand its relevance to energy circulation and physiological energy management.

Regarding energy content, the DM-specific Ec (Table 5.3) of the two organisms analysed here (6.63±2.67 kJ g $(DM)^{-1}$ and 7.16 ± 2.55 kJ (g $DM)^{-1}$) is slightly above the Ec described for other gelatinous zooplankton in Doyle et al. (2007) (1.96 – 5.95 kJ g $(DM)^{-1}$) and in range of the Ec of the Arctic high-lipid species in Percy & Fife (1981) (4.35 – 16.70 kJ g $(DM)^{-1}$) and the edible species reported by Khong et al. (2016), (4.08-11.81 kJ g $(DM)^{-1}$). Per WM the values of the organisms of our study would be amongst the highest in the gelatinous zooplankton due to the higher moisture of other gelatinous species. In the case of *P. physalis* previous analysis using bomb calorimetry compiled by Arai (1988) reported a Ec between 15.02-16.28 kJ (g $DM)^{-1}$. This is much higher than our result, but the value of 0.67 kJ (g $WM)^{-1}$, was also described in Arai 1988 for siphonophores and it is closer to our findings. It should be considered that Clarke et al. (1992) questioned the validity of both calorimetry and biochemically-based energy-analysis on gelatinous zooplankton. Doyle et al. (2007) argued that correcting for bound-water is necessary. It must also be considered that Doyle et al. (2007) showed evidence for disparity in energy densities between tissues of a same organism. This would mean that, in the case of *P. physalis* and *V. velella,* it would not be unreasonable to assume that certain parts of each organism could have higher energy density.

Regardless, some of these analyses are first measurements on these two ocean sailors. They fill data gaps from most gelatinous zooplankton studies. The data here provide new information for pleustonic bioenergetic models addressing both the impact of *P. physalis* and *V. velella,* both as predators and as prey. Examples are their ecological relationships with pleustonic gastropods, cirripeds and fish (Mansueti, 1963; Bieri, 1977; Betti et al., 2019; Griffin et al., 2019), loggerhead turtles (Frick et al., 2009) and, when stranded, sand crabs and, occasionally, birds (Matthews, 1955; Wenner, 1977; Phillips et al., 2017).

5.5. CONCLUSIONS

The water content in these two hydrozoans is lower than in other gelatinous zooplankton species (approximately between 85 and 91%). Probably due to the presence of hard chitin structures. This already shows that their nutritional content is different from non-pleustonic gelatinous prey. The biochemical composition follows the typical relative concentration with high protein, intermediate lipid concentration and low carbohydrate. The dry-mass- specific protein, lipid, and carbohydrate were high compared to published results for other gelatinous species. Only arctic gelatinous species had higher mass specific values for lipid and Ec. On a wet mass basis, the concentration of all the biochemical components in these two hydrozoans would be above most jellyfish. This makes both *P. physalis* and *V. velella,* high nutrient prey among gelatinous zooplankton. We hypothesised that *P. physalis* and *V. velella*'s biochemistry and Ec per WM should be equal to or greater than other jellyfish to explain the predation they experience. We find, that in general, our observations support this hypothesis.

The *R* for *V. velella* and *P. physalis* was low, but in range with the few other measurements from hydrozoans. The associated average Respiratory Carbon Demand was 0.72±0.38 mg C d^{-1} (g $WM)^{-1}$ for *P. physalis* and 0.69±0.33 mg C d^{-1} (g $WM)^{-1}$ for *V. velella*, and the potential RCD was 10 times higher. These RCD values generate another tool to quantify the impact of these jellyfish on prey.

We consider that our results support our hypothesis that respiratory metabolism of *V. velella* and *P. physalis* is lower than it is in pulsating gelatinous zooplankton due to their

wind-based propulsion. The low R:Φ ratio (~0.1), the lower protein-specific R (1.81 and 2.94 μL O2 h^{-1} (mg P)$^{-1}$ respectively), low respiration compared to their ingestion rates in the literature, a low HET/Ec (~2%) all fit our hypothesis. However, the relation may not be as clear since it does not completely explain the detection of a high Φ. Also, since we are working with organisms that contain a gas deposit within them, previously undocumented problems with incubations that could also explain a low R are hard to completely reject. Nevertheless, we stand by these first measurements of both the respiratory metabolism and biochemistry in *V. velella* and *P. physalis*.

REFERENCES

Alberty, R. A., & R. N. Goldberg, 1992. Standard Thermodynamic Formation Properties for the Adenosine 5′-Triphosphate Series. Biochemistry 31: 10610–10615.

Arai, M. N., 1988. Interactions of fish and pelagic coelenterates. Canadian Journal of Zoology 66: 1913–1927.

Arai, M. N., 2005. Predation on pelagic coelenterates: A review. Journal of the Marine Biological Association of the United Kingdom 85: 523–536.

Arai, M. N., J. A. Ford, & J. N. C. Whyte, 1989. Biochemical composition of fed and starved *Aequorea victoria* (Murbach et Shearer, 1902) (Hydromedusa). Journal of Experimental Marine Biology and Ecology 127: 289–299.

Araya, J. F., J. A. Aliaga, & M. E. Araya, 2016. On the distribution of *Physalia physalis* (Hydrozoa: Physaliidae) in Chile. Marine Biodiversity 46: 731–735, http://dx.doi.org/10.1007/s12526-015-0417-6.

Azzaro, M., T. T. Packard, L. S. Monticelli, G. Maimone, A. C. Rappazzo, F. Azzaro, F. Grilli, E. Crisafi, & R. La Ferla, 2019. Microbial metabolic rates in the Ross Sea: the ABIOCLEAR Project. Nature Conservation 34: 441–475.

Bailey, T. G., M. J. Youngbluth, & G. P. Owen, 1995. Chemical composition and metabolic rates of gelatinous zooplankton from midwater and benthic boundary layer environments off Cape Hatteras, North Carolina, USA. Marine Ecology Progress Series 122: 121–134.

Barangé, M., M. Zabala, T. Riera, & J. M. Gili, 1989. A general approach to the in situ energy budget of *Eudendrium racemosum*(Cnidaria, Hydrozoa) in the Western Mediterranean. Scientia Marina 53: 423–427.

Barnes, H., & J. Blackstock, 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilun method for ‘total’lipids. Journal of Experimental Marine Biology and Ecology Elsevier 12: 103–118.

Beers, J. R., 1966. Studies on the Chemical Composition of the Major Zooplankton Groups in the Sargasso Sea Off Bermuda. Limnology and Oceanography 11: 520–528.

Betti, F., M. Bo, F. Enrichetti, M. Manuele, R. Cattaneo-Vietti, & G. Bavestrello, 2019. Massive strandings of *Velella velella* (Hydrozoa: Anthoathecata: Porpitidae) in the Ligurian Sea (North-western Mediterranean Sea). European Zoological Journal 86: 343–353.

Bieri, R., 1966. Feeding Preferences and Rates of the Snail, *Ianthina prolongata*, the Barnacle, *Lepas anserifera*, the Nudibranchs, *Glaucus atlanticus* and *Fiona pinnata*, and the Food Web in the Marine Neuston. Publications of the Seto Marine Biological Laboratory 14: 161–170.

Bieri, R., 1977. the Ecological Significance of Seasonal Occurrence and Growth Rate of *Velella* (Hydrozoa). Publications of the Seto Marine Biological Laboratory 24: 63–76.

Biggs, D. C., 1977a. Respiration and ammonium excretion by open ocean gelatinous zooplankton. Limnology and Oceanography 22: 108–117.

Biggs, D. C., 1977b. Field studies of fishing, feeding, and digestion in siphonophores. Marine Behaviour and Physiology 4: 261–274.

Blank, R. J., & R. K. Trench, 1986. Nomenclature of Endosymbiotic Dinoflagellates. Taxon 35: 286–294.

Bligh, E. G., & W. J. Dyer, 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology NRC Research Press 37: 911–917.

Bondyale-Juez, D. R., T. T. Packard, M. A. Viera-Rodríguez, & M. Gómez, 2017. Respiration: comparison of the Winkler technique, O2 electrodes, O2 optodes and the respiratory electron transport system assay. Marine Biology Springer Berlin Heidelberg 164: 1–11, https://doi.org/10.1007/s00227-017-3271-1.

Bone, Q., & E. R. Trueman, 1982. Jet Propulsion of the Calycophoran Siphonophores Chelophyes and Abylopsis. Journal of the Marine Biological Association of the United Kingdom 62: 263–276.

Carter, G. S., 1931. Aquatic and Aerial Respiration in Animals. Biological Reviews 6: 1–35.

Chance, B., & G. Williams, 1955a. Respiratory Enzymes in Oxidative Phosphorylation II Difference spectra. Journal Biological Chemistry 217: 395–408.

Chance, B., & G. R. Williams, 1955b. Respiratory enzymes in oxidative phosphorylation. I. The respiratory chain. The Journal of biological chemistry 217: 383–394.

Chance, B., & G. R. Williams, 1955c. Respiratory enzyme in oxidative phosphorylation III The steady state. Journal of Biological Chemistry 217: 409–428.

Clarke, A., L. J. Holmes, & D. J. Gore, 1992. Proximate and elemental composition of gelatinous zooplankton from the Southern Ocean. Journal of Experimental Marine Biology and Ecology 155: 55–68.

Cleland, W., 1967. Enzyme kinetics. Annual review of biochemistry Annual Reviews 4139 El Camino Way, PO Box 10139, Palo Alto, CA 94303-0139, USA 36: 77–112.

Costello, J. H., S. P. Colin, B. J. Gemmell, J. O. Dabiri, & K. R. Sutherland, 2015. Multi-jet propulsion organized by clonal development in a colonial siphonophore. Nature Communications Nature Publishing Group 6: 1–6.

De Coen, W. M., & C. R. Janssen, 1997. The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed Daphnia populations. Journal of Aquatic Ecosystem Stress and Recovery Springer 6: 43–55.

Doyle, T. K., G. C. Hays, C. Harrod, & J. D. R. Houghton, 2014. Ecological and societal benefits of jellyfish Jellyfish blooms. Springer: 105–127.

Doyle, T. K., J. D. R. Houghton, R. McDevitt, J. Davenport, & G. C. Hays, 2007. The energy density of jellyfish: Estimates from bomb-calorimetry and proximate-composition. Journal of Experimental Marine Biology and Ecology 343: 239–252.

Dubischar, C. D., E. A. Pakhomov, & U. V. Bathmann, 2006. The tunicate *Salpa thompsoni* ecology in the Southern Ocean. II. Proximate and elemental composition. Marine Biology 149: 625–632.

Dubois, M., K. Gilles, J. K. Hamilton, P. A. Rebers, & F. Smith, 1956. A colorimetric method for the determination of sugars. Nature 168: 167.

Ferguson, S. J., 2010. ATP synthase: From sequence to ring size to the P/O ratio. Proceedings of the National Academy of Sciences 107: 16755–16756.

Ferrer, L., & A. Pastor, 2017. The Portuguese man-of-war: Gone with the wind. Regional Studies in Marine Science Elsevier B.V. 14: 53–62, http://dx.doi.org/10.1016/j.rsma.2017.05.004.

Fleming, N. E. C., C. Harrod, & J. D. R. Houghton, 2013. Identifying potentially harmful jellyfish blooms using shoreline surveys. Aquaculture Environment Interactions 4: 263–272.

Frick, M. G., K. L. Williams, A. B. Bolten, K. A. Bjorndal, & H. R. Martins, 2009. Foraging ecology of oceanic-stage loggerhead turtles *Caretta caretta*. Endangered Species Research 9: 91–97.

Gainey, R. L., 1972. The chemical analysis of *Velella lata* float. University of the Pacific.

Gemmell, B. J., J. H. Costello, & S. P. Colin, 2014. Exploring vortex enhancement and manipulation mechanisms in jellyfish that contributes to energetically efficient propulsion. Communicative and Integrative Biology 7: e29014.

Gemmell, B. J., K. T. Du Clos, S. P. Colin, K. R. Sutherland, & J. H. Costello, 2020. The most efficient metazoan swimmer creates a 'virtual wall' to enhance performance. Animal Behavior and Cognition .

Ghilardi, M., 2019. *Velella velella* (Cnidaria: Hydrozoa) in the Bay of Ranobe, south-west Madagascar. Plankton and Benthos Research 14: 206–209.

Gnaiger, E., N. Aasander Frostner, E. Abdul Karim, N. A. Abumrad, D. Acuna-Castroviejo, R. C. Adiele, B. Ahn, S. S. Ali, L. Alton, M. G. Alves, F. Amati, N. D. Amoedo, I. Andreadou, M. Arago, C. Aral, O. Arandarcikaite, E. Al., & L. Zvejniece, 2019. Mitochondrial respiratory states and rate. .

Griffin, D. C., C. Harrod, J. D. R. Houghton, & I. Capellini, 2019. Unravelling the macro-evolutionary ecology of fish-jellyfish associations: life in the "gingerbread house." Proceedings. Biological sciences 286: 1–9.

Gross, J., B. Dumsha, & N. Glazer, 1958. Comparative biochemistry of collagen some amino acids and carbohydrates. BBA - Biochimica et Biophysica Acta 30: 293–297.

Guglielmo, L., A. Granata, & R. Guglielmo, 2015. Orden Euphausiacea. Ibero Diversidad Etimológica 86A: 1–20.

Harris, R. ., T. Nishiyama, & J. Paul, 1986. Carbon, nitrogen and caloric content of eggs, larvae, and juveniles of the walleye pollock, *Theragra chalcogramma*. Journal Fish Biology 29: 87–98.

Hays, G. C., T. K. Doyle, & J. D. R. Houghton, 2018. A Paradigm Shift in the Trophic Importance of Jellyfish?. Trends in Ecology and Evolution 33: 874–884.

Hernández-León, S., & M. Gómez, 1996. Factors affecting the respiration/ETS ratio in marine zooplankton. Journal of Plankton Research 18: 239–255.

Hoover, A., & L. Miller, 2015. A numerical study of the benefits of driving jellyfish bells at their natural frequency. Journal of Theoretical Biology 374: 13–25.

Iguchi, N., H. Iwatani, K. Sugimoto, S. Kitajima, N. Honda, & O. Katoh, 2017. Biomass, body elemental composition, and carbon requirement of *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) in the southwestern Japan Sea. Plankton and Benthos Research 12: 104–114.

Ikeda, T., 1972. Chemical composition and nutrition of zooplankton in the Bering Sea In Takenouti, A. Y. (ed), Biological oceanography of the northern North Pacific Ocean. Idemitsu Shoten, Tokyo. Idemitsu Shoten, Tokyo: 433–442.

Ikeda, T., 2014. Synthesis toward a global model of metabolism and chemical composition of medusae and ctenophores. Journal of Experimental Marine Biology and Ecology Elsevier B.V. 456: 50–64, http://dx.doi.org/10.1016/j.jembe.2014.03.006.

Jenkins, R. L., 1983. Observations on the Commensal Relationship of *Nomeus gronovii* with *Physalia*

physalis . American Society of Ichthyologists and Herpetologists 1983: 250–252.

Joseph, J. D., 1979. Lipid composition of marine and estuarine invertebrates: Porifera and cnidaria. Progress in Lipid Research 18: 1–30.

Karl, D. M., 2014. Solar energy capture and transformation in the sea. Elementa: Science of the Anthropocene 2:.

Khong, N. M. H., F. M. Yusoff, B. Jamilah, M. Basri, I. Maznah, K. W. Chan, & J. Nishikawa, 2016. Nutritional composition and total collagen content of three commercially important edible jellyfish. Food Chemistry Elsevier Ltd 196: 953–960, http://dx.doi.org/10.1016/j.foodchem.2015.09.094.

Knight, J. A., S. Anderson, & J. M. Rawle, 1972. Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. Clinical Chemistry 18: 199–202.

Lane, C. E., E. Pringle, & A. M. Bergere, 1965. Amino acids in extracellular fluids of *Physalia physalis* and *Aurelia aurita*. Comparative biochemistry and physiology Elsevier 15: 259–262.

Larimer, J. L., & E. A. Ashby, 1962. Float gases, gas secretion and tissue respiration in the Portuguese man-of-war, *Physalia*. Journal of Cellular and Comparative Physiology 60: 41–47.

Larson, R. J., & G. R. Harbison, 1989. Source and fate of lipids in polar gelatinous zooplankton. Arctic 42: 339–346.

Lilley, M. K. S., & F. Lombard, 2015. Respiration of fragile planktonic zooplankton: Extending the possibilities with a single method. Journal of Experimental Marine Biology and Ecology 471: 226–231.

Lindley, J. A., 1999. Dry weight carbon and nitrogen content of some euphausiids from the north Atlantic Ocean and the Celtic Sea. Journal of Plankton Research 21: 2053–2066.

Lopes, A. R., M. Baptista, I. C. Rosa, G. Dionísio, J. Gomes-Pereira, J. R. Paula, C. Figueiredo, N. Bandarra, R. Calado, & R. Rosa, 2016. "Gone with the wind": Fatty acid biomarkers and chemotaxonomy of stranded pleustonic hydrozoans (*Velella velella* and *Physalia physalis*). Biochemical Systematics and Ecology 66: 297–306.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, & R. J. Randall, 1951. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry 193: 265–275.

Lucas, C. H., 1994. Biochemical composition of *Aurelia aurita* in relation to age and sexual maturity. Journal of Experimental Marine Biology and Ecology 183: 179–192.

Lucas, C. H., 2009. Biochemical composition of the mesopelagic coronate jellyfish *Periphylla periphylla* from the Gulf of Mexico. Journal of the Marine Biological Association of the United Kingdom ULPGC. Biblioteca Universitaria 89: 77–81.

Lucas, C. H., K. A. Pitt, J. E. Purcell, M. Lebrato, & R. H. Condon, 2011. What's in a jellyfish? Proximate and elemental composition and biometric relationships for use in biogeochemical studies. Ecology 92: 1704.

Mackie, G. O., P. R. Pugh, & J. E. Purcell, 1988. Siphonophore Biology. Advances in Marine Biology 24: 97–262.

Maldonado, F., T. T. Packard, & M. Gómez, 2012. Understanding tetrazolium reduction and the importance of substrates in measuring respiratory electron transport activity. Journal of Experimental Marine Biology and Ecology 434–435: 110–118.

Malej, A., J. Faganeli, & J. Pezdič, 1993. Stable isotope and biochemical fractionation in the marine pelagic food chain: the jellyfish *Pelagia noctiluca* and net zooplankton. Marine Biology: International Journal on Life in Oceans and Coastal Waters 116: 565–570.

Mansueti, R., 1963. Symbiotic behavior between small fishes and jellyfishes, with new data on that between the stromateid, *Peprilus alepidotus*, and the scyphomedusa, *Chrysaora quinquecirrha*. Copeia JSTOR 40–80.

Mapstone, G. M., 2014. Global diversity and review of Siphonophorae (Cnidaria: Hydrozoa). PLoS ONE 9: e87737.

Markwell, M. A. K., S. M. Haas, L. L. Bieber, & N. E. Tolbert, 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Analytical Biochemistry 87: 206–210.

Marsh, J. B., & D. B. Weinstein, 1966. Simple charring method for determination of lipids. Journal of Lipid Research 7: 574–576.

Martínez, I., A. Herrera, M. Tames-espinosa, D. R. Bondyale-juez, V. Romero-kutzner, T. T. Packard, & M. Gómez, 2020. Protein in marine plankton: A comparison of spectrophotometric methods. Journal of Experimental Marine Biology and Ecology Elsevier 526: 151357.

Matthews, D., 1955. Feeding Habits of the Sand Crab *Hippa pacifica* (Dana). Pacific Science IX: 382–386.

Moran, L. A., H. R. Horton, K. G. Scrimgeour, & M. D. Perry, 2012. Principles of biochemistry. Pearson Boston.

Mortillaro, J. M., K. A. Pitt, S. Y. Lee, & T. Meziane, 2009. Light intensity influences the production and translocation of fatty acids by zooxanthellae in the jellyfish *Cassiopea sp.* Journal of Experimental

Marine Biology and Ecology 378: 22–30.

Munro, C., Z. Vue, R. R. Behringer, & C. W. Dunn, 2019. Morphology and development of the Portuguese man of war, *Physalia physalis*. Scientific Reports Springer US 9: 1–12, http://dx.doi.org/10.1038/s41598-019-51842-1.

Nelson, M. M., C. F. Phleger, B. D. Mooney, & P. D. Nichols, 2000. Lipids of gelatinous antarctic zooplankton: Cnidaria and Ctenophora. Lipids 35: 551–559.

Nival, P., S. Nival, & I. Palazzoli, 1972. Données sur la respiration de différents organismes communs dans le plancton de Villefranche-sur-Mer. Marine Biology 17: 63–76.

Ochoa, S., 1943. Efficiency of aerobic phosphorylation in cell-free heart extracts. Journal of Biological Chemistry 151: 493–505.

Osma, N., M. Aristizabal, I. Fernández-Urruzola, T. T. Packard, & M. Gómez, 2016. Influence of Starvation on Respiratory Metabolism and Pyridine Nucleotide Levels in the Marine Dinoflagellate *Oxyrrhis marina*. Protist Elsevier GmbH. 167: 136–147.

Owens, T. G., & F. D. King, 1975. The measurement of respiratory electron-transport-system activity in marine zooplankton. Marine Biology 30: 27–36.

Packard, T. T., 1971. The Measurement of Respiratory Electron-transport Activity tn Marine Phytoplankton. Journal of Marine Research 29: 235–244.

Packard, T. T., & J. P. Christensen, 2004. Respiration and vertical carbon flux in the Gulf of Maine water column. Journal of Marine Research 62: 93–115.

Packard, T. T., N. Osma, I. Fernández-Urruzola, L. A. Codispoti, J. P. Christensen, & M. Gómez, 2015. Peruvian upwelling plankton respiration: Calculations of carbon flux, nutrient retention efficiency, and heterotrophic energy production. Biogeosciences 12: 2641–2654.

Pardy, R. L., & C. Dieckmann, 1975. Oxygen consumption in the symbiotic hydra *Hydra viridis*. Journal of Experimental Zoology 194: 373–378.

Pauly, D., W. Graham, S. Libralato, L. Morissette, & M. L. Deng Palomares, 2009. Jellyfish in ecosystems, online databases, and ecosystem models. Hydrobiologia 616: 67–85.

Percy, J. A., & F. J. Fife, 1981. The Biochemical Composition and Energy Content of Arctic Marine Macrozooplankton. Arctic 34: 307–313.

Pereira, R., M. A. Teodósio, & S. Garrido, 2014. An experimental study of *Aurelia aurita* feeding behaviour: Inference of the potential predation impact on a temperate estuarine nursery area. Estuarine, Coastal and Shelf Science 146: 102–110.

Phillips, N., L. Eagling, C. Harrod, N. Reid, V. Cappanera, & J. Houghton, 2017. Quacks snack on smacks: Mallard ducks (*Anas platyrhynchos*) observed feeding on hydrozoans (*Velella velella*). Plankton and Benthos Research 12: 143–144.

Pickwell, G. V, 1966. Physiological dynamics of siphonophores from deep scattering layers: size of gas-filled floats and rate of gas production. US Navy Electronics Laboratory.

Pierce, J., 2006. Aquarium design for the Portuguese man-of-war *Physalia physalis*. International Zoo Yearbook 40: 221–231.

Pires, R. F. T., N. Cordeiro, J. Dubert, A. Marraccini, P. Relvas, & A. Dos Santos, 2018. Untangling *Velella velella* (Cnidaria: Anthoathecatae) transport: A citizen science and oceanographic approach. Marine Ecology Progress Series 591: 241–251.

Prieto, L., D. MacÍas, A. Peliz, & J. Ruiz, 2015. Portuguese Man-of-War (*Physalia physalis*) in the Mediterranean: A permanent invasion or a casual appearance? Scientific Reports Nature Publishing Group 5: 1–7.

Purcell, J. E., 1984. Predation on fish larvae by *Physalia physalis*, the Portuguese man of. Marine Ecology Progress Series 19: 189–191.

Purcell, J. E., D. R. Bondyale-Juez, V. Romero-Kutzner, I. Martínez, R. Caprioli, M. Tames-Espinosa, J. Almunia, E. Alonso, T. T. Packard, & M. Gómez, 2019. Food supply effects on the asexual reproduction and respiratory metabolism of *Aurelia aurita* polyps. Hydrobiologia 846: 135–146.

Purcell, J. E., E. Clarkin, & T. K. Doyle, 2012. Foods of *Velella velella* (Cnidaria: Hydrozoa) in algal rafts and its distribution in Irish seas. Hydrobiologia 690: 47–55.

Purcell, J. E., V. Fuentes, D. Atienza, U. Tilves, D. Astorga, M. Kawahara, & G. C. Hays, 2010. Use of respiration rates of scyphozoan jellyfish to estimate their effects on the food web. Hydrobiologia 645: 135–152.

Purcell, J. E., & P. Kremer, 1983. Feeding and metabolism of the siphonophore *Sphaeronectes gracilis*. Journal of Plankton Research 5: 95–106.

Purcell, J. E., G. Milisenda, A. Rizzo, S. A. Carrion, S. Zampardi, S. Airoldi, G. Zagami, L. Guglielmo, F. Boero, T. K. Doyle, & S. Piraino, 2015. Digestion and predation rates of zooplankton by the pleustonic hydrozoan *Velella velella* and widespread blooms in 2013 and 2014. Journal of Plankton Research 37: 1056–1067.

Roberts, G. A. F., 1992. Chitin Chemistry. Journal of Chemical Information and Modeling. .

Rutter, W. J., 1967. Protein determinations in embryos In Wilt, F. H., & N. K. Wessels (eds), Methods in

developmental biology. New York: 671–683.

Schmidt, S., 2009. Shall We Really Do It Again? The Powerful Concept of Replication Is Neglected in the Social Sciences. Review of General Psychology 13: 90–100.

Schneider, G., 1989. Estimation of food demands of *Aurelia aurita* medusae populations in the kiel bight/western baltic. Ophelia 1: 17–27.

Schuchert, P., 2010. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Capitata Part 2. Revue Suisse de Zoologie 117: 337–555.

St-Amand, L., R. Gagnon, T. T. Packard, & C. Savenkoff, 1999. Effects of inorganic mercury on the respiration and the swimming activity of shrimp larvae, *Pandalus borealis*. Comparative Biochemistry and Physiology - C Pharmacology Toxicology and Endocrinology 122: 33–43.

Stenvers, V., X. Chi, & J. Javidpour, 2020. Seasonal variability of the fatty acid composition in *Aurelia aurita* (Cnidaria: Scyphozoa): implications for gelativore food web studies. Journal Of Plankton Research 00: 1–13.

Stillway, L. W., 1974. Nematocyst lipids of the Portuguese man-of-war *Physalia physalis*. Comparative Biochemistry and Physiology -- Part B: Biochemistry and 48: 35–38.

Stillway, L. W., 1976. Fatty acids of the portuguese man-of-war *Physalia physalis*. 53: 535–537.

Tamkun, M. M., & D. A. Hessinger, 1981. Isolation and partial characterization of a hemolytic and toxic protein from the nematocyst venom of the Portuguese man-of-war, Physalia physalis. Biochimica et Biophysica Acta (BBA)-Protein Structure Elsevier 667: 87–98.

Thiebot, J. B., & J. C. McInnes, 2020. Why do marine endotherms eat gelatinous prey?. ICES Journal of Marine Science 77: 58–71.

Uye, S., & H. Shimauchi, 2005. Population biomass, feeding, respiration and growth rates, and carbon budget of the scyphomedusa *Aurelia aurita* in the Inland Sea of Japan. Journal of Plankton Research 27: 237–248.

Walve, J., & U. Larsson, 1999. Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. Journal of Plankton Research 21: 2309–2321.

Wenner, A., 1977. Food Supply, Feeding Habits, and Egg Production in Pacific Mole Crabs (*Hippa pacifica* Dana). 31: 39–47.

Zagalsky, P. F., & P. J. Herring, 1977. Studies on the blue astaxanthin-proteins of *Velella velella* (Coelenterata: Chondrophora). Philosophical transactions of the Royal Society of London. Series B, Biological sciences 279: 289–326.

Zeman, S. M., M. Corrales-Ugalde, R. D. Brodeur, & K. R. Sutherland, 2018. Trophic ecology of the neustonic cnidarian *Velella velella* in the northern California Current during an extensive bloom year: insights from gut contents and stable isotope analysis. Marine Biology Springer Berlin Heidelberg 165: 1–13, https://doi.org/10.1007/s00227-018-3404-1.

CHAPTER 6

EFFECT OF AGE AND STARVATION ON RESPIRATION, LIPID AND PROTEIN IN *Aurelia aurita*

ABSTRACT

In this study we studied the biochemical and metabolic changes that occur in the jellyfish *Aurelia aurita* as it transitioned from ephyra (24h after strobilation) to medusa (66 days) and, also, similar changes in juveniles under food deprivation. Lipid and protein composition, physiological respiration (R) and enzymatic potential respiration (Φ) were measured, in addition to the typical biometrics, wet mass and diameter. Our observations found that around the third week of development ephyrae completed their transition to juvenile medusae. This transition was identified as shifts in the major biochemical components (lipid and protein), in the continuity of the umbrella and in the ratio of the physiological respiration to its potential. The results showed a much higher growth rate, a higher respiratory rate and a, never shown before, higher lipid-to-protein content in the first days of the ephyrae. The high lipid content provided energy and a buoyancy to the newly formed ephyrae. Juvenile *A. aurita* survived up to 58 days under food deprivation with several changes in its respiratory metabolism. These observations in ephyra may have similar implication on the metabolism and lipid composition of the *A. aurita* strobila.

6.1. INTRODUCTION

Research on jellyfish is increasing and as a result we are understanding, not only their impacts and problems, but also their ecological and societal benefits (Doyle et al., 2014). Researchers now contemplate the alterations to ecosystems and human activities caused by stinging cells and by the formation of blooms (Cegolon et al., 2013; Lucas et al., 2014), including their competition and predation on fish (Pauly et al., 2009), and also now consider the importance of jellyfish as prey (Thiebot & McInnes, 2020), as habitats and nurseries (Haddock, 2004), as a source of carbon flux (Lebrato et al., 2019) and their role as sea ambassadors in aquariums worldwide (Doyle et al., 2014). Besides, it has been debated in the last decades whether jellyfish are benefitted by anthropogenic impacts (Purcell et al., 2007; Richardson et al., 2009; Condon et al., 2012; Duarte et al., 2013; Pitt et al., 2018) and therefore increasing globally. Apart from the regional examples of jellyfish increase (Brotz et al., 2012) the idea of jellyfish surviving changing conditions is reinforced by the fact that their simple design has survived for 500 million years of change according to the fossil record (Young & Hagadorn, 2010) and by experiments showing jellyfish tolerance to low O_2, starvation amongst other stressors (Lilley et al., 2014; Algueró-Muñiz et al., 2016).

The metagenetic lifecycle of some scyphozoan and hydrozoan jellyfish may have contributed to their success (Pitt et al., 2013). Species with this life cycle have displayed enormous resilience, regeneration abilities and in special case life cycle reversal (Gilchrist, 1937; Piraino et al., 1996; He et al., 2015; Abrams & Goentoro, 2016).

Aurelia aurita was the species used in this studies investigation. This organism is a commonly studied metagenetic (Miyake et al., 1997; Ceh et al., 2015), bloom forming (Dong, 2019), prey and predator (Frick et al., 2009; Pereira et al., 2014; Takao et al., 2014) species of pelagic and coastal ecosystems (Kotova et al., 2015). Here, a batch of cultured *A. aurita* was monitored as they transition from ephyrae to juvenile medusa. Biometrics such as mass and size have been previously studied in this species (Frandsen & Riisgård, 1997; Uye & Shimauchi, 2005). However, research that include such young stages and transition to juvenile jellyfish are less common (Lucas, 1994; Båmstedt et al., 1999; Kamiyama, 2018). Additionally, the evolution of the protein and lipid content of the organisms was determined. This serves to complement the findings in Lucas (1994) by adding the lipid results for organism smaller than 1cm, absent in the literature, by observing in higher detail the biochemical changes in the first days of the ephyrae. The organic composition also informs about the nutritional value of the life stages as prey for different predators.

In addition, the respiratory response to the effects of food deprivation on the medusa were also analysed. The effects of starvation have been studied in jellyfish in several occasions to understand the process of shrinking in which the size of the organism decrease to accommodate the dietary restrictions or unfavourable environmental conditions (Hamner and Jenssen, 1974; Fu et al., 2014; Lilley et al., 2014; Goldstein and Riisgård, 2016). Jellyfish survival under starving conditions has been also documented (Richardson et al., 2009) and it is discussed as a mechanism that may allow certain jellyfish outlast other marine life under similar unfavourable conditions. The respiratory metabolic implications that accompany these processes have been less frequently described in the literature. Regulation of these respiratory processes may decrease the food demand and allow survival when food is less available. Respiration was measured through the analysis of O_2 consumption using optodes. Potential respiration (respiratory capacity) was measured by assaying the activity of the electron transport system (ETS) that controls respiration at a cellular level. The aim of this study was to determine the metabolic and biochemical changes that control and signal the transition from ephyra to medusa in *A. aurita* and to examine the respiratory changes this species suffer as a result of metabolic regulation under starving conditions.

6.2. MATERIALS AND METHODS

6.2.1. Sample rearing, size and biomass

The *A. aurita* samples were used for two different experiments. Strobilated polyps and juvenile medusae were obtained from Loro Parque Foundation's aquarium in Tenerife (Canary Islands, Spain) and came years ago from a culture stock of the aquarium of Madrid (Purcell et al., 2019). Strobilation was achieved by cooling the filtered sea water surrounding the polyps to 12ºC for 5-6 weeks with abundant food 3 times a week. Then, when strobilae in multiple polyps were fully developed, the temperature was raised to 20ºC. Some of the ephyrae, produced during strobilation were reared for 66 days at 21 ± 2ºC. During this time, the ephyrae were fed 24-48h *Artemia*-nauplii *ad libitum* once daily. Rearing took place in a 10L air-kreisel (Air kresiel tank, Ø 300mm, depth 100mm, Schuran) with the filtered seawater changed every 3 days. By day, 7 of the remaining ephyra, in the original tank, were carefully transferred to a 40L traditional kreisel (Breeding kreisel tank, Ø 300mm, depth 200mm, Schuran), at 21 ± 2ºC, in which 50% of the filtered seawater was changed weekly and 100% was changed monthly. Physiological respiration (R) was measured during the organisms' growth and transition from ephyra to medusa. The mean diameter (D) of the ephyra was determined photographically, in the case of the smaller ephyra pictures were taken using a stereoscopic microscope (Leica, TL3000 Ergo). When samples could not be photographed properly with the stereoscopic microscope the 13MP primary camera of a Huawei Y6II was used to take pictures. Pictures were analysed using Microsoft Powerpoint measuring several diameters of the samples central disk and umbrella depending on the age and morphology of the sample. For each measurement 3 pseudoreplicates were collected from the kreisel. Different numbers of individuals were pooled to gain enough biomass to perform the measurements: 4 one day old ephyrae, 3 four days old ephyra and then 2 individuals until day 21. The experiment procedure started with the R measurements, then wet mass (WM), and D were determined before storage in microvials or small bags, depending on their size. These samples were stored at -80ºC until enzymatic and biochemical composition analysis. During the 66 day culture no individual died except when frozen after the experiments.

The juvenile medusae for the starvation experiments were maintained in individual 2L Florence flasks with bubbles to promote circulation as in air-kreisels. These medusas were starved for 58 days at 18-20ºC with seawater changed (100%), at least twice a week. Physiological respiration was measured in these samples periodically during the starvation period. WM of these samples was only measured in the final day to limit organism manipulation. Then these samples were stored for enzyme and biochemical composition analysis.

6.2.2. Physiological respiration measurement (R)

R was determined from the decreasing levels of O_2 in incubated samples. The O_2 was measured using an optode similar to the one used by Lilley and Lombard (2015); the optode spot had been already installed inside the incubation bottle. The incubations lasted 2-4h and O_2 concentration was measured every 10-20min. Ephyrae were incubated in glass tubes (4 - 6mL), juvenile medusae from day 13 to 40 in BOD bottles (~58mL), from day 40 to 66 in 250mL glass jars with a layer of Parafilm© under the lid. In all of these incubations, bubbles were rigorously avoided. These different vessels facilitated detection of O_2 consumption in an adequate ratio of organism-biomass to seawater-volume (Purcell et al., 2010). Multiple organisms were incubated in the case of smaller sizes (<40mg WM) as described in the previous section. All incubations were performed in darkness at 20°C±0.2°C for the reared ephyra and medusa and 18ºC±0.2°C for the starved medusa.

The incubation vessels were gently inverted several times before measuring, to allow homogenous distribution of O_2. To compare the maximum oxygen consumption with the rates associated with the

enzymatic activity, i.e. potential respiration (Φ), the reared ephyrae and medusa were fed 1h before R measurements *Artemia* 24-48h nauplii *ad libitum*. The jellyfish in the starvation experiment were not fed to study the response of prolonged food deprivation on their respiratory metabolism.

6.2.3. Electron transport system (ETS) analysis

The methodology used followed the studies of (Packard et al., 1974; Hernández-León & Gómez, 1996; Packard & Christensen, 2004; Purcell et al., 2019). The samples stored at -80ºC were first sonified with ultra-sound in 0.1M phosphate buffer solution (Packard, 1971). The ratio between the targeted homogenate volume and the WM was 2-10mL per gram of sample, however, for ephyra the ratio reached 200mL per g to obtain enough volume for the assays. Then the homogenate was centrifuged at 4000rpm for 10 min (Gómez et al., 1996). 100µL of the supernatant was then mixed in a cuvette with 300 µL of substrate solution (NADH and NADPH, 1.7mM in the same buffer solution) and 100 µL of the indicator INT solution (2 mg INT (Acros Organics, Geel, Belgium) per mL deionized H_2O). The INT acted as an artificial electron acceptor and was reduced to formazan. The formazan dyed the solution red and the reaction was monitored using a spectrophotometer at 490nm. The rate of change in absorbance can be converted to rate of formazan production using the corresponding extinction coefficient of our INT solution, in this case 12.8 mM^{-1} cm^{-1}. The rate of formazan production can be converted to the stoichiometrically-equivalent O_2-reduction rate associated with the respiratory enzymatic system, i.e. potential respiration (Φ). The remaining homogenate from the analysis was used for proximate composition analysis.

6.2.4. Proximate composition (protein & lipid)

A more concentrated homogenate would have been necessary to measure the low concentrations of carbohydrates. Therefore, only protein and lipid content were determined. The determination of these organic components was based on the spectrophotometric methods proposed by Lowry et al. (1951) and Bligh and Dyer (1959) with the following procedures.

To measure the protein content the procedure used in Martínez et al. (2020) was followed. 100µL of homogenate were mixed with 500 µL of the Rutter solution proposed by Rutter (1967) with the addition of Dodecyl sulphate sodium salt (SDS) as suggested by Markwell et al. (1978). After 10mins, 50 µL of Folin solution were added and the solution was well mixed with a vortex and left in the darkness for 40min for the reaction to stabilised. Then, the solution had different intensities of blue depending on the concentration of protein. The absorbance of the solution was measured at 750nm spectrophotometrically. Bovine serum albumin was used as the standard.

To measure lipid, it was extracted following the procedure of Bligh and Dyer (1959) using chloroform and methanol. Then the mixtures were centrifuged for 10 min at 2600 g and 4ºC. 250µL of the resulting bottom phases were pipetted to test tubes and 500 µL of H_2SO_4 (95%) were gently added. These test tubes' content, without mixing, were charred at 200ºC for 15min following De Coen and Janssen (1997). After cooling down for 5min, the tubes´ content were well mixed and 40 µL of this mixed solutions were extracted and in another test tubes mixed with 1mL of phosphor-vanillin reagent and incubated for 15min at 37ºC. The solutions were then cooled for 5min and their absorbance was measured at 525nm spectrophotometrically. Olive oil (0-4.8 mg mL^{-1}) dissolved in chloroform was used as the standard.

6.2.5. Statistical analysis and graph design

The regression analysis of the data was performed with R version 3.5.3 (R Core Team, 2017). During statistical analysis the significance of the model proposed and the R^2 were tested using the R tools for linear models. Linear assumptions were tested using residual analysis and Q-Q analysis. The best fitted function was used to study the evolution of the parameters measured during the culture. WM

results were used to model growth rate based on WM changes. The power and exponential function fitting of the results were compared being this last one what it is normally assumed. Figures were developed using Microsoft Excel.

6.3. RESULTS

6.3.1. Biometrics and growth

The allometric relation between D (in mm) and WM (in mg) as *A. aurita* transitioned from ephyra to medusa (66 days), was described in Fig. 6.1 by the equation $WM = 0.49D^{2.36}$.

Age (d)	D (mm)	WM (mg)
1	1.58±0.1	2.53±0.76
4±1.4	4.1±1.2	12.36±3.7
6±1.4	5.39±0.66	12.48±9.01
9±1.4	6.75±1.25	33.22±10.77
13±1.4	7.03±1.03	55.17±9.9
17±1.4	8.65±0.61	93.5±13.49
21±1.4	13.7±1.14	174.73±36.47
27±1.4	16.75±1.77	297.87±37.34
34±1.4	21.65±4.19	696.33±106.59
40±1.4	22.79±4.29	1173.43±331.39
44±1.4	20.06±7.03	739.77±510.93
51±1.4	31.77±6.75	1719.2±346.34
58±1.4	32.3±7.05	1776.17±741
66±1.4	34.36±9.01	2435.9±960.49

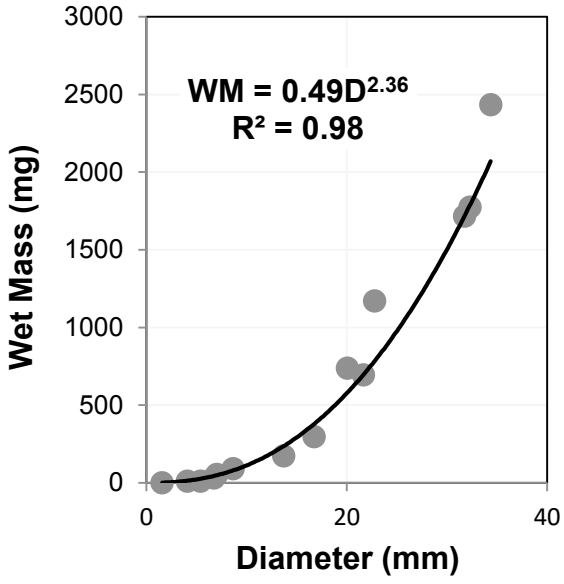


Figure 6.1. Compilation of the average and standard deviation of the central disk diameter (D) and wet mass (WM). These results, fitted to a power function, follow the equation $WM=0.49D^{2.36}$ with the R^2 and the regression parameters.

The final size of D in the largest remaining young medusa was 40.7mm. The starting size of lappet-tips D in the ephyra (total body length) was 3.6 ± 0.2 mm and 1.6±0.1 mm of the central disc D (Fig. 6.2A). The measurements were made as recommended by Båmstedt et al. (1999) and Fu et al. (2014) for *A. aurita* ephyra. Between day 15 and 17 the central disk and the edges of the umbrella are very close and the distinction between metephyra and young medusa is complex (Kinoshita et al., 1997). The D, in mm, of the organisms increased isometrically with time (t), in days (Fig. 6.2B), i.e. it is more or less linear, $D=0.48 \cdot t+3.52$. Meanwhile WM ranged from 2.13mg – 3.75g and the allometric increase in wet mass (WM, mg) (Fig. 6.2C) with time was better described by a power function ($WM=0.96 \cdot t^{1.76}$). With few exceptions there was more or less continuous increase in D and WM with time. However, the dispersion of WM results increased widely from day 20 until the last day of experiments.

Traditionally instantaneous specific growth rate (μ in d^{-1}) has been determined from biomass increases as an exponential function of time ($WM = WM_0 \cdot e^{\mu t}$). From Olesen et al., 1994, (Eqn. 6.1) describes the calculation of μ as:

$$\text{Ln}\,(W_t / W_0)/(t-t_0), \qquad (6.1)$$

where W_0 and W_t are the mean WM of the organisms on day 0 and day t, respectively. From Båmstedt et al. (1999) and Lilley et al. (2014b) one can find a similar equation:

$$\mu = \text{Ln}\,(W_2 / W_1)/(t_2-t_1), \qquad (6.2)$$

where W_2 and W_1 are the mean WM of the organisms on two consecutive days (t_2 and t_1). Using the exponential equation in Fig. 6.2C, a μ of 10% d^{-1} was obtained. From eqn. 6.1, the corresponding μ was 10.3 ± 1.3 % d^{-1} if we used the first and final day of measurements following Olesen et al. (1994) or Frandsen and Riisgård (1997). If instead μ was calculated between each consecutive observation (Eqn. 6.2) the average μ results in 13.1 ± 15.6 % d^{-1} with values ranging from 52.8 % d^{-1} between the first and second observation, less than 0.5 % d^{-1} between the last observations and even a negative observation -11.5 % d^{-1} between day 40 and 44.

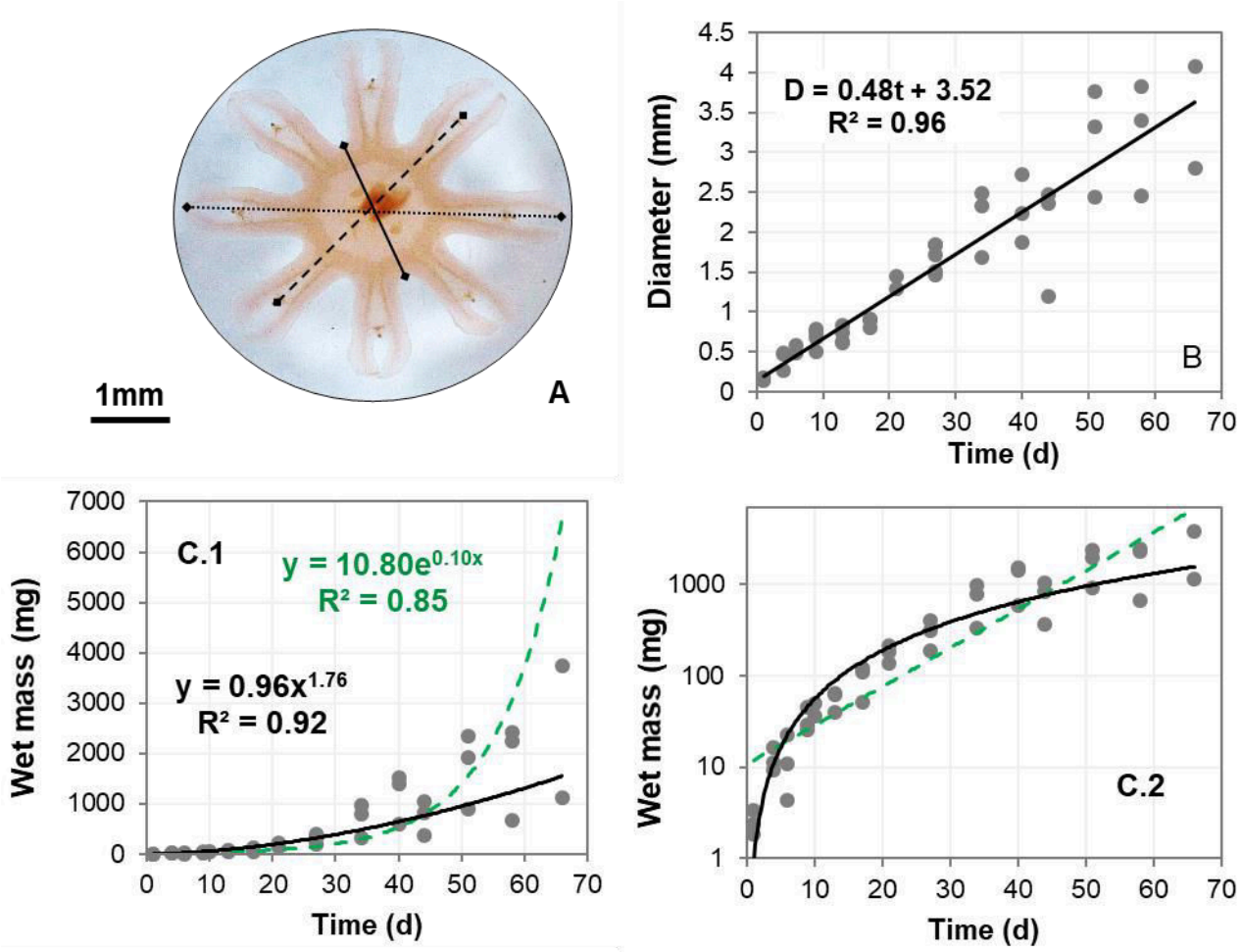


Figure 6.2. Diameter (D) and wet mass (WM) during days 1-66 of *Aurelia aurita* cultured. A. Measurements in ephyra: central disc D (solid line), rhopalia or disc D (dashed line) and over lappet or total body length (dotted line) (Båmstedt et al., 1999; Fu et al., 2014). D in ephyrae was central disc diameter and in juvenile medusa umbrella diameter. B. Linear increase of D with time (t) in days. Data fitted to a linear function with regression analysis parameters P<0.01 F=336.8 DF=43C.1. Evolution of WM with t, fitted exponential function (dashed green line, regression analysis parameters: P<0.01, F=234.2, DF=41) and fitted power function (solid black line, Regression analysis parameters: P<0.01, F=441.4, DF=41); C.2. is the same as C.1. but with log scale in the y axis for details of the first days. A total of 43 organisms.

However, both formulas are based on the assumption that growth followed an exponential function ($WM=WM_0 \cdot e^{\mu t}$), which in this case does not seem to fit the observed increase of WM with time (Fig. 6.2C). Here, a different approach was used to determine growth rate that fits the regression analysis in Fig. 6.2C. The increase in WM with time fitted a power function also known as parabolic growth (Zotin, 2015). To determine the rate of change of WM with time the power function of Fig. 6.2C was

differentiated (dWM/dt) and divide by the WM at time t (WM_t) to obtain the specific growth rate (μ'), ie. μ' = (dWM/dt)/WM_t. So, remembering that time is in days, we start with:

$$WM\ (mg) = a\ t(d)^b \qquad (6.3)$$

Then we differentiate with respect to time:

$$dWM/dt\ (mg\ d^{-1}) = a{\cdot}b\ t(d)^{b-1} \qquad (6.4)$$

Finally, dividing the first derivative (on the right of eqn 6.4) by WM_t, we get μ' in d^{-1}:

$$\mu' = a{\cdot}b\ t(d)^{b-1}/\ WM_t \qquad (6.5)$$

The resulting μ, based on the power function in Fig. 6.2C, is shown in Fig. 6.3. μ'was highest in small sizes and during the first days of culture (70.8 ± 20.5% d^{-1} between day 1 and 4) much higher than in the rest of the observations. Then the growth rate dropped slightly and stayed above 20% d^{-1} until day 13 where it stayed between 20 and 7% d^{-1} until day 24. Thereafter, it kept dropping until it reached the lowest value, 2.3% d^{-1}, on the last day.

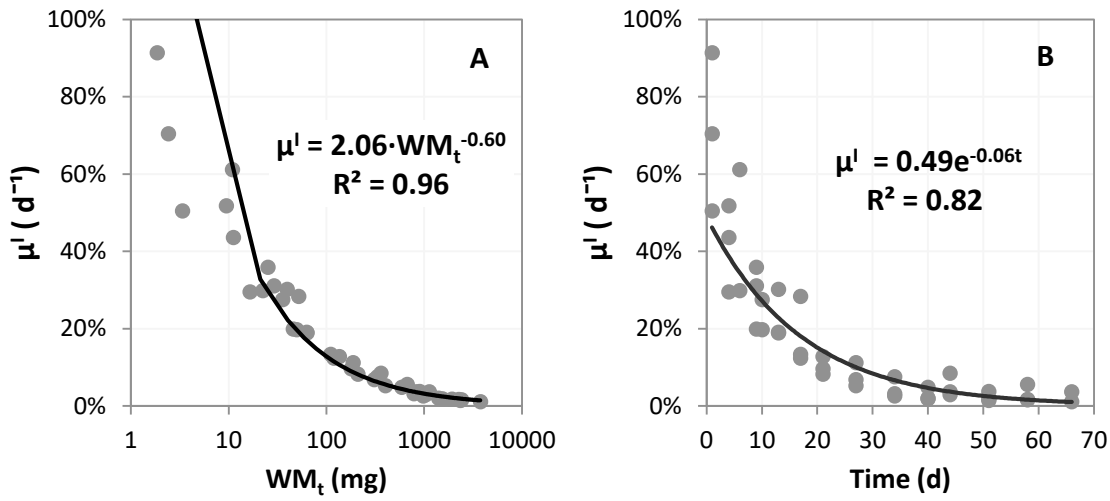


Figure 6.3. A. Specific growth rate (μ') based on equation 5 for different wet masses (WM_t) fitted to a power function (Regression analysis parameters: P<0.01, F=1028, DF=41). Beware of the x-axis' log scale. **B.** μ' with time (t) in days fitted to an exponential function (Regression analysis parameters: P<0.01, F=183.4, DF=41). A total of 43 organisms.

6.3.2. Respiratory metabolism

The relationship between R and WM and R and Φ were fitted to their respective allometric regression (Figs. 6.4A, B). The resulting power functions were R (μl O_2 h^{-1}) = 10.23·WM(g)$^{0.62}$ and R (μl O_2 h^{-1}) = 0.87·Φ (μl O_2 h^{-1})$^{0.73}$, respectively. The coefficient of determination with WM is higher. However, it must be mentioned that the Φ results for the ephyrae on the second day of experiments were high and the R unexpectedly low, hence those results do not follow the general trends observed. One of these trends is that smaller organisms had higher WM specific respiratory activity in both R and Φ (Fig. 6.4C). This is a well-known physiological property (Roberts, 1957). Similarly, younger organisms had higher respiratory activity than the last medusa stages (Fig. 6.4D). Furthermore, having both the physiological (R) and the enzymatic (Φ) respiration we can calculate the R:Φ ratio which shows the proportion of the potential maximum activity the organism is using for respiration.

Using this, we can observe that in the younger stages, with the exception of the second day, the R:Φ ratio is high (1.32±0.84, first 10 days) while in older stages many results are below 0.5 (0.43±0.18 from day 13 to 66) (Fig. 6.4C). Showcasing a higher activity in younger life stages or possibly evidencing a nutritional deficiency in older stages.

Additionally, R:Φ ratios exceeding 1.0 argue that there are biases or errors in the Φ assay and/or the R measurements, that the selection of the Φ assay is in error, or that in young organisms, there are unknown, but important O_2 consuming enzyme reactions not measured.

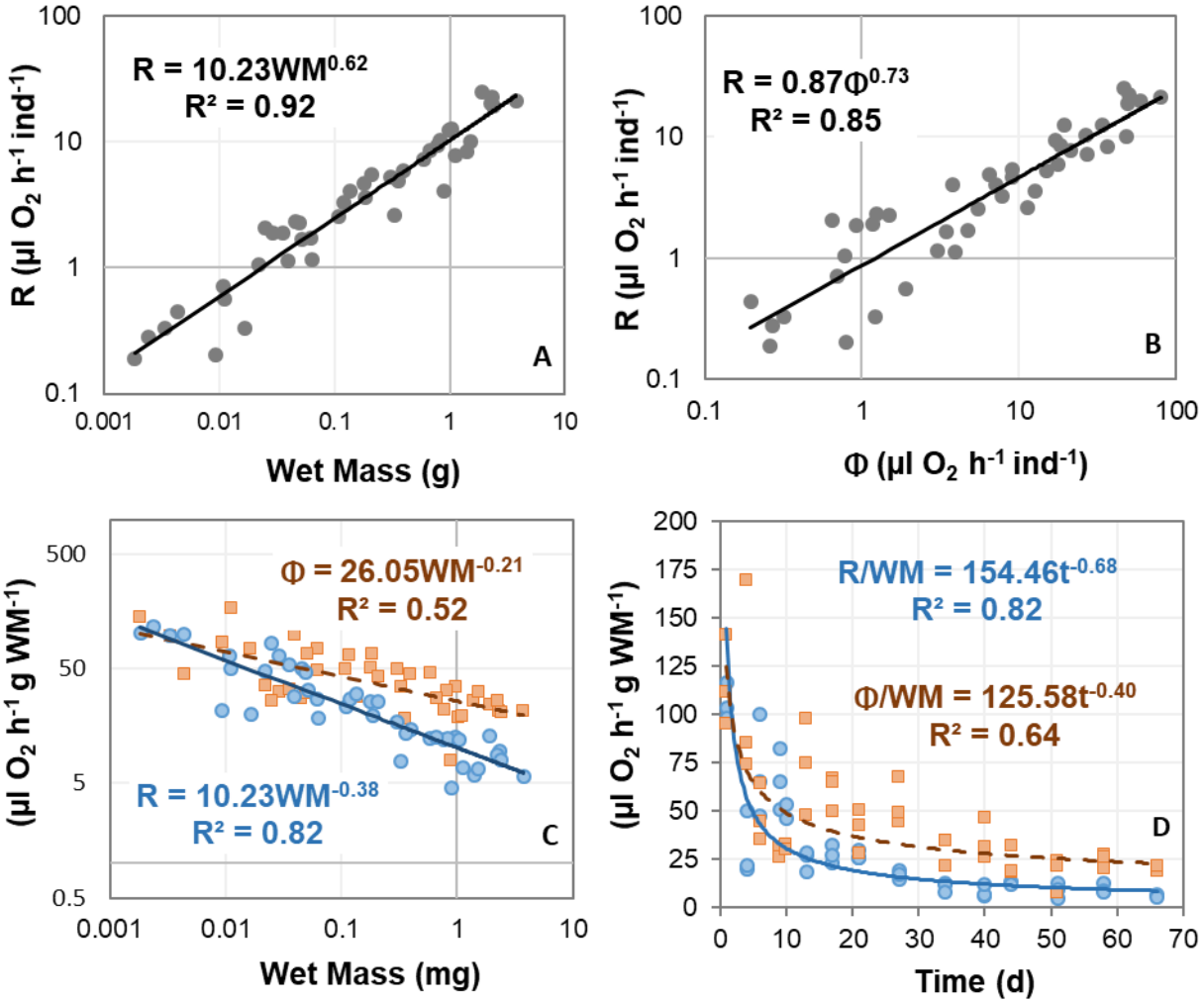


Figure 6.4. Respiratory activity during the culture. **A.** Regression analysis of physiological respiration (R) with wet mass (WM) with the corresponding power function fitting (Regression analysis parameters: P<0.01, F=500.6, DF=41). Beware of the x and y-axis' log scale. **B.** Regression analysis of physiological respiration (R) with potential respiration (Φ) with corresponding power function fitting (Regression analysis parameters: P<0.01, F=224.6, DF=41). **C.** Relation between WM-specific R (blue circles, solid line) and Φ (orange squares, dashed line) with WM with the corresponding power functions fitting (Respective regression analysis parameters: P<0.01, F=192.1, DF=41; P<0.01, F=44.91, DF=41). **D.** Evolution of WM-specific R (blue circles, solid line) and Φ (orange squares, dashed line) with time with the corresponding power function fitting (Respective regression analysis parameters: P<0.01, F=120.1, DF=41; P<0.01, F=49.12, DF=41). A total of 43 organisms. Beware log scale in A, B and C.

6.3.3. Lipid and protein composition

The lipid (L) and protein (P) content per WM were low (average <1%) as expected for gelatinous organism. P and L increased with size and mass presenting an allometric relationship with WM shown in the power functions in Fig. 6.5A. P shows a better fit than L. In contrast, P/WM and L/WM decrease rapidly with mass (Fig. 6.5B). The P/WM started at 0.78±0.13% in day 1 ephyrae and finished at 0.07±0.01% and L/WM started at 2.53±0.87% and finished at 0.02% (Fig. 6.5B). WM specific results may show a rapid decrease during the first 10 days due to an increase in water content which stabilised between day 13 and 17 (Fig. 6.5B). Interestingly, the lipids in the first days' ephyrae started at much higher levels than did their protein content (Fig. 6.5). The relative composition of the organic components shifted between days 13 and 17 to protein being the more concentrated organic component (Fig. 6.5B).

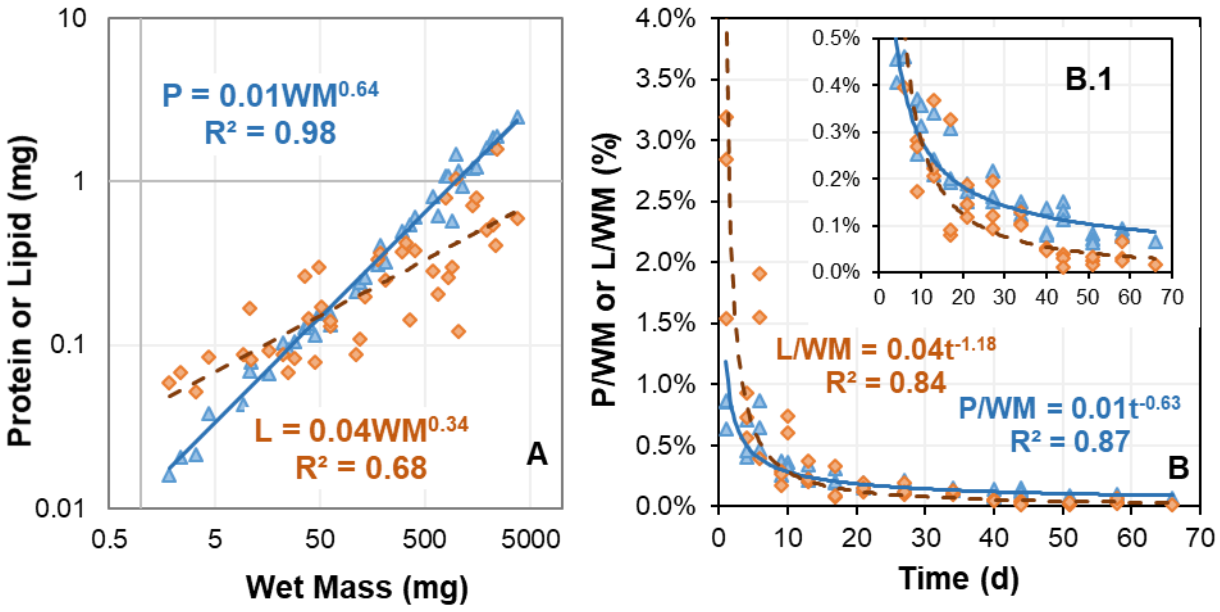


Figure 6.5. Protein (P) (Blue triangles, solid lines) and lipid (L) (Orange rhombus, dashed line) content in the *Aurelia aurita* cultured. **A.** Regression analysis of P and L content at different wet mass (WM) (Respective regression parameter: P<0.01, F=2645, DF=41, P<0.01, F=85.3, DF=41). Beware the log scale **B.** Evolution of P/WM and L/WM with time (t) fitted to a power function (Respective regression parameter: P<0.01, F=267.6, DF=41, P<0.01, F=207.6, DF=40). B.1. Amplified section of figure B from 0 to 0.5% y-axis to facilitate understanding at low levels of protein and lipid.

6.3.4. Starvation

The 5 starved organisms survived for at least 58 days except for one that looked unhealthy by day 37. The physiological O_2 consumption (R) by the end of the starvation period was about 5% of the starting R and around 15% the average R. In Fig. 6.6, the general trend observed was a decrease in respiration with food deprivation. However, more precisely, it can be divided into two periods. The first R measurement soon after feeding was the highest. Then there was a quick first drop to around 50-60% from the first R measurement. The second period started at around day 6 when R decreased fairly rapidly between days 6 and 16. By day 20 the rate of R deceleration slowed down and slowly reached the lowest values at between days 40 and 60 remaining fairly stable. Shrinking was observed but not quantified. By the end of the starvation period the average WM was 1404.6±144.3mg. Biochemical composition in the starved sample was compared to fed samples of similar size and no significant difference in the concentration of protein or lipid was observed. However, these

concentrations may be lower because shrinking was observed and likely decreased both mass and biochemical content.

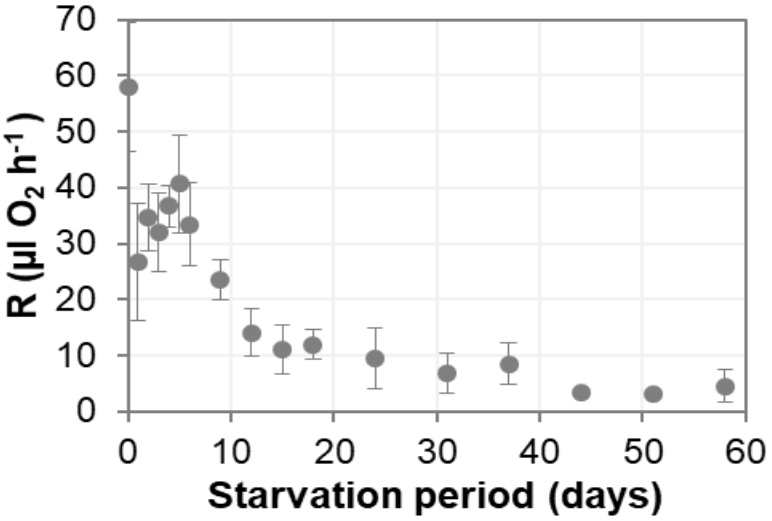


Figure 6.6. Evolution of physiological respiration (R) of *Aurelia aurita* after 0 to 58 days deprived of food. Points are the mean of the same 5 samples with their corresponding standard deviation.

6.4. DISCUSSION

6.4.1. Biometrics and growth

To be able to compare the results with similar observations in the literature, the biomass of ephyrae between 1 and 10mm was converted from WM to DM multiplying by 0.0391 (Lucas, 1994). For the same conversion in *A. aurita* of different sizes the factor 0.0342 from Lucas (1994), was used. To convert from DM to CM, the results were multiplied by 0.037, following Uye and Shimauchi (2005).

The allometric equation in Fig. 6.1 was similar to that of Olesen et al. (1994) (Table 6.1). After the particular conversions, the equation also resembled one in Uye and Shimauchi (2005) and in Båmstedt et al. (1994) (Table 6.1), even though in those studies the D ranges were <1-30cm approximately and the WM ranges were <1-1500g. Converted to µg of DM, our equation was similar to that of Båmstedt et al. (1999) (Table 6.1) with ephyrae <7mm, and to that of Kamiyama (2018) who worked with ephyrae and metephyrae of *Aurelia coerulea* <4mm in D.

Table 6.1. Compilation of *Aurelia spp*. power functions of mass (WM) and diameter (D) in the literature. Conversions were applied to the results in Fig. 6.1. The biomass of ephyrae between 1 and 10mm was converted from WM to DM multiplying by 0.0391. For the same conversion in older samples of different sizes the factor 0.03421 from Lucas (1994), was used. To convert from DM to CM, the results would be multiplied by 0.037 following Uye and Shimauchi (2005).

This study	Other Studies / Reference
$WM(mg)=0.49 \cdot D(mm)^{2.36}$	$WM(mg)=0.09 \cdot D(mm)^{2.79}$ / (Olesen et al., 1994)
$WM(g)=0.11 \cdot D(cm)^{2.36}$	$WM(g)=0.075 \cdot D(cm)^{2.86}$ / (Uye & Shimauchi, 2005) $WM(g)=0.095 \cdot D(cm)^{2.701}$ / (Båmstedt et al., 1994)
$DM(\mu g) = 19.04 \cdot D(mm)^{2.36}$	$DM(\mu g) = 22.33 \cdot D(mm)^{1.99}$ / (Båmstedt et al., 1999)
$CM (\mu g) =0.70 \cdot D(mm)^{2.36}$	$CM(\mu g) =1.19 \cdot D(mm)^{1.95}$ / (Kamiyama, 2018)

Besides the relation between size and mass, around day 17 (D≈10mm), the difference between the central disc's perimeter and the umbrella's perimeter were hard to distinguish. This corroborated the observations of Feitl et al. (2009) where the bell continuity reached 100% at 1cm. Such a morphological transition in the organism has implications regarding its Reynolds number and its functional interaction with the surrounding fluid.

While the increase in D was described by a linear function (Fig. 6.2C), the increase in WM followed an exponential or a power function (Thomas et al., 2010). This was very similar to the observations in Båmstedt et al. (1999) and Båmstedt et al. (2001) feeding samples with *Artemia* nauplii.

Several studies on jellyfish growth using biomass units showed the specific growth rate (μ), but few showed the exponential function describing the increase of biomass with time (Olesen et al., 1994; Frandsen & Riisgård, 1997; Møller & Riisgård, 2007; Lilley et al., 2014b). Our results, as a function of WM, fitted a power function rather than an exponential function. We will explore both μ and μ' as described in the results section.

Regarding μ, the growth experienced in the first days was much larger (52.8%d^{-1}) than during the rest of the experiment (between 32 – 0.5% d^{-1}). If we compare with μ results compiled by Frandsen and Riisgård (1997) (20-28% d^{-1}), they were smaller than during the first days and larger than our growth rates thereafter. The explanation could be that few experiments have been done using 1 day old ephyra and most have used longer time intervals between consecutive measurements. For example, Olesen et al. (1994) measured growth rate in ten days in samples with a starting D ~4mm and a final D ~9mm. In this conditions they would not observe the high ephyra initial growth rate and if we determined μ following eq.1 for the sizes and time period described (from day 1 to day 13) we would have obtained a μ value of 25.7% d^{-1}, closer to Olesen et al. (1994)'s observations. Similarly, Frandsen and Riisgård (1997) started at around 7.4 mm and ended at 9.5mm approximately and determined the growth rate in those 4 days resulting in 14 to 24% d^{-1}, missing the large growth rate of smaller sizes and starting to observe the lower growth rate at larger sizes. Conversely, Uye and Shimauchi (2005) determined growth rates in the wild after 90 days during the exponential growing period in 3 different years and obtained values ranging from 4.9 to 8.3% d^{-1}. Two of the few studies which have observed growth rates during the first days of the ephyra were Båmstedt et al. (1999) and Båmstedt et al. (2001). These articles reported higher growth rates during the first days of the ephyra ranging between 30 and 60% d^{-1}. However, Båmstedt et al. (2001) observed rapid growth rates for at least 10 days.

Except for the first days of the experiment, our results showed, in general, a lower μ than did other studies in the literature. Our results did not fit the exponential function described in the cited literature. One explanation may be that the growth has been insufficient due to the feeding or culture conditions. However, we proposed an analysis based on the resulting power function to obtain μ' displayed in Fig. 6.3. Using this analysis μ' was still higher in younger organisms, but fell in range with the observations in Båmstedt et al. (1999) and Båmstedt et al. (2001). Lilley et al. (2014b)'s Fig. 6.2 shows the evolution of μ with time in *Pelagia noctiluca*. The growth rate in smaller samples was markedly higher and there was a big drop as their size increases reaching specific growth rates below 5%d^{-1}. Møller and Riisgård (2007) also observed a much higher growth rate in organisms with a lower biomass and a quick drop as the biomass of the organisms increased. However, in both cases, μ from the smallest ephyrae only reached around 25% d^{-1}, so it is possible that the growth rates observed here for day 1 ephyrae, were abnormal. In any case, the results argue that specific growth rates in the first days of the ephyrae, were greater than during the remaining period from metephyra to juvenile medusa. Pitt et al. (2013) suggested that the high-water content in jellyfish may be key to allowing their rapid growth rates. This will be explored further on in the proximate composition section.

6.4.2. Respiratory Metabolism

From the multiple articles describing respiration in the genus *Aurelia* (Purcell et al., 2010), few centre on investigating the respiratory metabolism of ephyra. Probably the most unusual result regarding respiration is that the exponent of the equation relating WM and R is 0.62 (Fig. 6.4). Low exponents have been found before but in general, jellyfish are characterized by exponents close to 1 (Purcell et al., 2010). (Low values indicate high curvature of the power function.) Gambill and Peck (2014) compiled and plotted several results and observed functions of R and DM with an exponent closer to 0.65 for ephyrae while closer to 0.9 in medusae. If our results are converted to DM (mg) the resulting equation would have been R (µl O_2 h^{-1}) =1.09·$DM^{0.63}$ per h and R (µl O_2 d^{-1}) =26.12·$DM^{0.63}$ per day. Frandsen and Riisgård (1997) reported the function R (µl O_2 d^{-1}) =10.89·$DM(mg)^{0.86}$ reporting a higher exponent, but also working with samples ranged from ephyra with 0.4mg of DM to medusa of 2000mg DM; 20 times bigger than the biggest sample in our study. Kinoshita et al. (1997) found a low exponent (R (µl O_2 h^{-1}) =-0.91·$DM^{0.63}$) in a function much similar to ours. The authors speculated that allometric scaling defined ephyra respiration, while isometric scaling defined respiration in adult medusa; this was also observed in Frandsen and Riisgård (1997)'s data if the results are analysed separated by sizes. However, the isometric scaling was not observed when performing a regression analysis with this study's juvenile medusa, only. Kinoshita et al. (1997) also proposed that mass specific respiration in ephyrae was higher than in adults and cited the result of Mangum et al. (1972) on ephyra respiration (108.2 µl O_2 (g WM)$^{-1}$ h-1), very similar to our results for 24h ephyra, 106 ± 9.4 µl O_2 (g WM)$^{-1}$ h^{-1} (Fig. 6.4D). Kinoshita et al. (1997) suggested that ephyra and metephyra require a higher activity to swim due to their morphology. This higher activity in ephyra coincides with the higher R:Φ ratio (Fig. 6.3), i.e. higher use of the respiratory potential, and also coincides with the period of higher growth. Alternatively, respiratory activity may have decreased due to feeding. The organisms were fed *ad libitum* once per day. This may have been enough for the ephyra but not for juveniles, especially with non-enriched *Artemia*. Lack of food in larger sizes may also decrease the R:Φ ratio (Hernández-León & Gómez, 1996).

We predict that R during strobilation may be as high as the potential respiration (Φ) measured in Purcell et al. (2019), corresponding to the R of multiple ephyrae in formation and the polyp metabolism during the metamorphosis.

6.4.3. Proximate composition (lipid (L) and protein (P))

The article by Lucas (1994) is a very extensive analysis of proximate/biochemical components in *A. aurita* from ephyrae <10mm to medusa of 150mm. The P/DM results ranged from 2.07 – 28.56% and L/DM between 1.17-11.5%. The results, here presented, fall in that range except for the high lipid content in smaller samples (P/DM: 2.2 – 20.1%, L/DM: 0.5 – 64.6%). However, on average, per size range, our numbers are lower than her values except for L in small samples which are absent. Both studies show a rapid decrease of the biochemical concentration as size increased (Fig. 6.5). Some authors may suggest that this drop of lipid content below protein content could be partially due to the effect on fatty acids caused by feeding ephyrae with non-enriched *Artemia* (Chi et al., 2018). But this would not explain the high initial lipid content or the drop in both protein and lipid observed. The decrease in biochemical components was probably due to consumption for growth and energy during the first days of rapid growth and activity, as well as an increase in water content starting the development of the "faking giant" (Acuña et al., 2011; Pitt et al., 2013).

In Lucas (1994) the averages were grouped by size starting from 1-10mm, 10-20mm and so on. The lipid content in samples 1-10mm seemed to be absent. A shift in the biochemical components was observed here in that size range that could not be documented by Lucas (1994). Lipids were more abundant than proteins at the beginning and both decreased as the ephyrae grew. By the time medusa were juvenile, proteins were more concentrated than lipids. This was an inversion in the

relative concentration of these components (Fig. 6.5). The inversion was observed between days 13 and 17 when D was around 7-9mm. The greatest difference between the protein and lipid content was observed in the first days of the experiment. We consider that our results enrich the already thorough analysis in Lucas (1994).

According to the conversion to DM present in the literature (Lucas, 1994), the lipid content would have started here at around 64.6% DM in day 1 and around 18% DM by day 4. This high lipid content has never been documented before, except for the arctic samples of Percy and Fife (1981) (22.1% DM maximum). However, in arctic samples it was not higher than the protein.

L and P content dropped rapidly during the first days (L/WM: 2.5 to 0.7%, P/WM: 0.8 to 0.5%, Fig. 6.5). This coincided with the period of highest specific growth and the period that required higher activity to swim. Planula larvae have also been shown to have a high lipid content that serves as temporary food source (Nordström et al., 2003). It is possible that, apart from energy storage, the initial high lipid content may aid in the buoyancy of this larval stage (Kattner et al., 2007). The combination of an energy source and buoyancy boost may be key in the transitional period as the bell fills out to a full umbrella. In our results, around day 17, the bell was completely developed as well as completing the protein-lipid inversion at a D of around 10mm.

The disproportionate percentage of lipids per DM could suggest an error in the conversion to DM or in the lipid determination in these younger stages. If the measurements are correct it predicts that strobila stage of the polyps may accumulate lipids. The cold temperatures required for strobilation may provide the conditions needed to decrease metabolic energy consumption and convert the food consumed to lipid storage for the resulting ephyrae. Both strobila and ephyra have high food requirements (Lucas et al., 2012). Consumption of strobila and ephyrae may be nutritiously profitable for specific predators (Östman, 1997; Takao et al., 2014), especially if they produce up to 20 ephyrae during strobilation (Lucas et al., 2012) and considering the essential fatty acid (FA) composition described by Stenvers et al. (2020) for this species. Stenvers et al. (2020) observed in their results a higher FA content on average in their 3 analysed wild ephyrae of unknown age. However, the FA content measured was 102.08 μg $(g\ DM)^{-1}$, several orders of magnitude below the lipid measurements presented here since FAs are one component of the total lipids. This lipid content in newly formed ephyra if used as a food source helps explain the observations by Fu et al. (2014) where ephyrae survived starved for between 44 and 92 days depending on the temperature (15 – 9°C). This survivorship can have implications on the range in space and time of the newly formed ephyrae.

6.4.4. Starvation

Fig. 6.6 shows how respiration had dropped significantly by day 10. This resembles the rapid drop observed by Costello (1998) in the anthomedusa, *Cladonema californicum*. This drop took place slightly before the 10th day. Those specimens lasted for more than 25 days and in one of the replicates for more than 40 days. Arai (1986) did not observe any decrease in respiration during the first 3 days of food deprivation in the hydromedusa *Aequorea victoria* and neither did Costello (1998) for the first 3 days in larger samples. We observed an initial drop followed by a period of approximate stability and a bigger drop after approximately 6 days. Møller and Riisgård (2007) observed that the respiration responded promptly to feeding in *A. aurita*, it increased to a maximum in 4h and decreased 2.5h afterwards to pre-fed levels. Their maximum respiration was 3.5-fold the pre-fed respiration, which is higher than the response observed here in the first drop (2.3 approximately). We suggest the first drop shown in Fig. 6.6 corresponds to this first response. The second drop could be occurring concurrently with shrinking. Frandsen and Riisgård (1997) described in *A aurita* R during a 25-d starvation period. In the first 6 days, the R decreased slower and a bigger drop was not as noticeable until the 9th day and by day 25 R was less than half the initial R. Biomass behaved similarly, decreasing during the starvation period.

Besides cnidarians, the ctenophores, *Mnemiopsis leidyi* and *Beroe ovata,* in a 10 and 20-day starvation period, displayed a slow decrease in R and DM (Anninsky et al., 2005). In Regnault's (1981) experiment with the shrimp, *Crangnon cragnon,* the results were similar. The R of that shrimp decreased without a big drop in the first 5 days. Then, it suffered a much larger drop between day 9-12 followed by a period of slower, fairly stable decrease until day 30 of the starvation period. Their R measurements started 12h after feeding, so the first drop may have been missed. It must be mentioned that in their case, the R at day 30 was 37% that of R on day 1. Our R by day 30 compared to day 1 was around 20%; and 13% by day 58.

Hamner and Jenssen (1974) documented a continuous decrease in size of *A. aurita* for more than 120 days. They also started to observe the decrease in size after, approximately, 6 days of starvation. According to their observation, by day 60, WM was 32% the WM in day 6 a. Therefore, some of our decrease in R could be explained by shrinkage, however there must have been further decrease in the metabolic activity to achieve the low R detected (Fig. 6.6).

A. aurita polyps have also been shown to decrease their wet mass and R under starvation conditions (Purcell et al., 2019). Hence, this process may occur in both sessile and planktonic life stages as a response to unfavourable conditions.

6.5. CONCLUSIONS

Aurelia aurita ephyrae displayed changes in their anatomy, physiology and biochemistry as they transition to juvenile medusa. Here, we show how the specific growth rate was higher in ephyra than in juvenile medusa, but especially much higher in one day old ephyra. We also proposed an alternative determination of growth rate when growth displays a power function. Higher specific respiratory metabolism close to their specific respiratory potential was detected in recently born ephyra. The lipid content of the ephyrae exceeded protein content. This was the first observation of this kind reported in the literature. We suggest that the combination of these factors ephyrae survive during their first days, a period of crucial development and higher swimming activity. The lipids serve as a food source and buoyancy support. The transition from ephyra to juvenile medusa took place around the second and third week as the umbrella perimeter reached 100% continuity, while growth and respiration rate decreased and protein began to exceed lipid content. All parameter changes kept stabilising as the juvenile stage developed.

Once a juvenile, the species was able to decrease its respiratory metabolism under starvation condition. The combination of this metabolic regulation combined with shrinking in size provided *A. aurita* a mechanism to decrease its food requirement and stay alive even after a starvation period of 58 days.

REFERENCES

Abrams, M. J., & L. Goentoro, 2016. Symmetrization in jellyfish: Reorganization to regain function, and not lost parts. Zoology Elsevier GmbH. 119: 1–3, http://dx.doi.org/10.1016/j.zool.2015.10.001.

Acuña, J. L., Á. López-Urrutia, & S. Colin, 2011. Faking giants: the evolution of high prey clearance rates in jellyfishes. science American Association for the Advancement of Science 333: 1627–1629.

Algueró-Muñiz, M., C. L. Meunier, S. Holst, S. Alvarez-Fernandez, & M. Boersma, 2016. Withstanding multiple stressors: ephyrae of the moon jellyfish (*Aurelia aurita*, Scyphozoa) in a high-temperature, high-CO 2 and low-oxygen environment. Marine Biology Springer Berlin Heidelberg 163: 1–12.

Anninsky, B. E., G. A. Finenko, G. I. Abolmasova, E. S. Hubareva, L. S. Svetlichny, L. Bat, & A. E. Kideys, 2005. Effect of starvation on the biochemical compositions and respiration rates of ctenophores *Mnemiopsis leidyi* and Beroe ovata in the Black Sea. Journal of the Marine Biological Association of the United Kingdom 85: 549–561.

Arai, M. N., 1986. Oxygen consumption of fed and starved *Aequorea victoria* (Murbach and Shearer, 1902) (Hydromedusae). Physiological Zoology 59: 188–193.

Båmstedt, U., J. Lane, & M. B. Martinussen, 1999. Bioenergetics of ephyra larvae of the scyphozoan jellyfish *Aurelia aurita* in relation to temperature and salinity. Marine Biology 135: 89–98.

Båmstedt, U., M. B. Martinussen, & S. Matsakis, 1994. Trophodynamics of the two scyphozoan jellyfishes, *Aurelia aurita* and *Cyanea capillata*, in western Norway. ICES Journal of Marine Science 51: 369–382.

Båmstedt, U., B. Wild, & M. B. Martinussen, 2001. Significance of food type for growth of ephyrae *Aurelia aurita* (Scyphozoa). Marine Biology 139: 641–650.

Bligh, E. G., & W. J. Dyer, 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology NRC Research Press 37: 911–917.

Brotz, L., W. W. L. Cheung, K. Kleisner, E. Pakhomov, & D. Pauly, 2012. Increasing jellyfish populations: Trends in Large Marine Ecosystems. Hydrobiologia 690: 3–20.

Cegolon, L., W. C. Heymann, J. H. Lange, & G. Mastrangelo, 2013. Jellyfish stings and their management: A review. Marine Drugs 11: 523–550.

Ceh, J., J. Gonzalez, A. S. Pacheco, & J. M. Riascos, 2015. The elusive life cycle of scyphozoan jellyfish - Metagenesis revisited. Scientific Reports Nature Publishing Group 5: 1–13, http://dx.doi.org/10.1038/srep12037.

Chi, X., J. Javidpour, U. Sommer, & D. Mueller-Navarra, 2018. Tracking fatty acids from phytoplankton to jellyfish polyps under different stress regimes: A three trophic levels experiment. Frontiers in Ecology and Evolution 6:.

Condon, R. H., W. M. Graham, C. M. Duarte, K. A. Pitt, C. H. Lucas, S. H. D. Haddock, K. R. Sutherland, K. L. Robinson, M. N. Dawson, M. B. Decker, C. E. Mills, J. E. Purcell, A. Malej, H. Mianzan, S. Uye, S. Gelcich, & L. P. Madin, 2012. Questioning the Rise of Gelatinous Zooplankton in the World's Oceans. BioScience 62: 160–169.

Costello, J., 1998. Physiological response of the hydromedusa *Cladonema californicum* Hyman (Anthomedusa: Cladonemidae) to starvation and renewed feeding. Journal of Experimental Marine Biology and Ecology 225: 13–28.

De Coen, W. M., & C. R. Janssen, 1997. The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed Daphnia populations. Journal of Aquatic Ecosystem Stress and Recovery Springer 6: 43–55.

Dong, Z., 2019. Blooms of the Moon Jellyfish *Aurelia* : Causes, Consequences and Controls. World Seas: an Environmental Evaluation. Elsevier-Academic Press, https://doi.org/10.1016/B978-0-12-805052-1.00008-5.

Doyle, T. K., G. C. Hays, C. Harrod, & J. D. R. Houghton, 2014. Ecological and societal benefits of jellyfish Jellyfish blooms. Springer: 105–127.

Duarte, C. M., K. A. Pitt, C. H. Lucas, J. E. Purcell, S. Uye, K. L. Robinson, L. Brotz, M. B. Decker, K. R. Sutherland, A. Malej, L. Madin, H. Mianzan, J. M. Gili, V. Fuentes, D. Atienza, F. Pagès, D. Breitburg, J. Malek, W. M. Graham, & R. H. Condon, 2013. Is global ocean sprawl a cause of jellyfish blooms?. Frontiers in Ecology and the Environment 11: 91–97.

Feitl, K. E., A. F. Millett, S. P. Colin, J. O. Dabiri, & J. H. Costello, 2009. Functional morphology and fluid interactions during early development of the Scyphomedusa World Seas: An Environmental Evaluation *Aurelia aurita*. Biological Bulletin 217: 283–291.

Frandsen, K. T., & H. U. Riisgård, 1997. Size dependent respiration and growth of jellyfish, *Aurelia aurita*. Sarsia 82: 307–312.

Frick, M. G., K. L. Williams, A. B. Bolten, K. A. Bjorndal, & H. R. Martins, 2009. Foraging ecology of oceanic-stage loggerhead turtles *Caretta caretta*. Endangered Species Research 9: 91–97.

Fu, Z., M. Shibata, R. Makabe, H. Ikeda, & S. I. Uye, 2014. Body size reduction under starvation, and the point of no return, in ephyrae of the moon jellyfish *Aurelia aurita*. Marine Ecology Progress Series 510: 255–263.

Gambill, M., & M. A. Peck, 2014. Respiration rates of the polyps of four jellyfish species: Potential thermal triggers and limits. Journal of Experimental Marine Biology and Ecology Elsevier B.V. 459: 17–22, http://dx.doi.org/10.1016/j.jembe.2014.05.005.

Gilchrist, F. G., 1937. Budding and Locomotion in the Scyphistomas of *Aurelia*. The Biological Bulletin 72: 99–124.

Goldstein, J., & H. U. Riisgård, 2016. Population dynamics and factors controlling somatic degrowth of the common jellyfish, *Aurelia aurita*, in a temperate semi-enclosed cove (Kertinge Nor, Denmark). Marine Biology Springer Berlin Heidelberg 163: 1–12.

Gómez, M., S. Torres, & S. Hernzández-León, 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. South African Journal of Marine Science 17: 15–20.

Haddock, S. H. D., 2004. A golden age of gelata: past and future research on planktonic ctenophores and cnidarians.

Hydrobiologia 530: 549−556.

Hamner, W. M., & R. M. Jenssen, 1974. Growth , Degrowth , and Irreversible Cell Differentiation in *Aurelia aurita*. American Zoologist 14: 833–849.

He, J., L. Zheng, W. Zhang, & Y. Lin, 2015. Life cycle reversal in *Aurelia sp.1* (Cnidaria, Scyphozoa). PLoS ONE 10: 1–14.

Hernández-León, S., & M. Gómez, 1996. Factors affecting the respiration/ETS ratio in marine zooplankton. Journal of Plankton Research 18: 239–255.

Kamiyama, T., 2018. Planktonic ciliates as food for the scyphozoan *Aurelia coerulea*: feeding and growth responses of ephyra and metephyra stages. Journal of Oceanography Springer Japan 74: 53–63.

Kattner, G., W. Hagen, R. F. Lee, R. Campbell, D. Deibel, S. Falk-Petersen, M. Graeve, B. W. Hansen, H. J. Hirche, S. H. Jónasdóttir, M. L. Madsen, P. Mayzaud, D. Müller-Navarra, P. D. Nichols, G. A. Paffenhöfer, D. Pond, H. Saito, D. Stübing, & P. Virtue, 2007. Perspectives on marine zooplankton lipids. Canadian Journal of Fisheries and Aquatic Sciences 64: 1628–1639.

Kinoshita, J., J. Hiromi, & S. Kadota, 1997. Do respiratory metabolic rates of the scyphomedusa *Aurelia aurita* scale isometrically throughout ontogeny in a sexual generation? Hydrobiologia 347: 51–55.

Kotova, A. V., O. A. Kovtun, O. I. Podgornaya, & L. S. Adonin, 2015. Mesoglein differences between two jellyfish populations of the genus *Aurelia*. International Aquatic Research Springer Berlin Heidelberg 7: 123–131, http://dx.doi.org/10.1007/s40071-015-0096-8.

Lebrato, M., M. Pahlow, J. R. Frost, M. Küter, P. de Jesus Mendes, J. C. Molinero, & A. Oschlies, 2019. Sinking of Gelatinous Zooplankton Biomass Increases Deep Carbon Transfer Efficiency Globally. Global Biogeochemical Cycles 33: 1764–1783.

Lilley, M. K. S., A. Elineau, M. Ferraris, A. Thiéry, L. Stemmann, G. Gorsky, & F. Lombard, 2014a. Individual shrinking to enhance population survival: Quantifying the reproductive and metabolic expenditures of a starving jellyfish, Pelagia noctiluca. Journal of Plankton Research 36: 1585–1597.

Lilley, M. K. S., M. Ferraris, A. Elineau, L. Berline, P. Cuvilliers, L. Gilletta, A. Thiéry, G. Gorsky, & F. Lombard, 2014b. Culture and growth of the jellyfish *Pelagia noctiluca* in the laboratory. Marine Ecology Progress Series 510: 265–273.

Lilley, M. K. S., & F. Lombard, 2015. Respiration of fragile planktonic zooplankton: Extending the possibilities with a single method. Journal of Experimental Marine Biology and Ecology 471: 226–231.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, & R. J. Randall, 1951. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry 193: 265–275.

Lucas, C. H., 1994. Biochemical composition of *Aurelia aurita* in relation to age and sexual maturity. Journal of Experimental Marine Biology and Ecology 183: 179–192.

Lucas, C. H., S. Gelcich, & S.-I. Uye, 2014. Living with Jellyfish: Management and Adaptation Strategies In Lucas, C. H., & K. A. Pitt (eds), Jellyfish Blooms. Dordrecht: 129–149.

Lucas, C. H., W. M. Graham, & C. Widmer, 2012. Jellyfish Life Histories: Role of Polyps in Forming and Maintaining Scyphomedusa Populations Advances in Marine Biology. Elsevier Ltd.: 133–196, http://dx.doi.org/10.1016/B978-0-12-394282-1.00003-X.

Mangum, C. P., M. J. Oakes, & J. M. Shick, 1972. Rate-temperature responses in scyphozoan medusae and polyps. Marine Biology 15: 298–303.

Markwell, M. A. K., S. M. Haas, L. L. Bieber, & N. E. Tolbert, 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Analytical Biochemistry 87: 206–210.

Martínez, I., A. Herrera, M. Tames-espinosa, D. R. Bondyale-juez, V. Romero-kutzner, T. T. Packard, & M. Gómez, 2020. Protein in marine plankton: A comparison of spectrophotometric methods. Journal of Experimental Marine Biology and Ecology Elsevier 526: 151357.

Miyake, H., K. Iwao, & Y. Kakinuma, 1997. Introduction Life History and Environment of *Aurelia aurita*. 273 South Pacific Study 17:, http://cpi.kagoshima-u.ac.jp/publications/southpacificstudies/sps/sps17-2/SouthPacificStudies17(2)pp273-285.pdf.

Møller, L. F., & H. U. Riisgård, 2007. Respiration in the scyphozoan jellyfish *Aurelia aurita* and two hydromedusae (*Sarsia tubulosa* and *Aequorea vitrina*): Effect of size, temperature and growth. Marine Ecology Progress Series 330: 149–154.

Nordström, K., R. Wallén, J. Seymour, & D. Nilsson, 2003. A simple visual system without neurons in jellyfish larvae. Proceedings of the Royal Society B: Biological Sciences 270: 2349–2354.

Olesen, N. J., K. Frandsen, & H. U. Riisgard, 1994. Population dynamics, growth and energetics of jellyfish *Aurelia aurita* in a shallow fjord. Marine Ecology Progress Series 105: 9–18.

Östman, C., 1997. Abundance, feeding behaviour and nematocysts of scyphopolyps (Cnidaria) and nematocysts in their predator, the nudibranch *Coryphella verrucosa* (Mollusca). Hydrobiologia 355: 21–28.

Packard, T. T., 1971. The Measurement of Respiratory Electron-transport Activity tn Marine Phytoplankton. Journal of Marine Research 29: 235–244.

Packard, T. T., & J. P. Christensen, 2004. Respiration and vertical carbon flux in the Gulf of Maine water column. Journal of Marine Research 62: 93–115.

Packard, T. T., D. Harmon, & J. Boucher, 1974. Respiratory electron transport activity in plankton from upwelled waters. Tethys 6: 213–222.

Pauly, D., W. Graham, S. Libralato, L. Morissette, & M. L. Deng Palomares, 2009. Jellyfish in ecosystems, online databases, and ecosystem models. Hydrobiologia 616: 67–85.

Percy, J. A., & F. J. Fife, 1981. The Biochemical Composition and Energy Content of Arctic Marine Macrozooplankton. Arctic 34: 307–313.

Pereira, R., M. A. Teodósio, & S. Garrido, 2014. An experimental study of *Aurelia aurita* feeding behaviour: Inference of the potential predation impact on a temperate estuarine nursery area. Estuarine, Coastal and Shelf Science 146: 102–110.

Piraino, S., F. Boero, B. Aeschbach, & V. Schmid, 1996. Reversing the life cycle: Medusae transforming into polyps and cell transdifferentiation in *Turritopsis nutricula* (Cnidaria, Hydrozoa). Biological Bulletin 190: 302–312.

Pitt, K. A., C. M. Duarte, C. H. Lucas, K. R. Sutherland, R. H. Condon, H. Mianzan, J. E. Purcell, K. L. Robinson, & S. I. Uye, 2013. Jellyfish Body Plans Provide Allometric Advantages beyond Low Carbon Content. PLoS ONE 8: 1–10.

Pitt, K. A., C. H. Lucas, R. H. Condon, C. M. Duarte, & B. Stewart-Koster, 2018. Claims That Anthropogenic Stressors Facilitate Jellyfish Blooms Have Been Amplified Beyond the Available Evidence: A Systematic Review. Frontiers in Marine Science 5: 1–11.

Purcell, J. E., D. R. Bondyale-Juez, V. Romero-Kutzner, I. Martínez, R. Caprioli, M. Tames-Espinosa, J. Almunia, E. Alonso, T. T. Packard, & M. Gómez, 2019. Food supply effects on the asexual reproduction and respiratory metabolism of *Aurelia aurita* polyps. Hydrobiologia 846: 135–146.

Purcell, J. E., V. Fuentes, D. Atienza, U. Tilves, D. Astorga, M. Kawahara, & G. C. Hays, 2010. Use of respiration rates of scyphozoan jellyfish to estimate their effects on the food web. Hydrobiologia 645: 135–152.

Purcell, J. E., S. I. Uye, & W. T. Lo, 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: A review. Marine Ecology Progress Series 350: 153–174.

R Core Team, 2017. R: A language and environment for statistical com-puting. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from ….

Regnault, M., 1981. Respiration and Ammonia Excretion of the Shrimp *Crangon crangon* L.: Metabolic Response to Prolonged Starvation. Journal of Comparative Physiology 141: 549–555.

Richardson, A. J., A. Bakun, G. C. Hays, & M. J. Gibbons, 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends in Ecology and Evolution 24: 312–322.

Roberts, J., 1957. Thermal acclimation of metabolism in the crab *Pachygrapsus crassipes* randall. I. The influence of body size, starvation, and molting. Physiological Zoology 30: 232–242.

Rutter, W. J., 1967. Protein determinations in embryos In Wilt, F. H., & N. K. Wessels (eds), Methods in developmental biology. New York: 671–683.

Stenvers, V., X. Chi, & J. Javidpour, 2020. Seasonal variability of the fatty acid composition in *Aurelia aurita* (Cnidaria: Scyphozoa): implications for gelativore food web studies. Journal Of Plankton Research 00: 1–13.

Takao, M., H. Okawachi, S. I. Uye, & H. Okawachi, 2014. Natural predators of polyps of *Aurelia aurita* s.l. (Cnidaria: Scyphozoa: Semaeostomeae) and their predation rates. Plankton and Benthos Research 9: 105–113.

Thiebot, J. B., & J. C. McInnes, 2020. Why do marine endotherms eat gelatinous prey?. ICES Journal of Marine Science 77: 58–71.

Thomas, G. B., M. D. Weir, J. Hass, F. R. Giordano, & R. Korkmaz, 2010. Thomas' calculus. Pearson (Addison-Wesley), London.

Uye, S., & H. Shimauchi, 2005. Population biomass, feeding, respiration and growth rates, and carbon budget of the scyphomedusa *Aurelia aurita* in the Inland Sea of Japan. Journal of Plankton Research 27: 237–248.

Young, G. A., & J. W. Hagadorn, 2010. The fossil record of cnidarian medusae. Palaeoworld "Elsevier B.V. and NANJING INSTITUTE OF GEOLOGY AND PALAEONTOLOGY, CAS" 19: 212–221, http://dx.doi.org/10.1016/j.palwor.2010.09.014.

Zotin, A. A., 2015. The United Equation of Animal Growth. American Journal of Life Sciences 3: 345.

CHAPTER 7

RESUMEN DEL CONTENIDO Y DEL ESTADO DE LA MATERIA (en castellano)

7.1. El oxígeno, la respiración y la vida.

El oxígeno (O_2) normalmente se encuentra como una molécula diatómica en forma gaseosa a temperatura ambiente. Esta simple molécula ha tenido grandes implicaciones para la aparición y desarrollo de la vida tal y como la conocemos en este planeta.

Seguramente la conexión más directa entre el oxígeno y la existencia de vida en este planeta es su participación en los procesos respiratorios, sin embargo, no siempre fue así. Siguiendo las descripciones de Canfield (2005), hace más de 2500 millones de años, la vida en la Tierra era procariota y empleaba primordialmente procesos anóxicos. Se especula que algunos microorganismos de esta época ya contaban con rutas metabólicas capaces de aprovechar bajas concentraciones de O_2 resultante de procesos químicos minoritarios. Serían estos los grandes beneficiarios del cambio que ocurriría. Hay gran incertidumbre sobre como exactamente transcurrió este proceso, pero lo que sí ha sido confirmado es que hace aproximadamente 2400 millones de años, a comienzos del proterozoico, la concentración de O_2 atmosférico había estado aumentando rápidamente en los últimos miles de años en lo que se conoce como la Gran Oxidación (Holland, 2006) (Fig. 7.1). Se sospecha que uno de los principales procesos responsables, o incluso el responsable principal, fue la fotosíntesis oxigénica. Se considera que las encargadas de este novedoso proceso fueron primitivas microcianobacterias bentónicas. Como bien relata Sánchez-Baracaldo (2015) a lo largo del proterozoico estas fueron evolucionando y diversificándose y aparecieron macrocianobacterias bentónicas que continuaron con el incremento de O_2; como también lo hicieron macro y microcianobacterias planctónicas que fueron apareciendo a finales del proterozoico dando paso al phanerozoico con una alta producción primaria en los océanos que tendría y sigue teniendo grandes consecuencias para la vida en la tierra. Knoll (2003) describe de manera excelente que la novedad de este proceso no radica en emplear la energía luminosa para la generación de materia orgánica y ATP, que ya realizaban otras bacterias fotosintéticas, sino en la generación como producto secundario de O_2 a partir de H_2O (fotólisis del agua).

El aumento de oxígeno provocado por las cianobacterias se vio limitado en principio a la zona fótica puesto que requería luz. Debido a las reacciones de oxidación, el oxígeno disminuyó la presencia de otros donantes de electrones usados por otros fotoautótrofos permitiendo un mayor dominio de las cianobacterias (Knoll et al., 2016). Sin embargo, la mezcla de aguas necesaria para que el aumento de oxígeno llegase mucho más allá de la zona fótica y alterase para siempre la composición atmosférica requeriría varios miles de años.

Ante este aumento de la concentración de O_2, autores como Margulis & Sagan (1995) plantean que tuvo lugar el llamado “holocausto de oxígeno” en el que sobrevivieron aquellos organismos capaces de implementar estrategias para aprovechar dicho O_2. Aquellos procariontes capaces de usar O_2 para la generación de energía se vieron

beneficiados. No obstante, Knoll et al. (2016) matiza estas afirmaciones añadiendo que junto con el oxígeno aumentaron también otros aceptores de electrones como sulfatos o nitratos, por lo que la diversidad de procesos anaerobios y quimiosintéticos también aumentó (Lane, 2017). En cualquier caso, el aumento de oxigeno permitió también que los organismos desarrollaran lo que conocemos como respiración aeróbica. Este proceso lograba generar energía mediante transportes de electrones que terminan con la reducción del O_2 a H_2O. Dicha reacción transcurría en estructuras moleculares aceptores de electrones conocidos como O_2Red. Brochier-Armanet et al. (2009) realiza un recorrido por los diversos grupos de bacterias y arqueas capaces de realizar la respiración aeróbica. De entre ellas es de particular interés, para la siguiente transición de la vida, las bacterias que contienen O_2red del tipo A ya que comparten un alto parecido genético con el material genético de las mitocondrias.

El cambio en la concentración de O_2 alteró la composición de las comunidades bacterianas, pero también propició las condiciones para un salto en la diversidad de la vida en el planeta. Hasta ahora solo se han mencionado organismos procariontes de complejidad celular simple y carentes de orgánulo ni núcleo celular. Pero tras la gran oxidación aparecieron también los organismos eucariotas con núcleo y orgánulos celulares entre los que se encuentran los cloroplastos y las mitocondrias. Las evidencias genéticas apuntan a que, durante el proterozoico, cuando el oxígeno sólo había aumentado ligeramente en las aguas superficiales, una célula procariota terminó en el interior de otra célula procariota y ni la primera fue digerida ni la segunda pereció. Habían logrado convivir en esta nueva unión (Lane, 2017; Roger et al., 2017). Esta unión resultó ser estable y ventajosa y con el tiempo esta nueva formación celular fue especializando su novedosa disposición formando lo que hoy conocemos como células eucariotas (Margulis & Sagan, 1995). A este proceso se le conoce como endosimbiosis (Sagan, 1967; Gray, 2017). Muchas evidencias apuntan a que la primera endosimbiosis que dio lugar a células eucariotas fue entre una proteobacteria y una arquea (Williams et al., 2013) donde la proteobacteria se convertiría en lo que hoy conocemos como mitocondria y la arquea hospedadora en la célula que alberga al resto de orgánulos y núcleo celular (Roger et al., 2017). La descripción exacta de este proceso de eucariogénesis y la identificación de los tipos de célula responsables continúa siendo investigada pero esta unión dio lugar a las células que forman hoy a los organismos del dominio Eukarya (Gabaldón, 2018; Martijn et al., 2018). Otros de los orgánulos originados por endosimbiosis posteriores son los plástidos como por ejemplo los cloroplastos (Archibald, 2015). En este nuevo tipo celular, los eucariontes, se ponen de manifiesto dos aspectos fundamentales de la vida: la cooperación entre diferentes y el oxígeno son clave para la vida. Esto se apoya en que entre los dos principales dominios (Bacteria y Arquea) lograron crear un nuevo dominio con propiedades extraordinarias y emergentes capaces de competir con el resto de formas de vida, sobrevivir y evolucionar hasta la actualidad. Por otro lado, tanto si hablamos de mitocondrias como cloroplastos estos dos orgánulos de origen endosimbiótico están relacionados, aunque de manera diferente, con el oxígeno y su papel en la transformación de energía.

Sin embargo, en la época cuando tuvo lugar la endosimbiosis, que culminaría en la formación de las mitocondrias, el oxígeno era poco abundante (Knoll et al., 2016). Por lo que el procesado de oxigeno no era necesariamente la única forma de producción energética de estos primeros endosimbiontes (Roger et al., 2017). La verdadera ventaja vendría que con el tiempo y con el aumento de O_2 en el medio las proteobacterias endosimbiontes fueron especializándose en la transformación energética a formas como el adenosin trifosfato (ATP) y cediendo parte de su material genético que ya no era necesario al núcleo o directamente perdiéndolo (Lane, 2017); ya que al estar viviendo en

el interior de otra célula ciertas estructuras se volvían superfluas. Esta especialización logro una eficiencia ventajosa a la hora de generar y gestionar la producción de ATP, es decir, aprovechar la energía (Lane & Martin, 2015).

Además de la formación de células eucariotas otro gran paso de la vida fue el desarrollo de una multicelularidad compleja. Ya a comienzos del proterozoico (hace entre 2.5 y 1.9 mil millones de años) se han identificado algunas formaciones filamentosas de cianobacterias y otro tipo de bacterias (Rokas, 2008) (Fig. 7.1). Incluso algunos protistas empezaron a mostrar formaciones filamentosas poco después de su aparición. Pero durante el último cuarto del proterozoico (hace mil millones de años) aumentó el oxígeno atmosférico e hidrosférico, la fuente para la trasnformación de energía; y se desarrollaron los medios para procesarla de manera eficiente. Esto permitió dar un paso hacía una mayor diversificación en la vida eucariota (Hedges et al., 2004). Aparecieron novedosas complejidades celulares, no solo en la forma de nuevos tipos celulares derivados de procesos evolutivos y endosimbióticos sino también conformaciones celulares más complejas. Se trataba de una multicelularidad con células especializadas a lo que nos referiremos como pluricelularidad. Las evidencias genéticas apuntan a que los ancestros de muchas de las formas de vida pluricelular actual, eran protistas con formas individuales que desarrollaron pluricelularidad entre 1000 y 400 millones de años (Ruiz-Trillo et al., 2007; Rokas, 2008). El registro fósil muestra fósiles no ambiguos de los linajes de plantas, animales y hongos desde hace 600 millones de años (Rokas, 2008).

No obstante, cabe matizar que el oxígeno no fue la única condición clave para la aparición de vida animal. Hay autores que sostienen ejemplos de animales con importantes metabolismos anaeróbicos y animales capaces de sobrevivir en bajas concentraciones de oxígeno como evidencia de que el oxígeno no era el único requisito para la aparición de vida eucariota pluricelular (Mills & Canfield, 2014). Cuando aparecieron los primeros animales las concentraciones de oxígeno eran bastante inferiores a las actuales. Por lo que cabe matizar que, aunque necesario, quedan otros condicionantes por ser investigados (Mills & Canfield, 2014). Sin embargo, aunque el aumento en la concentración de O_2 no haya sido el único factor que condicionó la aparición de vida animal si ha tenido implicaciones clave para la diversificación y evolución de dicha vida dependiente de procesos respiratorios (Knoll, 1991; Sperling et al., 2013).

Los tiempos y protagonistas exactos de cada paso en el proceso evolutivo animal es algo que continúa en constante investigación. Pero si se reconoce que el phylum Cnidaria es uno de los primeros grupos en desarrollarse con tejido diferenciado y un sistema nervioso básico y se desvió de la línea que luego correspondería a todos los grupos bilaterales (Cunningham et al., 2017). Este grupo, los cnidarios, se ha ido diversificando en corales, anemonas, medusas y sifonóforos, entre otros muchos (Park et al., 2012), algunos de los cuales protagonizan los estudios de este trabajo. Los cnidarios cuentan con unos diseños de un alto éxito evolutivo ya que han logrado perdurar con su estrategia hasta la actualidad. Por ejemplo, existen registros fósiles de medusas desde finales del proterozoico y comienzos del Cámbrico, hace 600-500 millones de años, muy similares a las formas actuales (Cartwright et al., 2007; Young & Hagadorn, 2010) (Fig. 7.1). Estos diseños simples pudieron surgir cuando la concentración de O_2 era menor y han logrado reproducirse y evolucionar sobreviviendo a numerosos cambios del planeta. Por tanto, este tipo de organismos nos pueden enseñar mucho sobre el funcionamiento de la vida animal a lo largo de la historia.

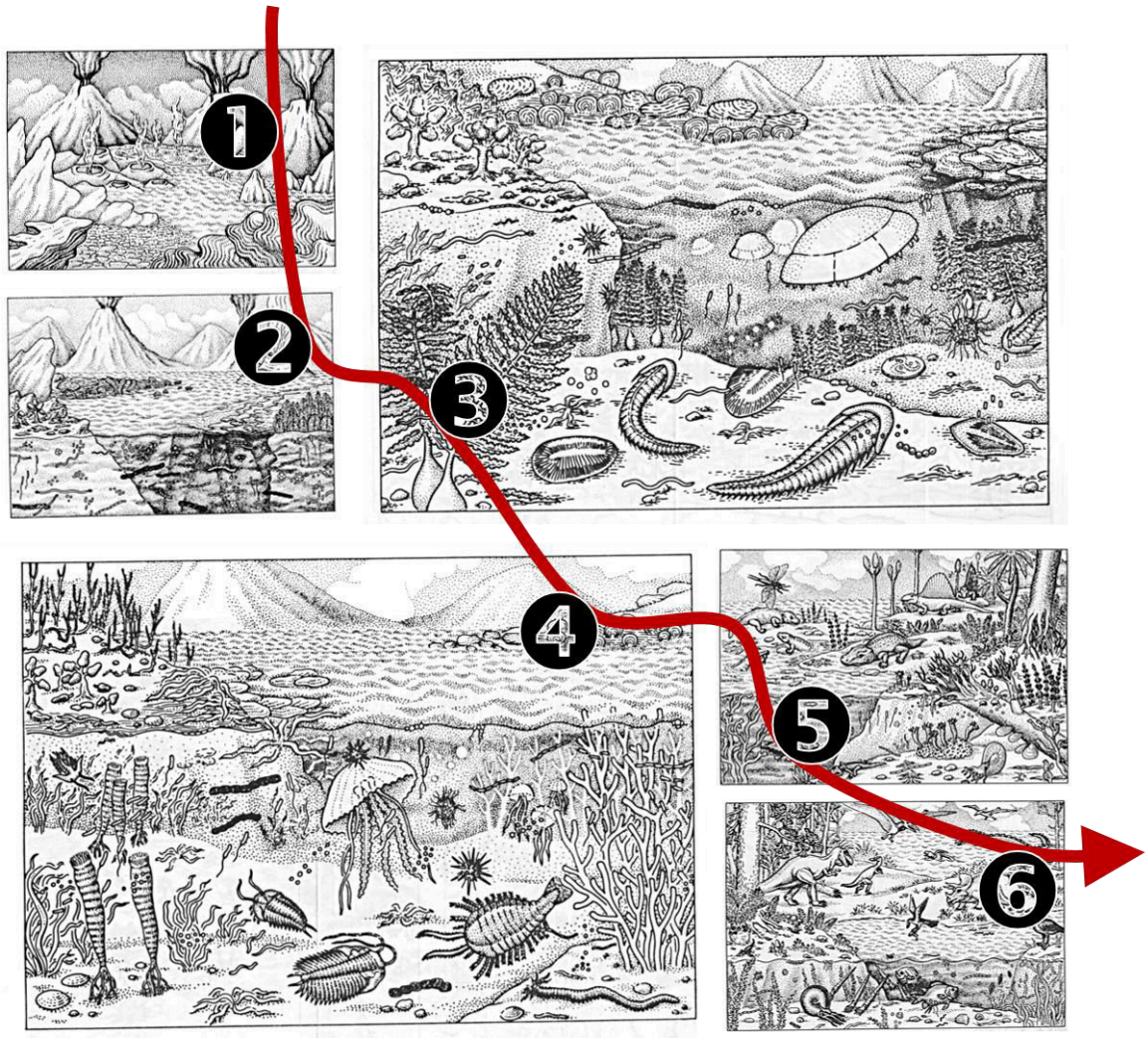


Figura 7.1. Viñetas resumen de la evolución de la vida en el planeta tierra de Margulis & Sagan (1995) desde hace alrededor de 3000 millones de años hasta hace 70 millones de años con énfasis en la aparición de los cnidarios (3 y 4). Esta figura enfatiza la importancia del oxígeno (2) y procesos asociados para la aparición de la vida eucariota en el planeta y entre estos primeros habitantes se encontraban las medusas reinando en el medio marino con una morfología y estrategia similar a la que hoy conservan. (1) Representación del Eón Arcaico, hace aproximadamente 4000 hasta hace 2500 años, con actividad hidrotermal y biofilms bacterianos que quedarían en el registro fósil como estromatolitos (Nutman et al., 2016). (2) Representación del principio del Eón Proterozoico, hace alrededor de 2500 millones de años, con la aparición de las cianobacterias y su producción fotosintética de O2. (3) Representación de finales del Eón Proterozoico, hace alrededor de 600 millones de años, con la aparición de las primeras formas de vida eucariotas y pluricelulares blandas. (4) Representación del comienzo del Eon Fanerozoico, Era Paleozoica, Periodo Cámbrico, hace alrededor de 500 millones de años, y el continuo desarrollo de la vida pluricelular con la aparición de estructuras duras y la emersión de formas de vida sencillas autótrofas y heterótrofas. (5) Representación de finales de la Era Paleozoica, hace alrededor de 300 millones de años, con el desarrollo de vertebrados y de vegetales marinos y terrestres. (6) Representación de la Era Mesozoica, hace entre 200 y 70 millones de año, con grandes reptiles aéreos, terrestres y marinos conviviendo con pequeños mamíferos y cuantiosa vida vegetal.

7.2. El rol de las medusas

Como ha sido descrito en el apartado anterior llegados al Cámbrico, y su famosa explosión de vida metazoa (Conway Morris, 2000), los cnidarios se diversificaron en sus distintas clases (Park et al., 2012). Entre las que se incluyen las formas de vida estrictamente sésiles como la clase Anthozoa (incluye anémonas y corales) y la menos conocida Staurozoa; los parasitos obligados de la clase Myxozoa; y las "medusas" de las clases Cubozoa (cubomedusas), Scyphozoa (escifomedusa o "verdaderas medusas") e Hydrozoa (incluye a hidromedusas y sifonóforos entre otros) (Collins, 2002). Estas últimas en algunas especies presentan ciclos de vida más complejos que combinan fases de vida sésiles y planctónicas. De entre todos estos, este trabajo se centrará principalmente en organismos de las clases Scyphozoa e Hydrozoa que serán introducidos más adelante. Las formas planctónicas de organismos del phylum Cnidaria se clasifican dentro de un grupo polifilético de uso no taxonómico llamado plancton gelatinoso que engloba a formas de vida planctónicas de phyla variados (tunicados, ctenoforos, cnidarios…) que comparten una serie de características entre la que destaca tener una fragilidad elevada debido a su alto contenido en agua (Purcell, 2009).

Durante estos primeros periodos de enorme diversificación marina el plancton gelatinoso, en especial las medusas, se debieron encontrar entre los principales superdepredadores marinos (Richardson et al., 2009) (Fig. 7.2). Sus estrategias de depredación y reproducción han debido resultar exitosas como para que no hayan cambiado mucho con respecto a las formas del registro fósil y sobrevivan en presencia de depredadores más modernos, que aparecieron millones de años después, como son los peces o los mamíferos marinos. Algunos autores han llegado a sugerir que debido a los efectos de origen antropogénico sobre los ecosistemas y el cambio climático que impacte negativamente sobre los peces podría resultar beneficioso para las medusas que han sobrevivido a grandes cambios del planeta y no dudarán en reconquistar su rol como depredadores pelágicos que antaño monopolizaban (Parsons & Lalli, 2002; Richardson et al., 2009) (Fig. 7.2). La realidad es que para muchos el rol depredador de las medusas en la cadena trófica ha pasado desapercibido debido a la atención prestada a depredadores no-gelatinosos. Sin embargo, las medusas y otros miembros del plancton gelatinoso puede que sean el mayor competidor de especies de peces zooplanctívoras y además sean depredadores de larvas y huevos de peces dificultando la recuperación de ecosistema dominados por medusas (Lynam et al., 2007; Pauly et al., 2009). Purcell et al. (1999) enumera una serie de características que convierten a las medusas en grandes competidores: las medusas se alimentan de forma continua sin saciarse a concentraciones naturales de alimento, capturan presas pequeña y grandes, depredan sobre estadios larvarios de sus competidores, cuando falta alimento pueden encogerse en lugar de fallecer, toleran mejor condiciones de baja concentración de oxígeno disuelto, se alimentan de forma pasiva reduciendo gastos energéticos de caza, aprovechan condiciones favorables para la formación rápida de grandes blooms y en muchas ocasiones cuentan con una sucesión rápida de generaciones de poca duración alternando entre etapas de reproducción sexual y etapas de reproducción asexual. Algunas de estas características serán abordadas más adelante.

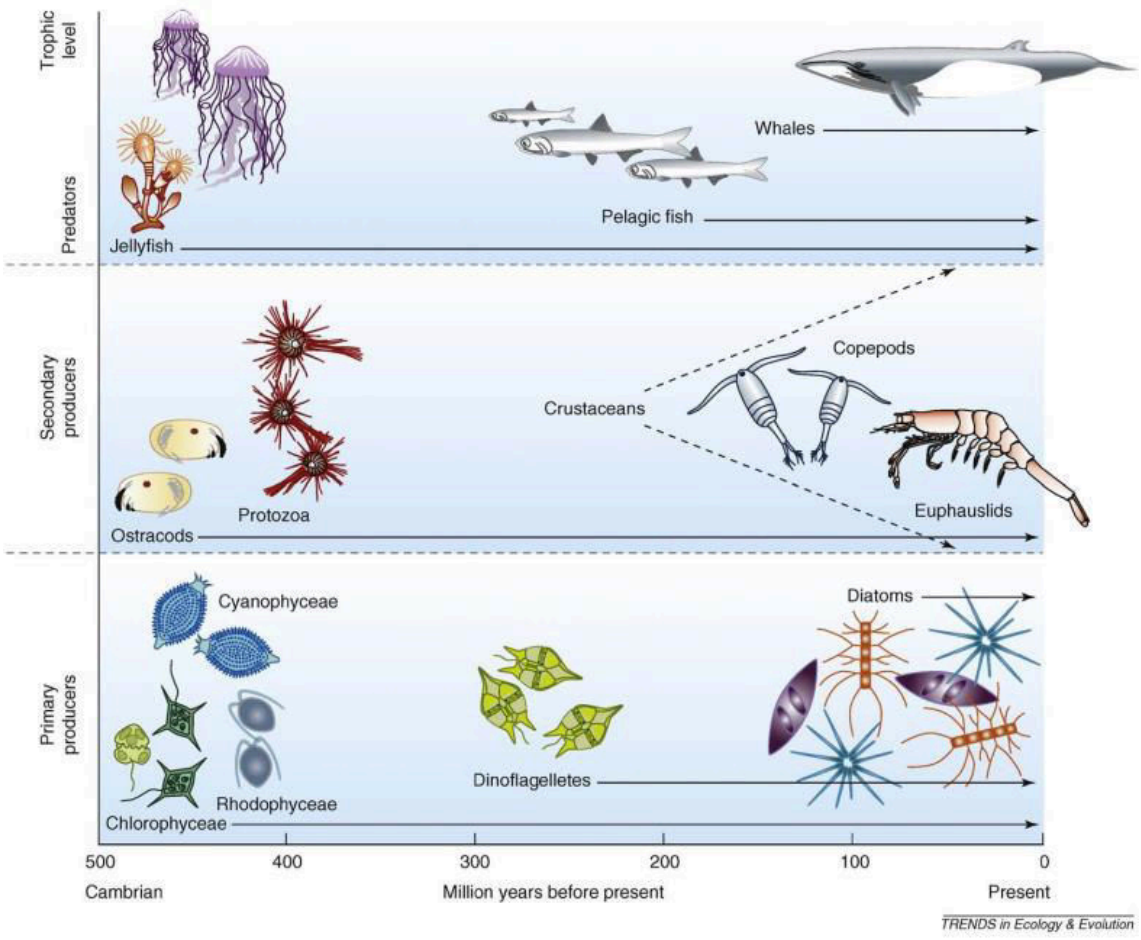


Figura 7.2. Obtenida de Richardson et al. (2009) a su vez modificada de Parsons 1979. Esquematización de la evolución de los principales representantes de los niveles tróficos marinos. Se muestra la transición de los cnidarios como depredador mayoritario a compartir el nicho trófico con competidores y depredadores más complejos. Pone de manifiesto la supervivencia de las medusas y como la retirada de competidores y depredadores puede crear el escenario idóneo para su retorno al podio.

Dentro de la perspectiva antropocentrista del ser humano también se han documentado problemas asociados a las medusas al margen de la competición y depredación sobre especies de interés pesquero. Purcell et al. (2007) recopila muchos de estos impactos sobre la actividad humana. En relación a la pesca, además de lo mencionado, los grandes blooms de medusas pueden dificultar la utilización de redes. Además, muchos de los cnidarios comparten una característica que es tener unas células urticantes, llamadas cnidocitos, que emplean para capturar a sus presas y para defensa. Las picaduras pueden ser de mayor o menor gravedad dependiendo de la especie, pero complican el manejo de capturas accidentales y pueden picar a pescadores. Cuando invaden áreas de recreo cercanas a la costa las picaduras pueden dañar a bañistas y afectar al turismo local. Estas picaduras también pueden tener un impacto negativo sobre la acuicultura. También se han documentado casos de obstrucción de entradas de agua marina de plantas eléctricas y plantas desaladoras. Todas estas situaciones suceden o se ven agravadas cuando se producen grandes agrupaciones de medusas.

De manera inversa también hay una gran discusión sobre como la actividad antropogénica impacta sobre las poblaciones de medusas produciendo un aumento

global de la población de plancton gelatinoso (Purcell et al., 2007; Richardson et al., 2009; Duarte et al., 2013). De manera resumida, las actividades humanas que podrían provocar este aumento son: la retirada de depredadores y competidores, la eutrofización, el transporte de especies invasoras y el aumento de sustrato en la costa para el asentamiento de fases de vida sésiles (pólipos) como son las embarcaciones, las estructuras para acuicultura, las boyas, las plataformas y en general el desarrollo costero urbanísticos y portuario. Mientras que otros autores argumentan que se trata de aumentos de poblaciones de medusas regionales o casos concretos sin que el aumento sea global y por tanto la conexión con actividades humanas es limitada no habiendo evidencias suficientes que la confirmen (Condon et al., 2012; Pitt et al., 2018). La monitorización continuada de la aparición o periodicidad de blooms de medusas y su estudio en múltiples regiones del planeta permitirá comprender mejor la evolución de sus poblaciones y nuestro impacto sobre las mismas.

Pero pensar en el plancton gelatinoso sólo como competidor, depredador, o problema, limitaría nuestro entendimiento de su rol en el ecosistema. Doyle et al. (2014) invita a reflexionar sobre los numerosos aspectos ecológicos, sociales y culturales positivos de las medusas. En relación a los humanos el plancton gelatinoso ha sido fuente de numerosas investigaciones para la extracción de productos con aplicaciones, medicas, cosméticas e industriales (Ohta et al., 2009; Kim et al., 2012; Avila Rodríguez et al., 2018; Verdes & Holford, 2018; Steinberger et al., 2019). Cabe destacar el descubrimiento de una proteína fluorescente verde (Green fluorescente Protein, GFP) descubierta en el género *Aequorea* que llevó a la obtención de un premio nobel por parte de sus descubridores y permitió el desarrollo de una "revolución en biotecnología" (Zimmer, 2009). Doyle et al. (2014) además propone que valoremos el disfrute, la curiosidad e interés por la naturaleza que despiertan los acuarios de medusas que son cada vez más comunes; y el conocimiento que obtenemos de estrategias vitales tan diversas como las que presenta el plancton gelatinoso. Incluso, afirma que la participación colectiva necesaria para la detección de varamientos de medusas y documentación de su distribución es una oportunidad para implicar a la población y crear una ciencia más participativa alrededor de este, a veces desestimado, miembro del océano.

Pero al margen de las aplicaciones humanas, a nivel ecológico el plancton gelatinoso es un miembro más de la comunidad planctónica. Como tal, muchas de sus especies cumplen funciones enriquecedoras para los sistemas que habitan. En la actualidad se contempla el papel de las medusas como fuentes de transporte de carbono a través de los llamados "jellyfalls" (Lebrato et al., 2019), como indicadores de contaminación marina (Macali & Bergami, 2020) y como zonas de comensalismo de peces y crustáceos sirviendo de guarderías y alimento simultáneamente (Mansueti, 1963; Purcell & Arai, 2001; Griffin et al., 2019). Esto último es importante, ya que, como muchos autores afirman en la literatura durante mucho tiempo el plancton se ha estudiado de forma crustaceocéntrica y las medusas han sido relegadas a papeles secundarios en las cadenas tróficas (Haddock, 2004). Sin embargo, se describen cada vez más especies que se alimentan de plancton gelatinoso: gasterópodos, cefalópodos, crustáceos, peces, tiburones, tortugas, aves, mamíferos, (Arai, 2005; Pauly et al., 2009; Choy et al., 2017), incluso el ser humano, con estimaciones de pesca actuales de hasta 900 000 toneladas anuales (Brotz & Pauly, 2017). La motivación detrás de esta depredación natural está siendo discutida en la actualidad (Hays et al., 2018; Thiebot & McInnes, 2020). Investigar la composición de miembros del plancton gelatinoso podría ser clave para entender no solo el rol del plancton gelatinoso como presa y no solo como depredador.

En este trabajo sobretodo nos centraremos en explorar estas dos caras (depredador y presa) de algunos miembros del plancton gelatinoso. Lo haremos a través del estudio de

su metabolismo y composición bioquímica. En el proceso aprenderemos sobre aspectos fisiológicos de dichos organismos que nos permitirán hacer estimación y desarrollar hipótesis a nivel ecológico.

7.3. La respiración: su función y estudio en medusas

Comprender las necesidades metabólicas de los organismos permite averiguar información sobre la demanda de alimento asociada. El alimento consumido por el organismo será empleado en varios procesos como el crecimiento y la reproducción, pero, sin duda, parte de ese alimento deberá ir destinado a la respiración (Schneider, 1989; Uye & Shimauchi, 2005). Durante la respiración se emplean sustancias orgánicas procedentes del alimento digerido para transformar la energía contenida y formar adenosin-trifosfato (ATP) y calor. Durante dicha reacción también se necesita oxígeno. Las sustancias orgánicas son oxidadas produciendo también agua (H_2O) y dióxido de carbono (CO_2). Se trata de una reacción RedOx muy similar a la que transcurre en una combustión. Sin embargo, esta se produce de una manera mucho más controlada, mediada por enzimas, lo cual permite lograr la transformación de energía y procesarla en forma de ATP (Lane, 2015). El papel del oxígeno en este proceso es fundamental y por ello su aumento en la atmósfera, descrito anteriormente, es tan importante para el desarrollo de vida compleja en la tierra. Gracias a él los organismos tienen un mecanismo bioquímico eficiente con el que liberar la energía de su alimento. Exceptuando casos puntuales, la mayoría de metazoos realizan algún tipo de respiración aeróbica. Algunos de estos organismos han llegado a desarrollar órganos especializados en captar oxígeno del medio como por ejemplo las branquias o los pulmones. En el caso de los cnidarios, incluyendo las medusas, carecen de órganos especializados para el intercambio de gases respiratorios y obtienen el oxígeno por difusión cutánea (Fig. 7.3). Es decir, las capas celulares expuestas al medio exterior tienen un intercambio de oxígeno con el medio que les rodea capaz de abastecer la demanda de oxígeno de todo el organismo. Pese a tener este sistema respiratorio simple pueden alcanzar grandes tamaños gracias al bajo número de células combinado con un alto contenido en agua (Acuña et al., 2011).

Además de oxígeno, los organismos necesitan alimento que puede obtenerse del exterior (heterotrofía) o ser producido en el organismo a partir de una fuente de energía (autotrofía). Los organismos fotosintéticos como las algas, el phytoplancton y las plantas son fotoautótrofos y producen su propio alimento a partir de la luz gracias a la fotosíntesis (Benedict, 1978). Algunos cnidarios cuentan con microalgas endosimbiontes, es decir algas unicelulares que viven en el interior del organismo de forma indispensable (Verde & McCloskey, 1998; Fransolet et al., 2012). Es muy conocido el caso de los corales hermatípicos pero otras cnidarios como especies de anemonas, de medusas o de hidras tienen este tipo de simbiosis con microalgas que pueden tratarse de zooclorelas o zooxantelas. Uno de los organismos que será introducido más adelante, *Velella velella* cuenta también con la presencia de zooxantellas. En este tipo de simbiosis complejas entre organismos el oxígeno juego un papel importante. Sin entrar en mucho detalle, durante el proceso fotosintético se produce oxígeno además de carbono orgánico, que sirve de alimento, a partir de H_2O, CO_2 y la fuente energética que en este caso es el sol. En el caso de los heterótrofos, como son la mayoría de los metazoa, el alimento se obtiene de la digestión de presas; pudiendo ser animales, vegetales o material orgánico. En el caso de los cnidarios la mayoría tienen un sistema de digestión simple llamado cavidad gastrovascular. Esta solo tiene un orificio de entrada y salida a diferencia de sistemas digestivos más complejos (Yonge, 1937).

El oxígeno difundido al interior y las sustancias orgánicas, obtenidas de la digestión del alimento o producidas por procesos autotróficos, son transportadas por el interior del organismo a cada una de las células que lo conforman. Una vez en la célula tienen lugar una serie de mecanismo regulados por enzimas para la liberación de energía a partir de estas sustancias orgánicas. Esto es lo que conocemos como respiración celular. Basándonos en Nelson et al. (2008) y Gnaiger et al. (2019) desglosaremos la respiración en 5 pasos desarrollando más aquellos más relevantes para este trabajo: Catabolismo de macronutirentes, generación de acetyl CoA, Ciclo de Krebs o de los ácidos tricarboxílicos, Sistema de Transporte de Electrones, Síntesis de ATP.

El catabolismo de macronutrientes dependerá de si la sustancia orgánica de la que se va a obtener energía es un glúcido, lípido o una proteína. Se trataría de aquellas transformaciones que tienen lugar antes de la entrada al orgánulo que está encargado de la transformación de la mayoría de la energía en forma de ATP, las mitocondrias. Durante este catabolismo se producen algunas moléculas de ATP y algunos Nicotinamida nucleótidos, como el NADH, cuya función será explicada más adelante. La participación de peroxisomas en estos procesos catabólicos puede ocasionar un consumo de oxígeno extramitocondrial. Tras este procesamiento las sustancias orgánicas derivadas entrarían en la mitocondria; si se trataba de un glúcido entrarían en la mitocondria moléculas de piruvato; de un ácido graso, cadenas lipídicas menores; y de una proteína; amino ácidos. El piruvato es oxidado para producir acetil-CoA y en el proceso también se produce CO_2 y se reduce el pridin nucleótido NAD^+ formando NADH. Los lípidos son oxidados en un proceso llamado β-oxidación que termina también con la formación de acetyl-CoA y la reducción de NAD^+ a NADH y flavin nucleótidos como FAD a $FADH_2$. En el caso de los amino ácidos, dependiendo del amino acido que sea, podrá ser convertido, en ocasiones con la participación de un proceso de desaminación (Fernández-Urruzola, 2015), en acetyl-CoA o entrará como una molécula intermedia del ciclo de Krebs del cual hablaremos a continuación (Fig. 7.3).

El acetyl-CoA entra en lo que nos referiremos como el inicio del ciclo de Krebs. Numerosas enzimas de este ciclo logran ir oxidando la molécula de acetil-CoA y sus sucesivas formas produciendo CO_2 junto con NADH, $FADH_2$ y alguna molécula de guanina-trifosfato (GTP). Concretamente se producen 3 moléculas de NADH y 1 de $FADH_2$ por cada acetyl-CoA. Este ciclo ha tenido lugar en el interior de la mitocondria. Las moléculas de NADH y $FADH_2$ producidas son utilizadas después en la membrana mitocondrial interna en lo que se denomina Sistema de Transporte de Electrones (*Electron Transport System*, ETS) (Fig. 7.3). Las enzimas del ETS oxidan a las moléculas de NADH y $FADH_2$ liberando en el proceso electrones que son transportados a través de la membrana mediante distintas reacciones dependientes del potencial estándar de reducción y reguladas por enzimas. El proceso comienza en el complejo I, o NADH deshidrogenasa, donde se libera el electrón a la vez que se oxida el NADH regenerando el NAD^+ en el interior de la matriz mitocondrial. De forma similar en el complejo II, también conocido como succinato deshidrogenasa, se oxida el $FADH_2$ regenerando el $FADH^+$ y liberando también un electrón al ETS. El transporte del electrón termina en el complejo IV o citocromo-oxidasa donde el electrón reacciona con el oxígeno reduciéndolo y formando agua usando protones (H^+) de alrededor. Por cada 4 electrones transportados se producen 2 moléculas de agua a partir de una molécula de O_2. El transporte de estos electrones provoca un bombeo de H^+ a la región intermembranal entre la membrana mitocondrial interna y la externa. El complejo I, III y IV son los que contribuyen a este bombeo de H^+ durante el flujo de electrones. Se bombean 10 H^+ por cada pareja de electrones que recorren el sistema (Fig. 7.3).

El bombeo de H^+ a la región intermembranal y el consumo de H^+ para la formación de H_2O en el interior genera un gradiente de pH entre esa región intermembrana y la matriz mitocondrial. Durante el último paso, la síntesis de ATP, la mitocondria transforma este gradiente en energía utilizando unas enzimas llamadas ATP sintasas, a veces llamado complejo V. Este complejo facilita el retorno de los H^+ acumulados en la región intermembrana a la matriz mitocondrial aprovechando el proceso para fosforilar adenosine difosofato (ADP) y formar ATP mediante el mecanismo descrito por Mitchell (1961). De esta manera almacena así energía en esta molécula que será transportada para su uso en otras regiones celulares. Aproximadamente, por cada 10 H^+ transportados se generan 3 moléculas de ATP. Dependiendo del nivel de acoplamiento el retorno de H^+ se empleará para la transformación de energía en forma de ATP o en forma de calor. Esto dependerá del nivel de participación de las proteínas de desacoplamiento en el retorno de los H^+(Nelson et al., 2008; Hill et al., 2016).

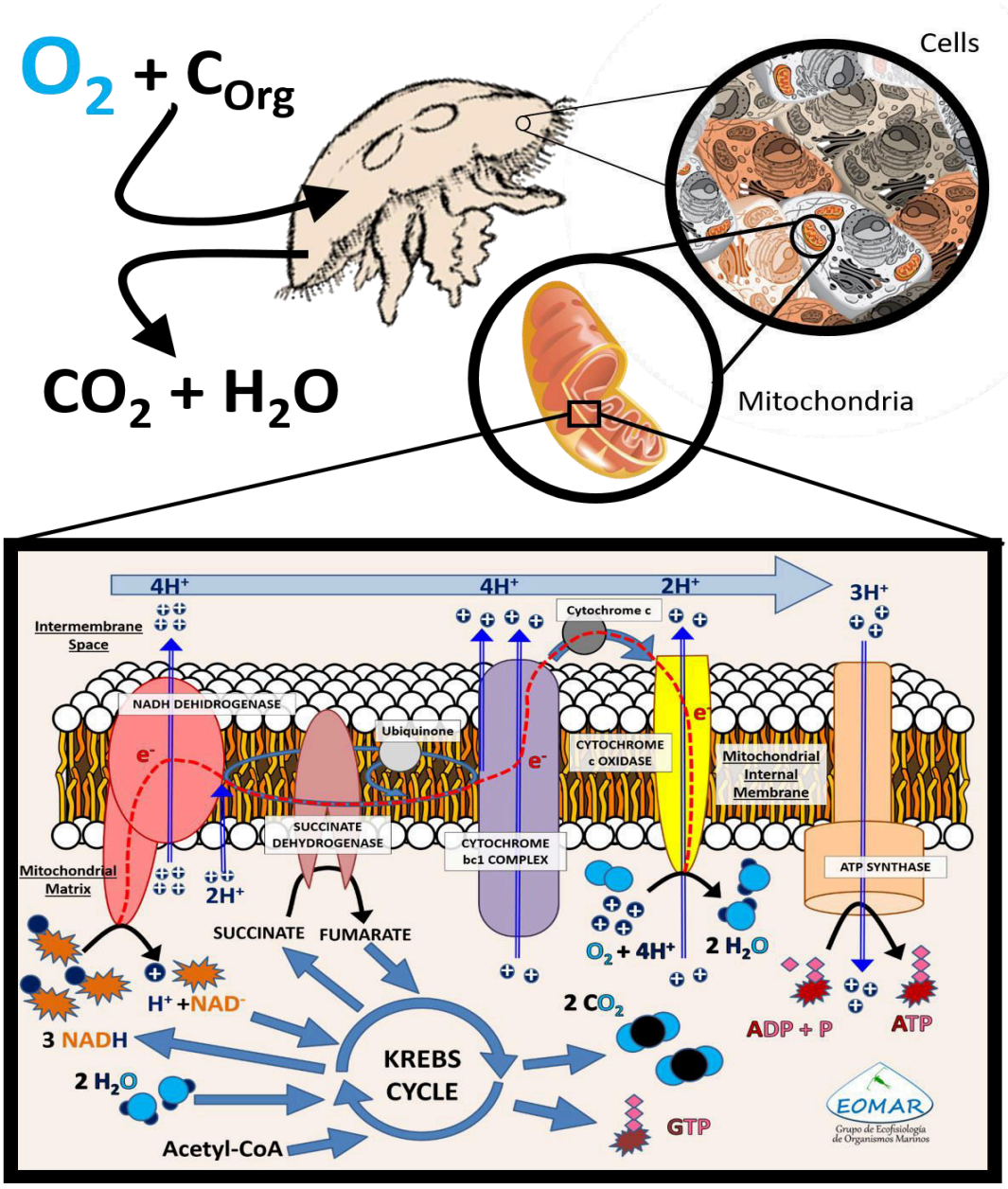


Figura 7.3. Esquema simplificado de la respiración fisiológica (R) y su control a nivel molecular en el sistema de transporte de electrones (ETS). Arriba, medusa y la reacción respiratoria con el consumo de oxígeno correspondiente. Circulo superior, células formando el tejido de la medusa. Círculo inferior, ilustración de una mitocondria. Recuadro inferior, esquema del ETS presente en la membrana mitocondrial interna. Comienza con la producción de CO_2 y NADH en el ciclo de Krebs a partir del acetil-CoA, continúa con la oxidación de NADH y la generación del flujo de electrones y termina con la reducción de O_2 formando H_2O. Durante el proceso se observa también el bombeo de protones (H^+) y la generación de ATP.

La liberación de energía en forma de ATP está en muchos organismos controlada por un sistema de transporte de electrones y un gradiente de protones. Esta respiración celular no es exclusivamente aeróbica ya que hay organismos que usan otros aceptores de electrones finales diferentes a O_2, como por ejemplo NO_3^-, NO_2^- o SO_4^{2-}, sobre todo en zonas de bajas concentraciones de O_2 (Vosjan, 1982). Sin embargo, la aparición de estas formas oxidadas estuvo relacionada con el aumento del O_2 al igual que la respiración aeróbica que requiere directamente oxígeno por ello fue tan revolucionario para la vida en el planeta el aumento del oxígeno del proterozoico descrito anteriormente. Estos procesos moleculares regulan la energía del organismo y condicionan la demanda de los principales sustratos de este proceso: la materia orgánica (alimento), que será oxidada, y el oxígeno, que será reducido. Entendiendo mejor este proceso vital logramos entender los mecanismos que controlan el consumo de oxígeno y de carbono con sus consecuencias macroscópicas y podemos estudiar el flujo de energía a través de los integrantes del sistema trófico.

Estas reacciones vitales, como ya se introdujo, son controladas por las mitocondrias y su resultado a escala del organismo es la ingesta de alimento, el consumo de O_2 y la producción de CO_2. En el caso de la ingesta, todos los organismos heterótrofos cuentan con medios para la ingesta de presas o materia orgánica del exterior que servirán de sustrato para el funcionamiento de los mecanismos metabólicos anteriormente descritos. También muchos organismos cuentan con órganos especializados para la captación de oxígeno y liberación de dióxido de carbono, sin embargo, esto dependerá de la complejidad estructural de dichos organismos. En el caso de los cnidarios su sencilla estructura tisular le permite carecer de órganos especializados y la captación de oxígeno se realiza por respiración integumentaria, es decir, por difusión a través de su escaso tejido en su cuerpo con alto contenido en agua (Graham, 1988). Thuesen et al. (2005) han observado que, en la mesoglea de escifozoos, a lo que se refieren como intragel, estas medusas son capaces de retener altas concentraciones de oxígeno. Esto les confiere la capacidad de mantener una actividad respiratoria elevada y sobrevivir en concentraciones de suboxia o hipoxia no aptas para multitud de organismos. Curiosamente la relación del phylum cnidaria y la respiración ha sido objeto de estudio desde hace muchos años, siendo uno de los representantes utilizados por Vernon (1895) en su exhaustivo estudio de intercambio respiratorio en invertebrados marinos, figurando incluso en su diagrama de la técnica utilizada (Fig. 7.4). Por el contrario, recientemente se ha descrito a un cnidario, en concreto un tipo de cnidario parasítico de la clase Myxozoa, que carece de partes del genoma mitocondrial relacionados con la respiración aeróbica por lo que podría ser el primer animal con metabolismo sin ETS completo (Fig. 7.4) (Yahalomi et al., 2020).

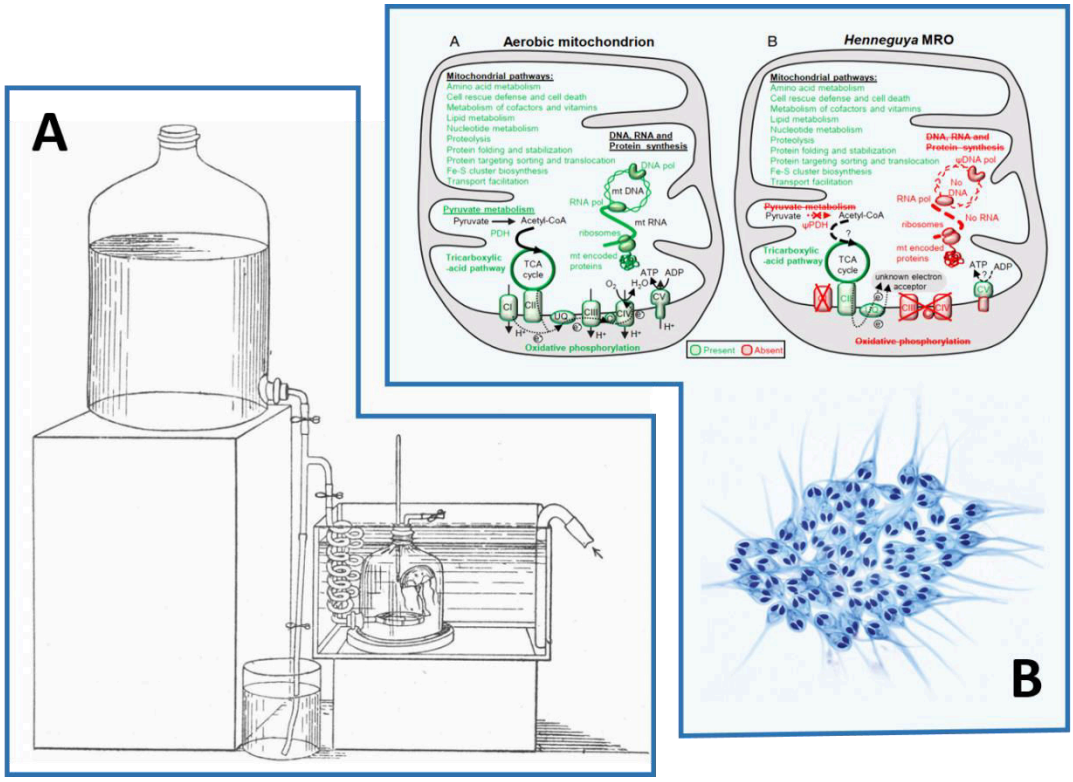


Figura 7.4. Visualización de trabajos seminales del estudio de la respiración en cnidarios. **A**. Diagrama de la instrumentación para medida del consumo de oxígeno en invertebrados empleada por Vernon (1895). Se puede observar que de entre los invertebrados trabajados usó la medusa en su diagrama. **B.** Imagen de Yahalomi et al. (2020) representando una mitocondria y sistema de transporte de electrones tradicional de un organismo metazoa junto a la mitocondria teórica del cnidario parasítico de la clase Myxozoa estudiado que presenta según su análisis genético la ausencia de los procesos en rojo. Abajo la imagen de portada de la publicación en PNAS del organismo en cuestión, *Henneguya salmonicola*, representado en muchos medios como "el primer animal que no necesita respirar".

Por tanto, puede que la fisiología respiratoria de este phylum albergue más claves para entender este proceso vital. Purcell et al. (2010) defendió el uso de medidas respiratorias en medusas como aproximación de su consumo de presas y así estimar impactos potenciales sobre las poblaciones depredadas a través de la demanda de carbono asociada (Uye & Shimauchi, 2005). Metodologías como los experimentos de tasas de ingestión o el análisis de contenido estomacal permitían identificar qué presas eran consumidas, pero acarreaban más dificultades a la hora de cuantificar el impacto sobre dichas presas. Las medidas respiratorias en cnidarios ofrecían algunas ventajas a la hora de cuantificar el impacto de blooms sobre las presas evitando las complicaciones de los experimentos de ingestión y siendo más rápidos que los análisis de contenido estomacal. No obstante, las incubaciones necesarias para determinar la respiración a través del consumo de oxígeno conllevan un problema que comparte con las otras técnicas: el muestreo de estos frágiles organismos vivos. Este muestreo acarrea una serie de dificultades logísticas especialmente cuando el organismo es de gran tamaño como ocurre en el caso de varias especies de escifozoos, algunas formadoras de blooms (Purcell et al., 2010; Iguchi et al., 2017). En general muchos integrantes del plancton gelatinoso tienen esta dificultad y es por ello que Purcell et al. (2010) incentivó el desarrollo de técnicas enzimáticas como las descritas por Packard (1985). Dichas técnicas enzimáticas estudian la actividad asociada a las enzimas respiratorias por lo que solo requiere del

tejido del organismo para determinar la actividad de las enzimas asociadas a esas células no necesariamente del organismo vivo. Esto será explicado en más detalle en el siguiente apartado.

7.4. Estudio del consumo de O_2 y de la actividad del ETS

Como ya se ha introducido el estudio de la respiración es clave para entender el funcionamiento de los seres vivos hasta el punto de permitir entender las necesidades alimenticias. Por lo general el metabolismo respiratorio se estudia a través de la medida del consumo de oxígeno, pero se podría trabajar también con la producción de CO_2 aunque esta es más compleja de medir disuelto en agua de mar (Mayzaud et al., 2005).

La medida del consumo de oxígeno en zooplancton puede realizarse mediante dos modalidades de incubaciones principalmente (Hernández-León & Ikeda, 2005). Una consiste en un flujo de agua constante conocido midiendo la concentración de oxígeno a la entrada y salida de la cámara cerrada que contiene al organismo. Esta técnica es muy efectiva para observar los cambios momentáneos en el consumo de oxígeno. Sin embargo, es más complicada de preparar y requiere de un calibrado riguroso que incorpore el ritmo de flujo entre otros parámetros. En el caso de plancton gelatinoso, el diseño de la cámara de incubación o el control del flujo requieren de más estudio para evitar que sean dañados durante la incubación. La otra técnica consiste en la incubación de organismos en recipiente cerrados sin intercambio de oxígeno con el exterior y evitando la presencia de aire en el interior de la incubación. En este caso se monitoriza la concentración de oxígeno en el interior de la cámara de incubación y la variación con la concentración de oxígeno será resultado del metabolismo del organismo incubado. No obstante, este tipo de incubaciones requieren horas y en algunos casos días dependiendo del volumen de la cámara y el tamaño del organismo con el que se trabaja. Las incubaciones se realizan a una temperatura concreta en oscuridad para evitar procesos fotosintéticos que pudiesen producir oxígeno. Conocida la variación de la concentración de oxígeno y el tiempo transcurrido de incubación se puede determinar la tasa de consumo de oxigeno ($\Delta[O_2]/\Delta t$) a la que nos referiremos como respiración fisiológica (R). Diversas técnicas pueden ser utilizadas para realizar el seguimiento de la concentración de O_2 durante la incubación(Ikeda et al., 2000). La más tradicional es la medida del oxígeno mediante la reacción química descrita por (Winkler, 1888) que al ser una reacción química requiere retirar parte del volumen de la incubación lo cual dificulta el proceso o en ocasiones implica que solo se mida al comienzo y al final de la incubación. Es por ello que se desarrollaron otros métodos de detección de la concentración de oxígeno a través de sensores. Para realizar el seguimiento de la concentración de O_2 sin necesidad de extraer agua existen instrumentos polarigráficos, que emplean una reacción electroquímica mediante electrodos, y métodos fotoquímicos como los optodos, que se basan en la desactivación (“quenching”) de la fluorescencia provocado por la concentración de O_2. De estas técnicas los electrodos son más comunes y los optodos más recientes. Todas las técnicas anteriormente descritas han sido empleadas para la determinación de la respiración en zooplancton, incluyendo medusas (Fig. 7.5) (Larson, 1987; Uye & Shimauchi, 2005; Lilley & Lombard, 2015).

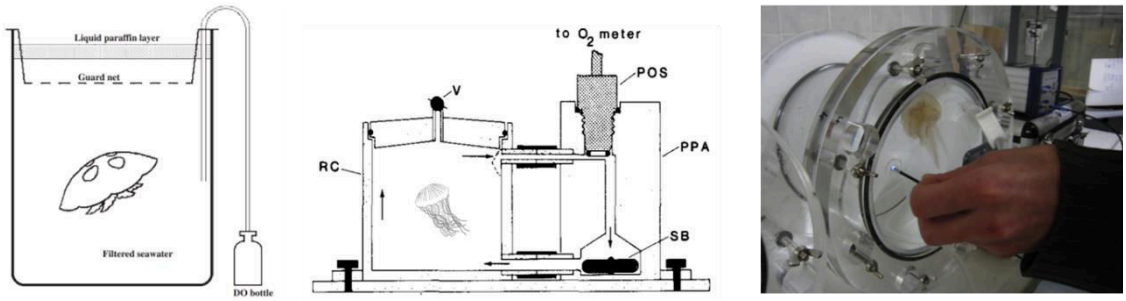


Figura 7.5. Recopilación de imágenes ejemplificando la variedad de técnicas más comunes para la medida del consumo de oxígeno. Izquierda, esquema de Uye & Shimauchi (2005) de su disposición para la incubación y extracción de agua para el análisis de O_2 por el método Winkler. Centro, esquema de la cámara de incubación de Larson (1987) con un sistema polarigráfico de medida de O_2 sin estancamiento de agua a través del electrodo (modificada para presentar la medusa). Derecha, imagen de Lilley & Lombard (2015) del proceso fotoquímico de medida con la fibra óptica y spot para determinar concentración de O_2 usando un optodo durante una incubación.

No obstante, las incubaciones están expuestas a efectos del confinamiento sobre el organismo que pueden afectar a su desempeño metabólico (Frandsen & Riisgård, 1997; Purcell et al., 2010) las incubaciones requieren de tiempo, requieren obtener al organismo vivo del medio y requiere grandes recipientes para organismos de gran tamaño. Por ello también se emplean proxys de la respiración como por ejemplo medir la actividad de las enzimas que controlan estos procesos respiratorios. Hay numerosas enzimas que se estudian con este propósito: succinato deshidrogenasa (SD) y NADH deshidrogenasa del ETS, lactato deshidrogenasa (LDH), pyruvato kinasa (PK)and citrato sintasa (CS) and malato deshidrogenasa (MDH) (Hernández-León & Ikeda, 2005). En este trabajo nos centramos solo en las enzimas del ETS que están más próximamente relacionadas con la reducción del O_2. En concreto el segundo capítulo de este trabajo realiza una comparativa entre las distintas técnicas para medir el consumo de oxígeno y determinar R y la medida del ETS para luego desarrollar el estudio de su aplicación en plancton gelatinoso.

De la medida de la actividad enzimática del ETS se obtiene la capacidad máxima de consumo de oxígeno por parte de las células del organismo a lo cual nos referiremos como respiración potencial (Φ) (Packard & Gómez, 2008). Para determinar esta actividad enzimática concreta se emplearon las metodologías basadas en aquellas para el estudio del ETS desarrolladas y modificadas por numerosos autores (Packard, 1971, 1985; Kenner & Ahmed, 1975; Owens & King, 1975; Gómez et al., 1996; Packard & Christensen, 2004; Maldonado et al., 2012). Comenzamos con la homogeneización del tejido que se desea estudiar para liberar las enzimas al medio. El medio de homogeneización consiste en una solución tamponada de fosfato 0.1M. Para homogeneizar se emplearon instrumentos como un sonicador o un homogeneizador de tipo Potter-Elvehjem. Luego este homogeneizado se centrifuga y el sobrenadante con las enzimas se mezcla con el sustrato de las enzimas que se desean estudiar en concentraciones que logren su actividad máxima. En el caso del estudio del ETS se emplea una solución de NADH y NADPH preparada también en buffer tamponado. Además, en el caso de este análisis se añade una solución de la sal de iodonitrotetrazolio (INT) (Nachlas et al., 1960; Caravelli et al., 2004). Este último actúa como aceptor artificial de electrones e indicador de la reacción ya que, al reducirse, el INT, genera formazán el cual tiñe la solución de rojo. Por tanto, se puede realizar un seguimiento de

la reacción con un espectrofotómetro. Dicha tasa de producción de formazán indica la tasa de actividad del sistema de electrones y permite estimar estequiométricamente la tasa potencial a la que se reduciría el O_2 en el sistema en lugar del formazán, es decir, la Φ. A dicha medida se le debe de restar la actividad observada en ausencia de enzima y también en ausencia de sustrato para corregir toda aquella actividad que no es estrictamente la de la reacción enzimática de interés a concentraciones correspondientes de sustrato. Basándonos en las pruebas realizadas por Owens & King (1975) y las recomendaciones para el desarrollo de técnicas enzimáticas descritas por Bisswanger (2014) se realizaron las pruebas descritas en el capítulo 3 para cerciorar la mejor implementación de las medidas de ETS en medusas.

7.5. Estudio de la composición bioquímica de las medusas y su rol como presa

Como se ha descrito en apartados anteriores el estudio de la respiración nos informa sobre las necesidades metabólicas del organismo y nos acerca a entender su demanda de alimento y por ende su impacto sobre presas. Hace cientos de millones de años puede que esto hubiese sido suficiente para posicionar el rol de las medusas como depredador final (Parsons, 1979). Con la aparición de nuevos grandes depredadores el rol de las medusas pareció pasar a segundo plano como organismos carentes de depredación o con una depredación muy específica o muy excepcional (Verity & Smetacek, 1996; Sommer et al., 2002). Sin embargo, numerosos autores han cuestionado este estigma de que el plancton gelatinoso sea un "callejón trófico sin salida" (Doyle et al., 2007; Hamilton, 2016). Es más, se han evidenciado múltiples relaciones tróficas que convierten a los organismos gelatinosos en un integrante vital de los sistemas tróficos y la explicación que subyace detrás de esta depredación observada es una cuestión de interés (Thiebot & McInnes, 2020). Ates (1991) defendió el papel como presa de las medusas y planteó que el menosprecio de éstas como presas podría deberse a la reticencia de ciertos observadores a aceptar que son un alimento útil y a las dificultades para identificar restos de éstas en el digestivo de sus depredadores debido a su fragilidad y rápida digestión. El aumento de evidencias filmográficas, análisis de nematocistos, análisis genéticos de contenidos estomacales y la continua revisión de la relación de muchos animales con el plancton gelatinoso han ido dando fuerza a su papel como alimento (Lamb et al., 2017; Thiebot & McInnes, 2020). El término gelatívoro o gelativoría ("gelativore") parece que se emplea por primera vez en una publicación en Drazen & Robison (2004). Este término engloba a todos aquellos organismos que consumen plancton gelatinoso. La aparición de este nuevo término es pertinente ya que los organismos gelatinosos no son presas convencionales como se irá describiendo en este apartado. Existen organismos descritos en la literatura con un elevado consumo y dependencia de presas gelatinosas como, por ejemplo: el pez luna, *Mola mola*; el pez mantequilla, *Peprilus triacanthus*; o la tortuga laud, *Dermochelys coriácea* (Arai, 2005; Heaslip et al., 2012). Aunque cabe matizar que en muchos casos la dieta de medusas no ha sido extensamente documentada y la compaginan con otras presas (Pope et al., 2010). Dejando a un lado a los famosos grandes consumidores, hay multitud de depredadores de plancton gelatinoso (Fig. 7.6). Tal vez una de las mejores recopilaciones de depredadores de plancton gelatinoso sea la de Arai (2005) donde se documenta la gelativoría por parte de otros organismos gelatinosos, tortugas, peces, condrictios (rayas y tiburones), moluscos, crustáceos, aves y humanos. Pauly et al. (2009) también recopiló evidencias de depredación sobre plancton gelatinoso por parte de aves, reptiles y una gran variedad de peces y crustáceos. Choy et al. (2017) describió la estructura de la red trófica que transcurría en altas profundidades

apoyándose en videos e incluía una enorme variedad de plancton gelatinoso que se depredaban entre sí e interactuaban también con peces, crustáceos y cefalópodos, entre otros, que habitaban a esas profundidades. Además de aquellos organismos que capturan a medusas vivas, hay multitud de peces y crustáceos que viven en simbiosis con algún organismo gelatinoso, al menos durante parte de su ciclo de vida, y hay evidencias de que estos a veces se alimentan de pedazos de su anfitrión (Mansueti, 1963; Rorke et al., 2015; Griffin et al., 2019). También existe toda una comunidad bentónica que se alimenta de los restos de medusas muertas cuando varan o se hunden, incluyendo a crustáceos, equinodermos y otros cnidarios bentónicos (Fig. 1.10) (Alamaru et al., 2009; Ates, 2017). Todo ello sin incluir la depredación añadida que sufren algunos organismos gelatinosos durante fases de vida sésiles como es el caso de los pólipos (Fig. 1.10) (Takao et al., 2014).

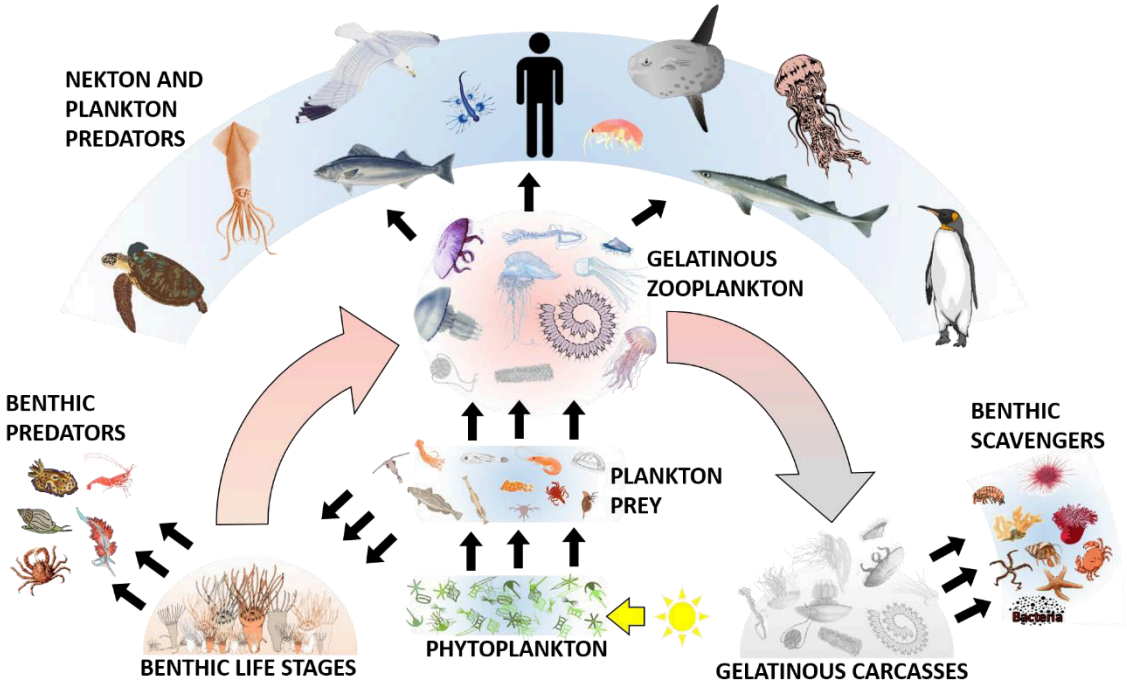


Figura 7.6. Esquema simplificado recopilando las interacciones tróficas descritas para el plancton gelatinoso. Flechas negras simbolizan flujos de depredación. Flechas coloreadas representan posibles transiciones entre momentos vitales del plancton gelatinoso. Los grupos representados describen la entrada energética al mar a través de los productores primarios, una versión muy simplificada de las presas planctónicas que incluye huevos, larvas e individuos pequeños del necton, una serie de representantes del plancton gelatinoso, algunos de los depredadores del plancton gelatinoso de distintos grupos, la fase de vida sésil bentónica que presentan algunos miembros del plancton gelatinoso con la interacción con sus depredadores y sus presas planctónicas representada, el hundimiento de plancton gelatinoso muerto y su consecuente depredación por descomponedores y carroñeros bentónicos. Los integrantes de los grupos están basados en diversos trabajos (Arai, 2005; Pauly et al., 2009; Riascos et al., 2012; Takao et al., 2014; Ates, 2017; Choy et al., 2017). Se han omitido, pero hay muchas interacciones simbióticas entre plancton gelatinoso y muchos otros individuos donde existe en ocasiones depredación mutua. Existen también interacciones adicionales entre integrantes de los grupos que no ha sido representadas como por ejemplo depredación interna entre los integrantes de las presas planctónica y entre los depredadores plancton y nectónicos, producción primaria de algas endosimbióticas en el interior de plancton gelatinoso a partir de energía solar y bucles microbianos. No se han representado en aras de la simplificación y centrar el esquema en la demanda de presas gelatinosas. Los organismos no están a escala.

No obstante, toda esta depredación desconcierta a la comunidad científica ya que se considera que el alto contenido en agua de las medusas lo convierte en una fuente pobre de alimento. Se consideró que la explicación podría radicar en que el plancton gelatinoso servía de alimento en ausencia de alternativas más nutritivas, pero estudios como Marques et al. (2016) demuestran que la demanda por medusas no es meramente circunstancial y beneficia a muchas especies oportunistas capaces de aprovechar la formación de grandes blooms de medusas. También se ha observado a pingüinos alimentarse de medusas de forma regular independientemente de la abundancia de las medusas y habiendo presas alternativas (Thiebot et al., 2017) y a peces en la cima de la cadena alimenticia, aunque haya presas nectónicas más energéticas (Cardona et al., 2012). Por ello se investigan las razones para la demanda en el consumo de medusas. Las hipótesis principales expuestas por Hays et al. (2018) y Thiebot & McInnes (2020) ofrecen explicaciones variadas para la demanda de plancton gelatinoso con alto contenido en agua. Algunas hipótesis están relacionadas con la facilidad de captura de estos organismos y su rápida digestión en comparación con otras presas. Otras se apoyan más en su enorme abundancia en muchas regiones. Otras sugieren que la motivación detrás de la depredación de plancton gelatinoso podría ser la ingesta estratégica de partes con mayor contenido energético como las gónadas. También se sugiere un cambio de paradigma en el enfoque investigador para el que las medusas han pasado de no ser alimento, a ser una comida poco nutritiva y ahora a ser una fuente de sustancias necesarias. Esta última hipótesis parece estar cogiendo fuerza e incentiva la investigación sobre la composición de las medusas depredadas (Stenvers et al., 2020). Para aumentar nuestra comprensión de este aspecto de la cadena trófica marina debemos comprender mejor la composición bioquímica y contenido energético de más integrantes del plancton gelatinoso y a lo largo de sus ciclos de vida. Por ello, en este trabajo se ha explorado también el contenido en proteínas, lípidos y carbohidratos de especies cuya composición ha sido menos estudiada en el capítulo 5, y, en detalle, a lo largo del ciclo de vida de una especie en el capítulo 6. Se buscó explorar las implicaciones que podría tener sobre la depredación de estos organismos.

Para la determinación del contenido en proteína, lípidos y carbohidratos (composición bioquímica) se emplearon diversas técnicas que trabajarían con el homogeneizado derivado del análisis enzimático. Las técnicas se encuentran descritas en más detalle en cada capítulo donde son aplicadas. Se pueden resumir en una reacción química controlada que colorea la solución y con un posterior análisis espectrofotométrico. La concentración es determinada basada en una curva de calibrado del componente orgánico de interés. La figura 7.7 resume de forma esquemática cada uno de los procedimientos.

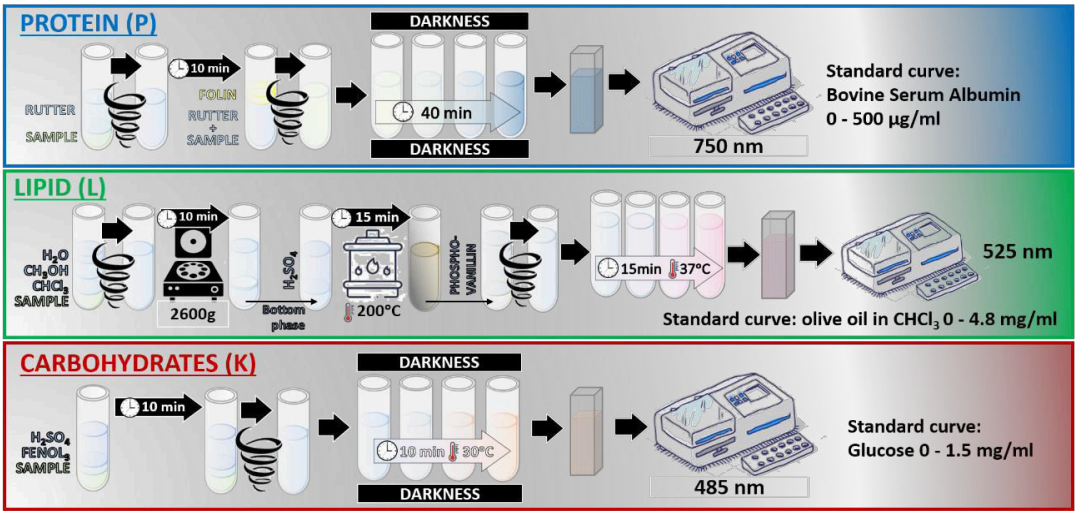


Figura 7.7. Recopilación de esquemas de los procedimientos para la determinación de proteínas, lípidos y carbohidratos basados en Lowry et al. (1951), Bligh & Dyer (1959) & Dubois et al. (1956) respectivamente.

Con la información del metabolismo respiratorio investigamos la motivación detrás de la depredación realizada por las medusas mientras que con la composición bioquímica aprendemos sobre la motivación detrás de la depredación sobre las medusas. Logramos con ello estudiar la obtención de energía de estos organismos junto con su contenido energético de una manera novedosa describiendo sistemas en términos de energía además de carbono (Karl, 2014; Chiaverano et al., 2018).

7.6. Resumen de los organismos usados en este trabajo

Para cumplir los propósitos de este trabajo se emplean una variedad relativamente amplia de organismos. En el capítulo 2 se emplean un representante de heterotrofía y un representante de autotrofía con el objetivo de comparar las técnicas para medir respiración a través del consumo de oxígeno y también con la señal del análisis ETS. En representación de los heterótrofos se usaron misidáceos concretamente la especie *Leptomysis lingvura*. En este caso se trata de una especie epibentónica de estos pequeños crustáceos que habitan formando grandes agregaciones en el norte de Gran Canaria donde fueron muestreados buceando con red. Estos se alimentan de detritus, fitoplancton, micro y mesozooplancton y son alimento para una gran variedad de peces (Mauchline, 1980). En el grupo de investigación donde se ha realizado la tesis se ha trabajado extensamente para lograr un conocimiento adecuado sobre el mantenimiento y medida de la respiración de estos organismos (Herrera-Ulibarri, 2013; Osma et al., 2016). Por ello *Leptomysis lingvura* fue un buen candidato para comparar técnicas de medida de respiración en organismos heterótrofos. En cambio, como representante de autótrofos se utilizó la clorofita *Ulva rigida*. Las especies del género *Ulva* (lechugas de mar) son unas macroalgas verdes del intermareal comunes globalmente (Zou et al., 2007) y en el caso de Gran Canaria de fácil muestreo y mantenimiento. Además, nuevamente, se trataba de un organismo con el que el laboratorio donde se realizó la tesis ya había trabajado (Asensio Elvira, 2013). La comparación buscaba sobre todo validar la aplicación de técnicas que luego serían empleadas para el estudio de la respiración en medusas. Examinar organismos heterótrofos y autótrofos cobra sentido si se tiene en cuenta que algunas especies gelatinosas albergar algas endosimbiontes.

Una vez seleccionada la técnica para medir el consumo de oxígeno y realizadas las pruebas descritas para la aplicación de las medidas enzimáticas se procedió en los capítulos 3, 4, 5 y 6 al estudio de la respiración en medusas. Las especies abordadas en este trabajo incluyen dos escifozoos y dos hidrozoos.

Los escifozoos estudiados fueron ambos del orden Semaeostomeae pero con ciclos de vida diferentes: ***Pelagia noctiluca*** y ***Aurelia aurita***. *Pelagia noctiluca* es una medusa holoplanctónica, es decir, pasa todo su ciclo de vida como parte del plancton (Ramondenc et al., 2019) (Fig. 7.8). Ha sido extensamente estudiada en el Mediterráneo (Canepa et al., 2014) donde forma grandes blooms y depreda sobre poblaciones de zooplancton y larvas de peces de forma no selectiva (Sabatés et al., 2010; Milisenda et al., 2018). También cuenta con nematocistos para la captura de sus presas provocando una picadura importante cuando impacta sobre bañistas (Mariottini et al., 2008). Por tanto, impacta sobre las poblaciones de fauna y actividades humanas locales. Dependiendo de la circulación mediterránea y atlántica se repiten periodos de gran agregación de *P. noctiluca* en aguas canarias (Rodríguez et al., 2015). También es presa de poblaciones de peces, tortugas y crustáceos (Milisenda et al., 2014; Couce-Montero et al., 2015).

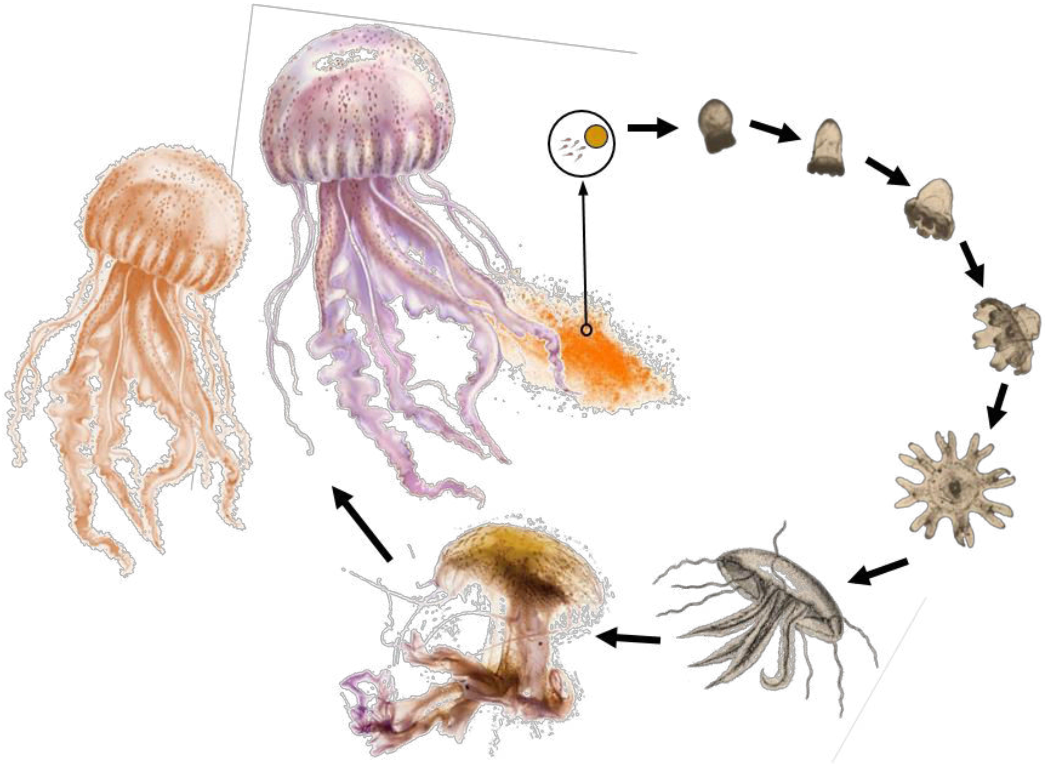


Figura 7.8. Ciclo de vida de *Pelagia noctiluca* (Sandrini et al., 1983; Canepa et al., 2014; Ramondenc et al., 2019).

Aurelia aurita es una especie muy comúnmente estudiada ya que su cultivo es relativamente sencillo y se emplea mucho en acuarios. Se trata de un escifozoo con un ciclo de vida complejo con un periodo de vida sésil y un periodo de vida planctónico, lo cual es relativamente común en escifozoos (Ceh et al., 2015) (Fig. 7.9). La fase de vida durante el periodo sésil se conoce como escifistoma o pólipo. Los pólipos se pueden reproducir de manera asexual y formar colonias del mismo individuo. También los pólipos bajo condiciones concretas comienzan una metamorfosis llamada estrobilación donde se segmentan formando una estróbila de la cual sale una fase larvaria planctónica

llamada éfira (Fig. 7.9) (Fuchs et al., 2014; Sukhoputova & Kraus, 2017). Las éfiras se desarrollan hasta formar la fase de vida adulta, la medusa. Las medusas adultas se reproducen sexualmente liberando gametos al medio que les rodea. Estos gametos fecundados desarrollan una fase embrionaria que culmina con la formación de una larva plánula que encuentra sustrato para formar el pólipo (Holst & Jarms, 2007). Existen descripciones de excepciones al ciclo donde la plánula se desarrolla en éfira sin una fase pólipo evidente y también se han documentado fenómenos de reversión del ciclo de vida (He et al., 2015). Los pólipos tienen una gran versatilidad; logran reproducirse por mecanismos variados (Gilchrist, 1937; Vagelli, 2007) y durante la estrobilación pueden producir hasta 20 éfiras por pólipo y sobrevivir para continuar multiplicándose y repetir el proceso (Lucas et al., 2012) (Fig. 7.9). Por tanto, son fases clave a la hora de controlar la formación de blooms. Sin embargo, son las medusas las que participan en las cadenas tróficas en océano abierto como depredador y presa (Schneider & Behrends, 1998). Además de su faceta cultivada y de su curioso ciclo de vida *Aurelia aurita* en la naturaleza forma grandes blooms con importantes consecuencias (Dong, 2019). Se trata nuevamente de un depredador de gran variedad de integrantes del zooplancton y ocasionalmente de larvas de peces (Sullivan et al., 1997; Titelman & Hansson, 2006). A su vez puede ser presa de peces, tortugas, crustáceos, equinodermos y otros cnidarios, incluyendo otros escifozoos (Ates, 2017).

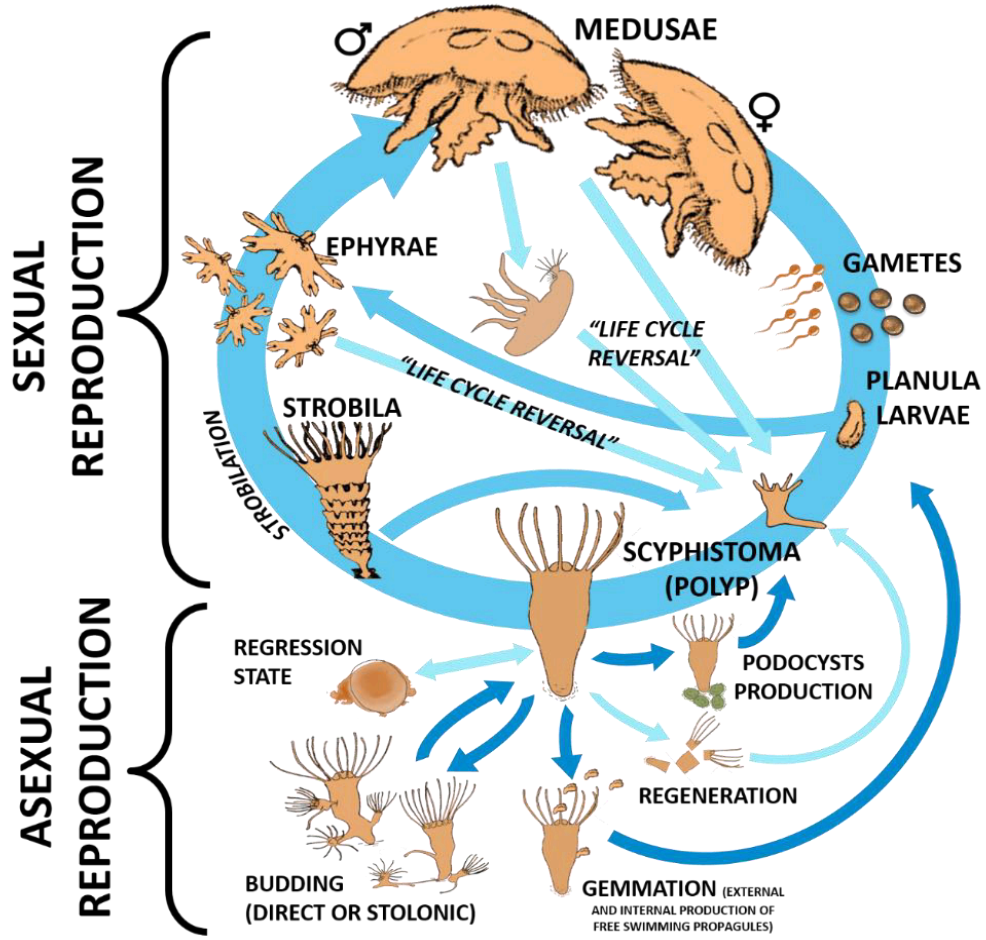


Figura 7.9. Recopilación de los procesos reproductivos observados en el género *Aurelia* basado en la literatura (Gilchrist, 1937; Hamner & Jenssen, 1974; Lucas, 2001; Vagelli, 2007; He et al., 2015; Hubot et al., 2017). Reuniendo el ciclo de reproducción sexual con fase medusa y fase pólipo y los diversos mecanismos de reproducción asexual de la fase pólipo.

Los hidrozoos estudiados fueron ***Physalia physalis*** y ***Velella velella***. Ambos son integrantes gelatinosos del pleuston, el ecosistema en la zona más superficial del océano en contacto con la atmósfera (Zamponi, 2011). Ambas especies forman colonias de individuos con alto grado de polimorfismo donde cada uno tiene una función especializada (Schuchert, 2010; Munro et al., 2019). La colonia está unida a una estructura flotante quitonosa llamada pneumatóforo o flotador que se trata de una estructura especializada de la colonia llena de gas y que permite a la colonia usar el viento atmosférico para desplazarse a lo largo del pleuston (Mackie, 1974; Iosilevskii & Weihs, 2009). Además de su excepcional nicho oceánico, presentan diversas características singulares. Ambos tienen una coloración azul debida a la presencia de unas chromatoproteinas concretas (Mackie et al., 1988). También como ya se ha comentado forman colonias polimórficas flotantes libres donde los individuos, o zooides, realizan funciones concretas. Este tipo de conformación llevó a Mackie & Boag (1963) a afirmar que se trataba de una forma de escapar de las limitaciones de un cuerpo diploblástico; donde otros organismos han evolucionado cuerpos triploblásticos y forman órganos a partir del mesodermo, estos individuos coloniales han construido estructuras parecidas a órganos a partir de la especialización de individuos de la colonia. Se trata del máximo aprovechamiento de las posibilidades fisiológico-anatómicas de la colonialidad (Mackie et al., 1988). Wilson, (1975) lo describió como una de las grandes historias de la evolución. Curiosamente estas especies fueron algunas de las primeras descritas debido a su naturaleza flotante lo cual las puso en contacto con la humanidad pronto; llegando a formar varamientos formados por numerosas colonias (Araya et al., 2016; Betti et al., 2019). Sin embargo, su fisiología y su papel en los ecosistemas no es estudiado con tanta frecuencia como con otros integrantes del plancton gelatinoso más fáciles de cultivar (Pierce, 2006).

Physalia physalis es del orden siphonophorae. A diferencia de otros sifonóforos se encuentra en su estado adulto arrastrado por el viento a través de las aguas superficiales pudiendo recorrer grandes distancias gracias su gran pneumatóforo (Ferrer & Pastor, 2017). Desde su situación flotante extienden largos zooides con palpones tentaculares ("tentacular palpons") especializados en la defensa y captura empleando nematocistos (Munro et al., 2019) (Fig. 7.10). Su toxina es potente y por ello se trata de una especie cuya picadura es de riesgo para los humanos e impacta sobre el turismo (Edwards & Hessinger, 2000; Prieto et al., 2015), especialmente, cuando forman grandes blooms. Normalmente dichas toxinas se emplean para la captura de sus presas tradicionales: peces pequeños, larvas de peces y más excepcionalmente cefalópodos y quetognatos (Purcell, 1984). Como se observó en muchas especies de medusas, *P. physalis* también sirve de huésped para algunas especies de peces comensales como por ejemplo las especies del género Nomeus (Jenkins, 1983). Además, son presa para depredadores integrantes del pleuston como los nudibranquios del género *Glaucus* o cirrípedos como *Lepas anserifera*, pero también son presas para tortugas y peces y cuando se encuentran varadas para cangrejos (Jones, 1968; Wenner, 1977; Frick et al., 2009). Su ciclo de vida y anatomía han sido revisados recientemente por Munro et al. (2019) poniendo de manifiesto la necesidad de más estudios sobre el funcionamiento de esta especie.

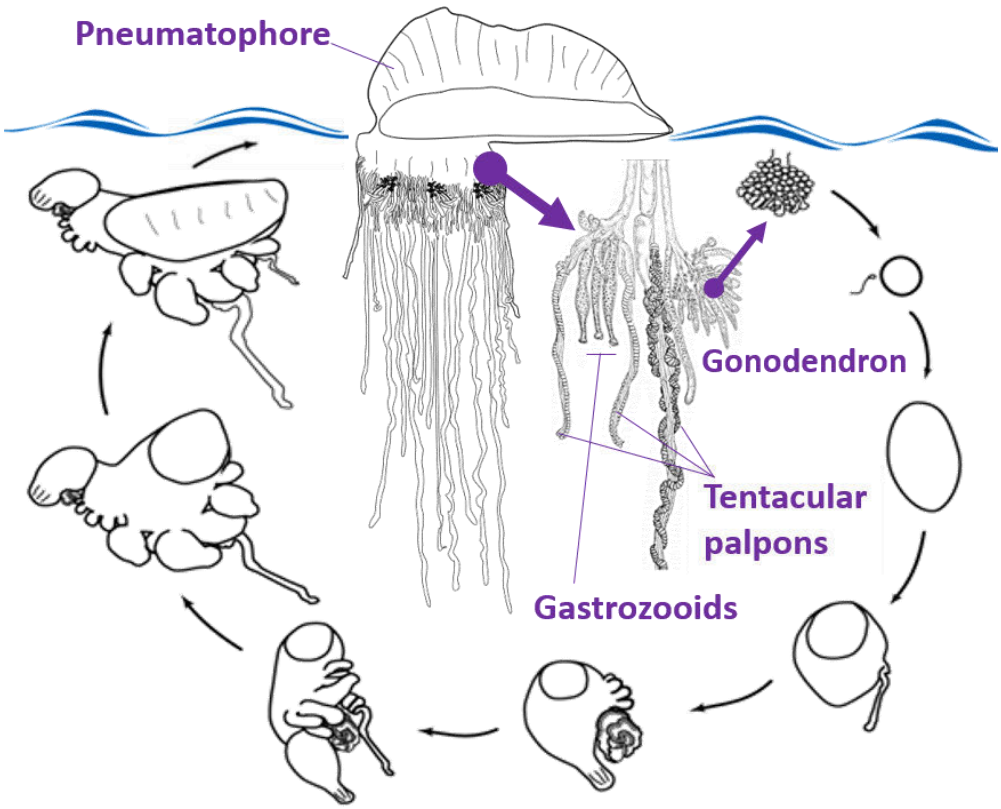


Figura 7.10. Anatomía de la colonia y ciclo de vida de *Physalia physalis*. (Lane, 1960; Munro et al., 2019)

Velella velella, en cambio, es una especie del orden Anthoatecata y no se conoce completamente su ciclo vital, pero si se han descrito dos fases de vida. Una fase "pólipo" durante la formación de la colonia de zooides que flotan suspendidos del flotador. De los zooides encargados de la reproducción se generan las fases de vida medusa de menor tamaño (Duarte et al., 2019) (Fig. 7.11). Esta fase medusa genera gametos y la fecundación y desarrollo embrionario no ha sido bien evidenciado, pero culmina con la formación de los estadios larvarios conaria y rataria (Schuchert, 2010). Y el posterior desarrollo de la colonia adulta. A diferencia de *P. physalis*, *V. velella* cuenta con microalgas endosimbiontes, zooxantelas, que están presentes también en la fase medusa. No obstante, también se ha documentado la presencia de nematocistos para la depredación de *V. velella* sobre zooplancton y huevos de peces (Zeman et al., 2018). Por otro lado, las colonias de *V. velella* son presas para múltiples depredadores. Algunos que cohabitan en el pleuston, como *Janthina palida* o *Lepas anserifera* (Bieri, 1966), otros no, como tortugas, peces, condrictios o aves (Phillips et al., 2017). Además, algunos crustáceos durante su etapa como larva megalopa parasitan estas colonias flotantes para recorrer grandes distancias estableciendo una relación simbiótica temporal (Purcell et al., 2012).

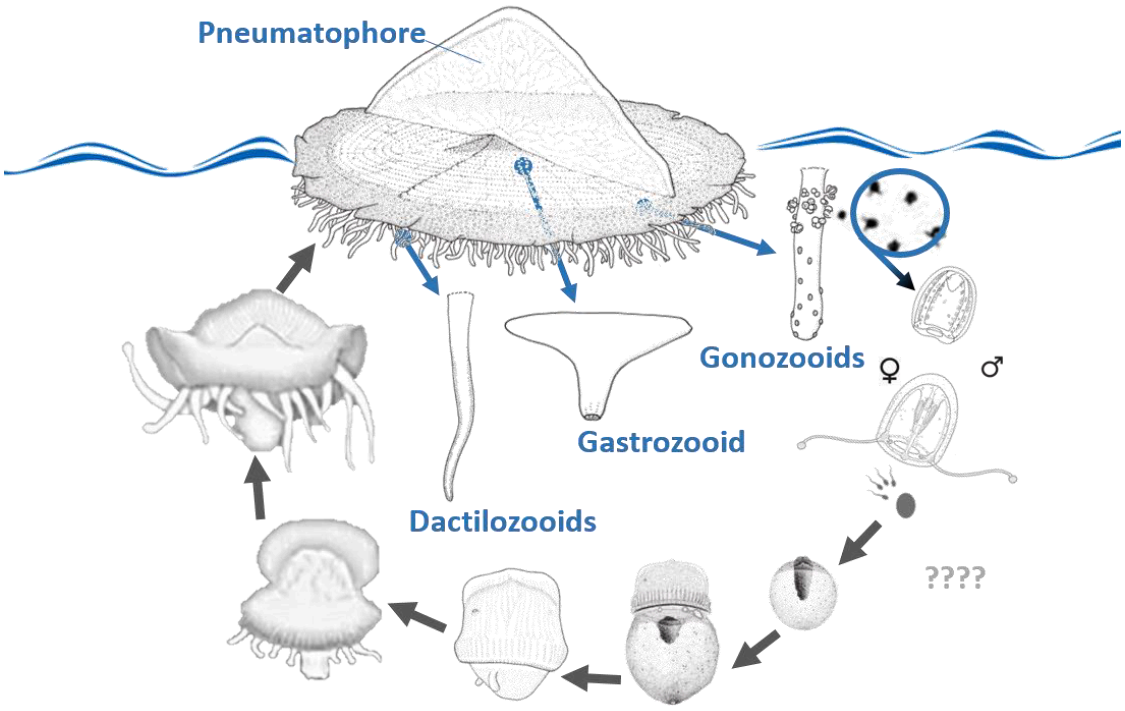


Figura 7.11. Anatomía de la colonia y ciclo de vida de *Velella velella* (Schuchert, 2010; Pires et al., 2018).

En ninguno de estos integrantes del plancton gelatinoso se han estudiado los procesos fisiológicos con las técnicas enzimáticas empleadas aquí. Tampoco se han usado datos de respiración para estudiar las contribuciones de estos individuos desde el punto de vista energético. Además, en concreto en el caso de *Physalia* y *Velella* la respiración y la composición muy rara vez ha sido medidas y en este trabajo se encuentran algunos de las primeras medidas en estas especies. También en el caso de *Aurelia* el seguimiento de su metabolismo y composición a lo largo de su desarrollo desde éfira a medusa ha revelado aspectos nunca antes documentados sobre los cambios que experimenta durante los primeros días en el plancton.

7.7. Objetivos de la tesis

El objetivo general de este trabajo es mejorar el entendimiento del rol de las medusas como depredador y como presa. Para el estudio de su impacto como depredador se desarrollan y prueban técnicas para el estudio de la actividad respiratoria que permiten, por ejemplo, estimar la demanda de carbono, y por tanto alimento, asociado. Para el estudio de su demanda como presa se analizó el contenido en proteínas, lípidos y carbohidratos de especies del plancton gelatinoso pudiendo determinar su contenido energético y entender mejor su aporte cuando son depredados. Además, se emplearon estas mismas metodologías para detectar cambios sufridos en el metabolismo respiratorio y composición durante el crecimiento y periodos de inanición.

Los objetivos de este trabajo se pueden desglosar en:

- Abordados en el capítulo 2:
    - Examinar el grado de similitud entre los resultados de distintas técnicas para medir el consumo de oxígeno. Además, verificar la relación entre

estos resultados y la técnica enzimática de estudio del ETS en organismos autótrofos y heterótrofos con el fin de aplicar estos conocimientos en el proceso de estudio de respiración en plancton gelatinoso.

- Abordados en el capítulo 3 y 4:
    - Tras la realización de las pruebas necesarias, desarrollar y aplicar la metodología para realizar medidas respiratorias de tipo tradicional y enzimáticas en miembros del plancton gelatinoso, utilizando *Aurelia aurita* y *Pelagia noctiluca* como organismos de prueba.

- Abordados en el capítulo 4 y 5:
    - Comparar la respiración obtenida por métodos de consumo de oxígeno y por técnicas enzimáticas en distintos organismos del plancton gelatinoso y comparar con el resto del zooplancton.
    - Estudiar las implicaciones de este metabolismo respiratorio sobre la demanda de carbón y flujo energético asociado al plancton gelatinoso para estimar el impacto sobre poblaciones de presas.
    - Analizar la relación entre la liberación de energía asociada a la respiración y el contenido energético como una nueva herramienta para estudiar el metabolismo.

- Abordados en el capítulo 5:
    - Medir por primera vez la composición bioquímica y contenido energético de integrantes del pleuston, *Velella velella* y *Physalia physalis* y comparar con el resto de plancton gelatinoso.

- Abordados en el capítulo 6:
    - Monitorizar la respuesta a la inanición prolongada sobre la respiración de *Aurelia aurita* que presenta una gran resistencia a la inanición.
    - Emplear las técnicas desarrolladas para observar los cambios experimentados a nivel de metabolismo respiratorio y composición bioquímica durante el periodo de transición de larva éfira de 24h a medusa juvenil de 54d.

7.8. Resumen por capítulo

A continuación, se muestran los resúmenes de cada uno de los capítulos relacionados con las investigaciones realizadas durante la tesis.

7.8.1. Resumen Capítulo 2

La respiración aeróbica es un proceso biológico de transformación de la energía que consume carbono orgánico y oxígeno. En el océano, el equilibrio entre la fotosíntesis y la respiración se reconoce como crítico para entender el impacto del océano en el CO_2 hidrosférico y atmosférico. Las técnicas para determinar la respiración pueden basarse en la química inorgánica, la electroquímica, la fotoquímica y la enzimología. En este trabajo, para comparar estos métodos, la respiración fisiológica fue medida simultáneamente por el método de Winkler (W), electrodos de O_2 (E), y optodos de O_2 (O). Estas técnicas detectaron el consumo de O_2 respiratorio (R), *in situ*, en cámaras de incubación en oscuridad. Las mediciones de la actividad del sistema de transporte de

electrones respiratorios (ETS) detectaron la respiración potencial (Φ), bioquímicamente. *Leptomysis lingvura*, un misidáceo marino, y *Ulva rigida*, una especie de alga verde fueron los dos organismos probados.

Los resultados de la respiración fisiológica de cada técnica no fueron significativamente diferentes (múltiples pruebas t de Student para muestras emparejadas, p-valor > 0.05) y estuvieron dentro del rango de medidas similares publicadas. La aplicación del análisis de regresión de Passing-Bablok puso de manifiesto la alta correlación entre los resultados y el análisis de Bland-Altman examinó la diferencia media ("sesgo") y los límites de concordancia entre los métodos. La respiración media estandarizada por masa seca de *L. lingvura* y *U. rigida* fue de 0,147 ± 0,037 y 0,023 ± 0,008 µmol O_2 h^{-1} (mg de masa seca)$^{-1}$, n=9, respectivamente. Las proporciones R:Φ fueron diferentes en los dos organismos. Sin embargo, la regresión lineal entre R y Φ para *L. lingvura* y *U. rigida* fue mejor (r^2 = 0,814 y 0,313) que la regresión lineal entre R y la biomasa seca (r^2 = 0,643 y 0,213).

7.8.2. Resumen Capítulo 3

Antes de hacer ensayos de ETS en medusas, había que determinar las condiciones óptimas para el ensayo. Esta es la práctica estándar con el análisis de enzimas. Se eligió usar *Aurelia aurita* en representación de las medusas que se planeaba estudiar. La concentración óptima de sustrato se determinó a partir de la afinidad del sustrato del ETS siguiendo la fórmula clásica de Michaelis-Menten. Se determinaron tanto los valores de la curva de Michaelis-Menten como la relación del pH con la reacción del ETS. A partir de la curva que describe la dependencia del pH [ETS = f(pH)] se identificó el pH óptimo del ensayo. El efecto de la temperatura en la actividad del ETS en estas muestras se estudió siguiendo la ecuación de Arrhenius a partir de la cual se determinó la energía de activación. Esto facilitó las correcciones de temperatura en el trabajo experimental. Además, se verificó la linealidad de la velocidad de reacción enzimática en función de la concentración de la enzima y la biomasa proteíca. Esto fue importante porque los resultados facilitaron la comparación de las mediciones realizadas a diferentes concentraciones de enzimas y diferentes biomasas. Los resultados se compararon con las condiciones de anteriores ensayos de ETS. Estas condiciones óptimas se emplearon luego para los análisis de ETS en medusas reportados en los capítulos siguientes.

7.8.3. Resumen Capítulo 4

Investigamos la respiración y la composición bioquímica de medusas, en concreto de dos especies de escifozoos, *Aurelia aurita* y *Pelagia noctiluca*. La actividad respiratoria se determinó mediante el análisis enzimático del sistema de transporte de electrones respiratorios (ETS). Con esta actividad del ETS se calculó estequiométricamente la respiración potencial (Φ), en unidades de oxígeno, y se comparó con la respiración aeróbica (*R*) determinada por optodos y con la biomasa húmeda (WM). El análisis de regresión entre *R,* Φ y WM, mostró una función de potencia adecuada para describir la relación entre estos parametros [*R* (µl O_2 h^{-1}) = 0,78 Φ (µl O_2 h^{-1}) ^{0,90} (r^2 = 0,97)]. *R* (µl O_2 h^{-1}) = 0,23 WM (g) ^{0,93} (r^2 = 0,97)].

La composición bioquímica se estudió mediante la medición del contenido de proteínas, lípidos y carbohidratos. Estos análisis revelaron que el contenido de lípidos específicos de WM es mayor en las etapas de vida más pequeñas y disminuye a medida que aumenta el tamaño, mientras que el contenido de proteínas se mantiene estable. El contenido de

estos componentes bioquímicos se utilizó para calcular el contenido energético basado en composición de ambas especies. Este parámetro no fue muy constante debido a los cambios en el contenido de lípidos. También se comparó con estudios que usaron bombas calorimétricas y el tejido de medusas.

La demanda de carbono respiratorio (*RCD*) y las necesidades mínimas de carbono normalizadas por masa (*MCR*) se determinaron, por separado, a partir de *R* y Φ. Se utilizaron para calcular el impacto del consumo de presas por parte de las medusas, así como para calcular la tasa de oxidación de carbono respiratorio cnidario global (2,27 - 3,15 Gt C y^{-1}). La tasa de transformación heterotrófica de la energía (*HET*), también se calculó a partir de las mediciones respiratorias. El *HET* potencial asociado a la actividad mitocondrial fue de 10,03±5,31 y 11,05±4,88 J d^{-1} g WM^{-1} para *A. aurita* y *P. noctiluca,* respectivamente. Estos valores están dentro del rango *HET* calculado a partir de los resultados *R* de la literatura para *A. aurita* y *P. noctiluca*. Sin embargo, estos son casi 1000 veces mayores que la tasa de producción de energía normalizado por masa del sol (0,017 J d^{-1} g^{-1}). Sin embargo, extrapolada a la abundancia de cnidarios planctónicos global la HET global asociada sólo equivale al 2-3% de la energía solar fijada por la producción primaria. Más cálculos empleando *HET* asociada a la respiración promoverían la modelización de la circulación de la energía, lo que permitiría avanzar en la comprensión de la dinámica del flujo de energía en los ecosistemas donde tradicionalmente se trabaja a partir de la modelización de la biomasa y la circulación elemental.

7.8.4. Resumen Capítulo 5

En este estudio arrojamos luz sobre el papel como presa y depredador de dos importantes representantes del amplio ecosistema pleustónico, *Physalia physalis* y *Velella velella*. Verificamos las implicaciones que su naturaleza a la deriva impulsada por el viento tiene en sus metabolismos y comprobamos si su composición coincide con la de otras especies gelatinosas. Para investigar su valor nutritivo como presa analizamos la composición bioquímica (biomasa húmeda y seca, proteínas (P), lípidos (L), carbohidratos (K)) de los dos hidrozoos y calculamos el contenido energético correspondiente (Ec). Para estudiar la relación de su fisiología con su propulsión pasiva y estimar su impacto como depredadores, estimamos la demanda de carbono asociada a su metabolismo respiratorio (respiración fisiológica (R) y respiración potencial (Φ)). El contenido de agua de estos organismos era inferior al de otros zooplancton gelatinosos, probablemente debido a la presencia de sus estructuras de quitina. La composición bioquímica por masa seca estaba dentro del rango del zooplancton gelatinoso en la literatura con un contenido ligeramente mayor de proteínas y Ec. La Ec por masa húmeda (WM) era en general más alta que la de otras especies gelatinosas, excluyendo los especímenes árticos. El metabolismo respiratorio por contenido de proteínas de ambos hidrozoos era en general más bajo que en las medusas pulsantes. El R:Φ fue bajo comparado con estudios anteriores sobre el zooplancton. La demanda respiratoria de carbono fue menor que la demanda calculada de carbono de los estudios de ingestión de presas. Hubo una relación entre la respiración y su estilo de vida a la deriva, bastante inactivo, en comparación con otros miembros más activos del gelatinoso zooplancton. El contenido de P, L y K de estos hidrozoos pleustónicos se asemeja al de otros integrantes del zooplancton gelatinosos, pero en general tienen un menor contenido de agua. Proponemos que estos resultados apoyaron ambas hipótesis. Estos resultados son las primeras medidas de la respiración publicadas para ambas especies. Es necesario seguir investigando para probar explicaciones alternativas a estas observaciones.

7.8.5. Resumen Capítulo 6

En este trabajo se exploraron los cambios bioquímicos y metabólicos que se producen en la medusa *Aurelia aurita en su transición* de ephyra (24 horas después de la estrobilización) a medusa (66 días) y, también, cambios que sufren los juveniles durante la privación de alimento. Se midió la composición lipídica y proteínica, la respiración fisiológica (R) y la respiración de potencial enzimático (Φ), además de la biometría típica, la masa húmeda y el diámetro. Nuestras observaciones apoyan que aproximadamente alrededor de la tercera semana de desarrollo las éfiras completan su transición a medusas juveniles. Esta transición se pudo identificar en los cambios de los principales componentes bioquímicos (lípidos y proteínas), en la continuidad de la campana y en la respiración fisiológica en comparación con su potencial. Los resultados mostraron una tasa de crecimiento mucho más alta, una mayor tasa respiratoria y un, nunca antes documentado, mayor contenido de lípidos que proteínas en los primeros días de las éfiras. El alto contenido en lípidos sirve como fuente de energía y como ayuda para la flotabilidad de los nuevas éfiras. Las medusas juveniles de *A. aurita* sobrevivieron hasta 58 días bajo privación de alimentos con varios cambios en su metabolismo respiratorio. Estas observaciones en éfiras pueden tener implicaciones similares en el metabolismo y la composición lipídica de la strobila de *A. aurita*.

REFERENCIAS

Acuña, J. L., Á. López-Urrutia, & S. Colin, 2011. Faking giants: the evolution of high prey clearance rates in jellyfishes. science American Association for the Advancement of Science 333: 1627–1629.

Alamaru, A., O. Bronstein, G. Dishon, & Y. Loya, 2009. Opportunistic feeding by the fungiid coral *Fungia scruposa* on the moon jellyfish *Aurelia aurita*. Coral Reefs 28: 865–865.

Arai, M. N., 2005. Predation on pelagic coelenterates: A review. Journal of the Marine Biological Association of the United Kingdom 85: 523–536.

Araya, J. F., J. A. Aliaga, & M. E. Araya, 2016. On the distribution of *Physalia physalis* (Hydrozoa: Physaliidae) in Chile. Marine Biodiversity 46: 731–735, http://dx.doi.org/10.1007/s12526-015-0417-6.

Archibald, J. M., 2015. Endosymbiosis and eukaryotic cell evolution. Current Biology Elsevier Ltd 25: R911–R921, http://dx.doi.org/10.1016/j.cub.2015.07.055.

Asensio Elvira, M. T., 2013. Respiratory metabolism in the marine macroalga, Ulva spp: exploratory studies with the respiratory electron transport system (ETS). Universidad de Las Palmas de Gran Canaria.

Ates, R. M. L., 1991. Predation on Cnidaria by vertebrates other than fishes. Hydrobiologia 216–217: 305–307.

Ates, R. M. L., 2017. Benthic scavengers and predators of jellyfish, material for a review. Plankton and Benthos Research 12: 71–77.

Avila Rodríguez, M. I., L. G. Rodríguez Barroso, & M. L. Sánchez, 2018. Collagen: A review on its sources and potential cosmetic applications. Journal of Cosmetic Dermatology 17: 20–26.

Benedict, C. R., 1978. Nature of Obligate Photoautotrophy. Annual Review of Plant Physiology 29: 67–93.

Betti, F., M. Bo, F. Enrichetti, M. Manuele, R. Cattaneo-Vietti, & G. Bavestrello, 2019. Massive strandings of *Velella velella* (Hydrozoa: Anthoathecata: Porpitidae) in the Ligurian Sea (North-western Mediterranean Sea). European Zoological Journal 86: 343–353.

Bieri, R., 1966. Feeding Preferences and Rates of the Snail, *Ianthina prolongata*, the Barnacle, *Lepas anserifera*, the Nudibranchs, *Glaucus atlanticus* and *Fiona pinnata*, and the Food Web in the Marine Neuston. Publications of the Seto Marine Biological Laboratory 14: 161–170.

Bisswanger, H., 2014. Enzyme assays. Perspectives in Science Elsevier 1: 41–55, http://dx.doi.org/10.1016/j.pisc.2014.02.005.

Bligh, E. G., & W. J. Dyer, 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology NRC Research Press 37: 911–917.

Brochier-Armanet, C., E. Talla, & S. Gribaldo, 2009. The multiple evolutionary histories of dioxygen reductases: Implications for the origin and evolution of aerobic respiration. Molecular Biology and Evolution 26: 285–297.

Brotz, L., & D. Pauly, 2017. Studying jellyfish fisheries: Toward accurate national catch reports and appropriate methods for stock asssessments In Mariottini, G. L. (ed), Jellyfish. : 313–329.

Canepa, A., V. Fuentes, A. Sabatés, S. Piriano, F. Boero, & J. M. Gili, 2014. *Pelagia noctiluca* in the Mediterranean In Pitt, K. A., & C. H. Lucas (eds), Jellyfish Blooms. Springer Science, Dordrecht: 1–304.

Canfield, D. E., 2005. The early history of atmospheric oxygen: Homage to Robert M. Garrels. Annual Review of Earth and Planetary Sciences 33: 1–36.

Caravelli, A., L. Giannuzzi, & N. Zaritzky, 2004. Effect of chlorine on filamentous microorganisms present in activated sludge as evaluated by respirometry and INT-dehydrogenase activity. Water Research 38: 2395–2405.

Cardona, L., I. Á. de Quevedo, A. Borrell, & A. Aguilar, 2012. Massive consumption of gelatinous plankton by mediterranean apex predators. PLoS ONE 7: e31329-1–14.

Cartwright, P., S. L. Halgedahl, J. R. Hendricks, R. D. Jarrard, A. C. Marques, A. G. Collins, & B. S. Lieberman, 2007. Exceptionally preserved jellyfishes from the Middle Cambria. PLoS ONE 2: e1121-1–7.

Ceh, J., J. Gonzalez, A. S. Pacheco, & J. M. Riascos, 2015. The elusive life cycle of scyphozoan jellyfish - Metagenesis revisited. Scientific Reports Nature Publishing Group 5: 1–13, http://dx.doi.org/10.1038/srep12037.

Chiaverano, L. M., K. L. Robinson, J. Tam, J. J. Ruzicka, J. Quiñones, K. T. Aleksa, F. J. Hernandez, R. D. Brodeur, R. Leaf, S. Uye, M. B. Decker, M. Acha, H. W. Mianzan, & W. M. Graham, 2018. Evaluating the role of large jellyfish and forage fishes as energy pathways, and their interplay with fisheries, in the Northern Humboldt Current System. Progress in Oceanography 164: 28–36.

Choy, C. A., S. H. D. Haddock, & B. H. Robison, 2017. Deep pelagic food web structure as revealed by in situ feeding observations. Proceedings. Biological sciences 284:.

Collins, A. G., 2002. Phylogeny of the Medusozoa and the evolution of cnidarian life cycles. Journal of Evolutionary Biology 15: 418–432.

Condon, R. H., W. M. Graham, C. M. Duarte, K. A. Pitt, C. H. Lucas, S. H. D. Haddock, K. R. Sutherland, K. L. Robinson, M. N. Dawson, M. B. Decker, C. E.

Mills, J. E. Purcell, A. Malej, H. Mianzan, S. Uye, S. Gelcich, & L. P. Madin, 2012. Questioning the Rise of Gelatinous Zooplankton in the World's Oceans. BioScience 62: 160–169.

Conway Morris, S., 2000. The Cambrian "explosion": Slow-fuse or megatonnage? Proceedings of the National Academy of Sciences of the United States of America 97: 4426–4429.

Couce-Montero, L., V. Christensen, & J. J. Castro, 2015. Effects of small-scale and recreational fisheries on the Gran Canaria ecosystem. Ecological Modelling Elsevier B.V. 312: 61–76, http://dx.doi.org/10.1016/j.ecolmodel.2015.05.021.

Cunningham, J. A., A. G. Liu, S. Bengtson, & P. C. J. Donoghue, 2017. The origin of animals: Can molecular clocks and the fossil record be reconciled?. BioEssays 39: 1–12.

Dong, Z., 2019. Blooms of the Moon Jellyfish Aurelia : Causes, Consequences and Controls World Seas: an Environmental Evaluation. Elsevier-Academic Press: 163–171, https://doi.org/10.1016/B978-0-12-805052-1.00008-5.

Doyle, T. K., G. C. Hays, C. Harrod, & J. D. R. Houghton, 2014. Ecological and societal benefits of jellyfish In Pitt, K. A., & C. H. Lucas (eds), Jellyfish blooms. Springer: 105–127.

Doyle, T. K., J. D. R. Houghton, R. McDevitt, J. Davenport, & G. C. Hays, 2007. The energy density of jellyfish: Estimates from bomb-calorimetry and proximate-composition. Journal of Experimental Marine Biology and Ecology 343: 239–252.

Drazen, J. C., & B. H. Robison, 2004. Direct observations of the association between a deep-sea fish and a giant scyphomedusa. Marine and Freshwater Behaviour and Physiology 37: 209–214.

Duarte, C. M., K. A. Pitt, C. H. Lucas, J. E. Purcell, S. Uye, K. L. Robinson, L. Brotz, M. B. Decker, K. R. Sutherland, A. Malej, L. Madin, H. Mianzan, J. M. Gili, V. Fuentes, D. Atienza, F. Pagès, D. Breitburg, J. Malek, W. M. Graham, & R. H. Condon, 2013. Is global ocean sprawl a cause of jellyfish blooms?. Frontiers in Ecology and the Environment 11: 91–97.

Duarte, I., S. Leandro, M. Ferreira, H. Pinto, J. Falcão, J. Reis, S. Maia, & S. Marques, 2019. Early development of Velella velella medusae in laboratory. Frontiers in Marine Science | International Meeting on Marine Research .

Dubois, M., K. Gilles, J. K. Hamilton, P. A. Rebers, & F. Smith, 1956. A colorimetric method for the determination of sugars. Nature 168: 350–356.

Edwards, L., & D. A. Hessinger, 2000. Portuguese Man-of-war (*Physalia physalis*) venom induces calcium influx into cells by permeabilizing plasma membranes. Toxicon 38: 1015–1028.

Fernández-Urruzola, I., 2015. Understanding the zooplankton ammonium excretion: from biogeochemical implications to intracellular regulatory mechanisms. Universidad de Las Palmas de Gran Canaria.

Ferrer, L., & A. Pastor, 2017. The Portuguese man-of-war: Gone with the wind. Regional Studies in Marine Science Elsevier B.V. 14: 53–62, http://dx.doi.org/10.1016/j.rsma.2017.05.004.

Frandsen, K. T., & H. U. Riisgård, 1997. Size dependent respiration and growth of jellyfish, *Aurelia aurita*. Sarsia 82: 307–312.

Fransolet, D., S. Roberty, & J. C. Plumier, 2012. Establishment of endosymbiosis: The case of cnidarians and Symbiodinium. Journal of Experimental Marine Biology and Ecology Elsevier B.V. 420–421: 1–7, http://dx.doi.org/10.1016/j.jembe.2012.03.015.

Frick, M. G., K. L. Williams, A. B. Bolten, K. A. Bjorndal, & H. R. Martins, 2009. Foraging ecology of oceanic-stage loggerhead turtles *Caretta caretta*. Endangered Species Research 9: 91–97.

Fuchs, B., W. Wang, S. Graspeuntner, Y. Li, S. Insua, E. M. Herbst, P. Dirksen, A. M. Böhm, G. Hemmrich, F. Sommer, T. Domazet-Lošo, U. C. Klostermeier, F. Anton-Erxleben, P. Rosenstiel, T. C. G. Bosch, & K. Khalturin, 2014. Regulation of polyp-to-jellyfish transition in *Aurelia aurita*. Current Biology 24: 263–273.

Gabaldón, T., 2018. Relative timing of mitochondrial endosymbiosis and the "pre-mitochondrial symbioses" hypothesis. IUBMB Life 70: 1188–1196.

Gilchrist, F. G., 1937. Budding and Locomotion in the Scyphistomas of *Aurelia*. The Biological Bulletin 72: 99–124.

Gnaiger, E., N. Aasander Frostner, E. Abdul Karim, N. A. Abumrad, D. Acuna-Castroviejo, R. C. Adiele, B. Ahn, S. S. Ali, L. Alton, M. G. Alves, F. Amati, N. D. Amoedo, I. Andreadou, M. Arago, C. Aral, O. Arandarcikaite, E. Al., & L. Zvejniece, 2019. Mitochondrial respiratory states and rate. .

Gómez, M., S. Torres, & S. Hernzández-León, 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. South African Journal of Marine Science 17: 15–20.

Graham, J. B., 1988. Ecological and evolutionary aspects of integumentary respiration: Body size, diffusion, and the invertebrata. Integrative and Comparative Biology 28: 1031–1045.

Gray, M. W., 2017. Lynn Margulis and the endosymbiont hypothesis: 50 years later. Molecular Biology of the Cell 28: 1285–1287.

Griffin, D. C., C. Harrod, J. D. R. Houghton, & I. Capellini, 2019. Unravelling the macro-evolutionary ecology of fish-jellyfish associations: life in the "gingerbread house." Proceedings. Biological sciences 286: 1–9.

Haddock, S. H. D., 2004. A golden age of gelata: past and future research on planktonic ctenophores and

cnidarians. Hydrobiologia 530: 549−556.

Hamilton, G., 2016. The secret lives of JELLYFISH. Nature 531: 432–434.

Hamner, W. M., & R. M. Jenssen, 1974. Growth , Degrowth , and Irreversible Cell Differentiation in *Aurelia aurita*. American Zoologist 14: 833–849.

Hays, G. C., T. K. Doyle, & J. D. R. Houghton, 2018. A Paradigm Shift in the Trophic Importance of Jellyfish?. Trends in Ecology and Evolution 33: 874–884.

He, J., L. Zheng, W. Zhang, & Y. Lin, 2015. Life cycle reversal in *Aurelia sp.1* (Cnidaria, Scyphozoa). PLoS ONE 10: 1–14.

Heaslip, S. G., S. J. Iverson, W. D. Bowen, & M. C. James, 2012. Jellyfish support high energy intake of leatherback sea turtles (dermochelys coriacea): Video evidence from animal-borne cameras. PLoS ONE 7: 1–7.

Hedges, S. B., J. E. Blair, M. L. Venturi, & J. L. Shoe, 2004. Size and complexity among multicellular organisms. BMC Evolutionary Biology 4: 1–9.

Hernández-León, S., & T. Ikeda, 2005. Zooplankton respiration. Respiration in Aquatic Systems. Oxford University Press, New York 57–82.

Herrera-Ulibarri, A., 2013. Identification, Abundance and Rearing of Mysids of Gran Canaria: aplication to laboratory and oceanographic respiratory metabolism studies. .

Hill, R. W., G. A. Wyse, & M. Anderson, 2016. Fisiologia Animal-2. Artmed Editora.

Holland, H. D., 2006. The oxygenation of the atmosphere and oceans. Philosophical Transactions of the Royal Society B: Biological Sciences 361: 903–915.

Holst, S., & G. Jarms, 2007. Substrate choice and settlement preferences of planula larvae of five Scyphozoa (Cnidaria) from German Bight, North Sea. Marine Biology 151: 863–871.

Hubot, N., C. H. Lucas, & S. Piraino, 2017. Environmental control of asexual reproduction and somatic growth of *Aurelia spp.* (Cnidaria, Scyphozoa) polyps from the Adriatic Sea. PLoS ONE 12: 1–16.

Iguchi, N., H. Iwatani, K. Sugimoto, S. Kitajima, N. Honda, & O. Katoh, 2017. Biomass, body elemental composition, and carbon requirement of *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) in the southwestern Japan Sea. Plankton and Benthos Research 12: 104–114.

Ikeda, T., J. J. Torres, S. Hernández-León, & S. P. Geiger, 2000. Metabolism. In 'ICES Zooplankton Methodology Manual'.(Eds ME Harris, RP Wiebe, PH Lenz, J. Skjoldal and HR Huntley.) pp. 455–532. Academic Press: London.

Iosilevskii, G., & D. Weihs, 2009. Hydrodynamics of sailing of the Portuguese man-of-war *Physalia physalis*. Journal of the Royal Society Interface 6: 613–626.

Jenkins, R. L., 1983. Observations on the Commensal Relationship of *Nomeus gronovii* with *Physalia physalis* . American Society of Ichthyologists and Herpetologists 1983: 250–252.

Jones, E. C., 1968. *Lepas anserifera* Linné (Cirripedia Lapadomorpha) feeding on fish and *Physalia*. Crustaceana 14: 312–313.

Karl, D. M., 2014. Solar energy capture and transformation in the sea. Elementa: Science of the Anthropocene 2: 1–6.

Kenner, R. A., & S. I. Ahmed, 1975. Correlation between oxygen utilization and electron transport activity in marine phytoplankton. Marine biology Springer 33: 129–133.

Kim, J. Y., S. M. Lee, & J. H. Lee, 2012. Biogas production from moon jellyfish (*Aurelia aurita*) using of the anaerobic digestion. Journal of Industrial and Engineering Chemistry The Korean Society of Industrial and Engineering Chemistry 18: 2147–2150, http://dx.doi.org/10.1016/j.jiec.2012.06.010.

Knoll, A. H., 1991. End of the Proterozoic Eon. Scientific American 265: 64–73.

Knoll, A. H., 2003. The geological consequences of evolution. Geobiology 1: 3–14.

Knoll, A. H., K. D. Bergmann, & J. V. Strauss, 2016. Life: The first two billion years. Philosophical Transactions of the Royal Society B: Biological Sciences 371: 1–13.

Lamb, P. D., E. Hunter, J. K. Pinnegar, S. Creer, R. G. Davies, & M. I. Taylor, 2017. Jellyfish on the menu: MtDNA assay reveals scyphozoan predation in the Irish sea. Royal Society Open Science 4:.

Lane, C. E., 1960. The Portuguese Man -of-War. Scientific American 202: 158–171.

Lane, N., 2015. The vital question: energy, evolution, and the origins of complex life. WW Norton & Company.

Lane, N., 2017. Serial endosymbiosis or singular event at the origin of eukaryotes?. Journal of Theoretical Biology Elsevier Ltd 434: 58–67.

Lane, N., & W. F. Martin, 2015. Eukaryotes really are special, and mitochondria are why. Proceedings of the National Academy of Sciences of the United States of America 112: E4823.

Larson, R. J., 1987. Respiration and Carbon Turnover Rates of Medusae from the NE Pacific. Comparative Biochemistry and Physiology Part A: Physiology 87: 93–100, https://www.sciencedirect.com/science/article/pii/0300962987904300.

Lebrato, M., M. Pahlow, J. R. Frost, M. Küter, P. de Jesus Mendes, J. C. Molinero, & A. Oschlies, 2019. Sinking of Gelatinous Zooplankton Biomass Increases Deep Carbon Transfer Efficiency Globally. Global Biogeochemical Cycles 33: 1764–1783.

Lilley, M. K. S., & F. Lombard, 2015. Respiration of fragile planktonic zooplankton: Extending the possibilities with a single method. Journal of Experimental Marine Biology and Ecology 471: 226–231.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, & R. J. Randall, 1951. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry 193: 265–275.

Lucas, C. H., 2001. Reproduction and life history strategies of the common jellyfish, Aurelia aurita, in relation to its ambient environment. Hydrobiologia 451: 229–246.

Lucas, C. H., W. M. Graham, & C. Widmer, 2012. Jellyfish Life Histories: Role of Polyps in Forming and Maintaining Scyphomedusa Populations Advances in Marine Biology. Elsevier Ltd.: 133–196, http://dx.doi.org/10.1016/B978-0-12-394282-1.00003-X.

Lynam, C. P., M. J. Gibbons, B. E. Axelsen, C. A. J. Sparks, J. Coetzee, B. G. Heywood, & A. S. Brierley, 2007. Jellyfish overtake fish in a heavily fished ecosystem. Current Biology 16: R492.

Macali, A., & E. Bergami, 2020. Jellyfish as innovative bioindicator for plastic pollution. Ecological Indicators 115: 1–8.

Mackie, G. O., 1974. Locomotion, flotation, and dispersal Coelenterate Biology: Reviews and New Perspectives. Academic Press New York: 313–357.

Mackie, G. O., & D. A. Boag, 1963. Fishing, feeding and digestion in siphonophores. Pubbl statz zool Napoli 33: 178–196.

Mackie, G. O., P. R. Pugh, & J. E. Purcell, 1988. Siphonophore Biology. Advances in Marine Biology 24: 97–262.

Maldonado, F., T. T. Packard, & M. Gómez, 2012. Understanding tetrazolium reduction and the importance of substrates in measuring respiratory electron transport activity. Journal of Experimental Marine Biology and Ecology 434–435: 110–118.

Mansueti, R., 1963. Symbiotic behavior between small fishes and jellyfishes, with new data on that between the stromateid, *Peprilus alepidotus*, and the scyphomedusa, *Chrysaora quinquecirrha*. Copeia JSTOR 40–80.

Margulis, L., & C. Sagan, 1995. Microcosmo. , 1–256.

Mariottini, G. L., E. Giacco, & L. Pane, 2008. The mauve stinger *Pelagia noctiluca* (Forsskål, 1775). Distribution, ecology, toxicity and epidemiology of stings. A review. Marine Drugs 6: 496–513.

Marques, R., C. Bouvier, A. M. Darnaude, J. C. Molinero, C. Przybyla, S. Soriano, J. A. Tomasini, & D. Bonnet, 2016. Jellyfish as an alternative source of food for opportunistic fishes. Journal of Experimental Marine Biology and Ecology Elsevier B.V. 485: 1–7, http://dx.doi.org/10.1016/j.jembe.2016.08.008.

Martijn, J., J. Vosseberg, L. Guy, P. Offre, & T. J. G. Ettema, 2018. Deep mitochondrial origin outside the sampled alphaproteobacteria. Nature 557: 101–105.

Mauchline, J., 1980. The biology of mysids and euphausiids. Advances in Marine Biology 18: 1–680.

Mayzaud, P., M. Boutoute, S. Gasparini, L. Mousseau, & D. Lefevre, 2005. Respiration in marine zooplankton - The other side of the coin: CO_2 production. Limnology and Oceanography 50: 291–298.

Milisenda, G., S. Rosa, V. L. Fuentes, F. Boero, L. Guglielmo, J. E. Purcell, & S. Piraino, 2014. Jellyfish as prey: Frequency of predation and selective foraging of *Boops boops* (vertebrata, actinopterygii) on the mauve stinger *Pelagia noctiluca* (cnidaria, scyphozoa). PLoS ONE 9: 1–10.

Milisenda, G., S. Rossi, S. Vizzini, V. L. Fuentes, J. E. Purcell, U. Tilves, & S. Piraino, 2018. Seasonal variability of diet and trophic level of the gelatinous predator *Pelagia noctiluca* (Scyphozoa). Scientific Reports 8: 12140.

Mills, D. B., & D. E. Canfield, 2014. Oxygen and animal evolution: Did a rise of atmospheric oxygen "trigger" the origin of animals? BioEssays 36: 1145–1155.

Mitchell, P., 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 189: 291–292.

Munro, C., Z. Vue, R. R. Behringer, & C. W. Dunn, 2019. Morphology and development of the Portuguese man of war, *Physalia physalis*. Scientific Reports Springer US 9: 1–12, http://dx.doi.org/10.1038/s41598-019-51842-1.

Nachlas, M. M., I. Margulies, & A. M. Seligman, 1960. A Calorimetric Succinic Method for Dehydrogenase the Estimation Activity *. The Journal of Biological Chemistry 235: 499–503.

Nelson, D. L., A. L. Lehninger, & M. M. Cox, 2008. Lehninger principles of biochemistry. Macmillan.

Nutman, A. P., V. C. Bennett, C. R. L. Friend, M. J. Van Kranendonk, & A. R. Chivas, 2016. Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures. Nature Nature Publishing Group 537: 535–538, http://dx.doi.org/10.1038/nature19355.

Ohta, N., M. Sato, K. Ushida, M. Kokubo, T. Baba, K. Taniguchi, M. Urai, K. Kihira, & J. Mochida, 2009. Jellyfish mucin may have potential disease-modifying effects on osteoarthritis. BMC Biotechnology 9: 1–11.

Osma, N., I. Fernández-Urruzola, M. Gómez, S. Montesdeoca-Esponda, & T. T. Packard, 2016. Predicting in vivo oxygen consumption rate from ETS activity and bisubstrate enzyme kinetics in cultured marine zooplankton. Marine Biology 163: 146.

Owens, T. G., & F. D. King, 1975. The measurement of respiratory electron-transport-system activity in marine zooplankton. Marine Biology 30: 27–36.

Packard, T. T., 1971. The Measurement of Respiratory Electron-transport Activity in Marine Phytoplankton. Journal of Marine Research 29: 235–244.

Packard, T. T., 1985. Measurement of electron transport activity of marine microplankton . In Williams, P. J., & H. W. Jannasch (eds), Advances in Aquatic Microbiology. Academic Press, New York: 207–261.

Packard, T. T., & J. P. Christensen, 2004. Respiration and vertical carbon flux in the Gulf of Maine water column. Journal of Marine Research 62: 93–115.

Packard, T. T., & M. Gómez, 2008. Exploring a first-principles-based model for zooplankton respiration. ICES Journal of Marine Science Oxford University Press 65: 371–378.

Park, E., D. S. Hwang, J. S. Lee, J. I. Song, T. K. Seo, & Y. J. Won, 2012. Estimation of divergence times in cnidarian evolution based on mitochondrial protein-coding genes and the fossil record. Molecular Phylogenetics and Evolution Elsevier Inc. 62: 329–345, http://dx.doi.org/10.1016/j.ympev.2011.10.008.

Parsons, T. R., 1979. Some ecological, experimental and evolutionary aspects of the upwelling ecosystem. South African Journal of Science. 75: 536–540.

Parsons, T. R., & C. M. Lalli, 2002. Jellyfish population explosions : Revisiting a hypothesis of possible causes. Mer. , 111–121.

Pauly, D., W. Graham, S. Libralato, L. Morissette, & M. L. Deng Palomares, 2009. Jellyfish in ecosystems, online databases, and ecosystem models. Hydrobiologia 616: 67–85.

Phillips, N., L. Eagling, C. Harrod, N. Reid, V. Cappanera, & J. Houghton, 2017. Quacks snack on smacks: Mallard ducks (*Anas platyrhynchos*) observed feeding on hydrozoans (*Velella velella*). Plankton and Benthos Research 12: 143–144.

Pierce, J., 2006. Aquarium design for the Portuguese man-of-war *Physalia physalis*. International Zoo Yearbook 40: 221–231.

Pires, R. F. T., N. Cordeiro, J. Dubert, A. Marraccini, P. Relvas, & A. Dos Santos, 2018. Untangling *Velella velella* (Cnidaria: Anthoathecatae) transport: A citizen science and oceanographic approach. Marine Ecology Progress Series 591: 241–251.

Pitt, K. A., C. H. Lucas, R. H. Condon, C. M. Duarte, & B. Stewart-Koster, 2018. Claims That Anthropogenic Stressors Facilitate Jellyfish Blooms Have Been Amplified Beyond the Available Evidence: A Systematic Review. Frontiers in Marine Science 5: 1–11.

Pope, E. C., G. C. Hays, T. M. Thys, T. K. Doyle, D. W. Sims, N. Queiroz, V. J. Hobson, L. Kubicek, & J. D. R. Houghton, 2010. The biology and ecology of the ocean sunfish *Mola mola*: A review of current knowledge and future research perspectives. Reviews in Fish Biology and Fisheries 20: 471–487.

Prieto, L., D. MacÍas, A. Peliz, & J. Ruiz, 2015. Portuguese Man-of-War (*Physalia physalis*) in the Mediterranean: A permanent invasion or a casual appearance? Scientific Reports Nature Publishing Group 5: 1–7.

Purcell, J. E., 1984. Predation on fish larvae by *Physalia physalis*, the Portuguese man of. Marine Ecology Progress Series 19: 189–191.

Purcell, J. E., 2009. Extension of methods for jellyfish and ctenophore trophic ecology to large-scale research. Hydrobiologia 616: 23–50.

Purcell, J. E., & M. N. Arai, 2001. Interactions of pelagic cnidarians and ctenophores with fish: a review. Hydrobiologia 451: 27–44.

Purcell, J. E., E. Clarkin, & T. K. Doyle, 2012. Foods of *Velella velella* (Cnidaria: Hydrozoa) in algal rafts and its distribution in Irish seas. Hydrobiologia 690: 47–55.

Purcell, J. E., V. Fuentes, D. Atienza, U. Tilves, D. Astorga, M. Kawahara, & G. C. Hays, 2010. Use of respiration rates of scyphozoan jellyfish to estimate their effects on the food web. Hydrobiologia 645: 135–152.

Purcell, J. E., A. Malej, & A. Benović, 1999. Potential Links of Jellyfish to Eutrophication and. Coastal and estuarine studies Wiley Online Library 55: 241–263.

Purcell, J. E., S. I. Uye, & W. T. Lo, 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: A review. Marine Ecology Progress Series 350: 153–174.

Ramondenc, S., M. Ferrieux, S. Collet, F. Benedetti, L. Guidi, & F. Lombard, 2019. From egg to maturity: A closed system for complete life cycle studies of the holopelagic jellyfish *Pelagia noctiluca*. Journal of Plankton Research 41: 207–217.

Riascos, J. M., M. Vergara, J. Fajardo, V. Villegas, & A. S. Pacheco, 2012. The role of hyperiid parasites as a trophic link between jellyfish and fishes. Journal of Fish Biology 81: 1686–1695.

Richardson, A. J., A. Bakun, G. C. Hays, & M. J. Gibbons, 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends in Ecology and Evolution 24: 312–322.

Rodríguez, G., G. Clarindo, & L. McKnight, 2015. Jellyfish outbreaks in coastal city beaches from a management perspective. Coastal Cities and their Sustainable Future 1: 277–288.

Roger, A. J., S. A. Muñoz-Gómez, & R. Kamikawa, 2017. The Origin and Diversification of Mitochondria. Current Biology 27: R1177–R1192.

Rokas, A., 2008. The origins of multicellularity and the early history of the genetic toolkit for animal development. Annual Review of Genetics 42: 235–251.

Rorke, O., S. D. Lavery, M. Wang, A. M. Waite, L. E. Beckley, P. A. Thompson, & A. G. Jeffs, 2015.

Hitching Rides and Stealing Bites. ICES Journal of Marine Science 72: 124–127.

Ruiz-Trillo, I., G. Burger, P. W. H. Holland, N. King, B. F. Lang, A. J. Roger, & M. W. Gray, 2007. The origins of multicellularity: a multi-taxon genome initiative. Trends in Genetics 23: 113–118.

Sabatés, A., F. Pagès, D. Atienza, V. Fuentes, J. E. Purcell, & J. M. Gili, 2010. Planktonic cnidarian distribution and feeding of *Pelagia noctiluca* in the NW Mediterranean Sea. Hydrobiologia 645: 153–165.

Sagan, L., 1967. On the origin of mitosing cells. Journal of Theoretical Biology 14: 225–274.

Sánchez-Baracaldo, P., 2015. Origin of marine planktonic cyanobacteria. Scientific Reports Nature Publishing Group 5: 14–17.

Sandrini, L. R., M. Avian, & C. Anatomy, 1983. Biological cycle of *Pelagia noctiluca*: morphological aspects of the development. Comparative and General Pharmacology 174: 169–174.

Schneider, G., 1989. Estimation of food demands of *Aurelia aurita* medusae populations in the kiel bight/western baltic. Ophelia 31: 17–27.

Schneider, G., & G. Behrends, 1998. Top-down control in a neritic plankton system by *Aurelia aurita* medusae — a summary. Ophelia 48: 71–82.

Schuchert, P., 2010. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Capitata Part 2. Revue Suisse de Zoologie 117: 337–555.

Sommer, U., H. Stibor, A. Katechakis, F. Sommer, & T. Hansen, 2002. Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production:primary production. Hydrobiologia 484: 11–20.

Sperling, E. A., C. A. Frieder, A. V. Raman, P. R. Girguis, L. A. Levin, & A. H. Knoll, 2013. Oxygen, ecology, and the Cambrian radiation of animals. Proceedings of the National Academy of Sciences of the United States of America 110: 13446–13451.

Steinberger, L. R., T. Gulakhmedova, Z. Barkay, M. Gozin, & S. Richter, 2019. Jellyfish-Based Plastic. Advanced Sustainable Systems 3: 1–6.

Stenvers, V., X. Chi, & J. Javidpour, 2020. Seasonal variability of the fatty acid composition in *Aurelia aurita* (Cnidaria: Scyphozoa): implications for gelativore food web studies. Journal Of Plankton Research 00: 1–13.

Sukhoputova, A. V., & Y. A. Kraus, 2017. Environmental factors inducing the transformation of polyp into medusae in *Aurelia aurita* (Scyphozoa). Russian Journal of Developmental Biology 48: 106–116.

Sullivan, B. K., C. L. Suchman, & J. H. Costello, 1997. Mechanics of prey selection by ephyrae of the scyphomedusa *Aurelia aurita*. Marine Biology 130: 213–222.

Takao, M., H. Okawachi, S. I. Uye, & H. Okawachi, 2014. Natural predators of polyps of *Aurelia aurita* s.l. (Cnidaria: Scyphozoa: Semaeostomeae) and their predation rates. Plankton and Benthos Research 9: 105–113.

Thiebot, J. B., J. P. Y. Arnould, A. Gómez-Laich, K. Ito, A. Kato, T. Mattern, H. Mitamura, T. Noda, T. Poupart, F. Quintana, T. Raclot, Y. Ropert-Coudert, J. E. Sala, P. J. Seddon, G. J. Sutton, K. Yoda, & A. Takahashi, 2017. Jellyfish and other gelata as food for four penguin species – insights from predator-borne videos. Frontiers in Ecology and the Environment 15: 437–441.

Thiebot, J. B., & J. C. McInnes, 2020. Why do marine endotherms eat gelatinous prey?. ICES Journal of Marine Science 77: 58–71.

Thuesen, E. V., L. D. Rutherford, P. L. Brommer, K. Garrison, M. A. Gutowska, & T. Towanda, 2005. Intragel oxygen promotes hypoxia tolerance of scyphomedusae. Journal of Experimental Biology 208: 2475–2482.

Titelman, J., & L. J. Hansson, 2006. Feeding rates of the jellyfish *Aurelia aurita* on fish larvae. Marine Biology 149: 297–306.

Uye, S., & H. Shimauchi, 2005. Population biomass, feeding, respiration and growth rates, and carbon budget of the scyphomedusa *Aurelia aurita* in the Inland Sea of Japan. Journal of Plankton Research 27: 237–248.

Vagelli, A., 2007. New observations on the asexual reproduction of *Aurelia aurita* (Cnidaria , Scyphozoa) with comments on its life cycle and adaptive significance. Invertebrate Zoology 4: 111–127.

Verde, E. A., & L. R. McCloskey, 1998. Production, respiration, and photophysiology of the mangrove jellyfish *Cassiopea xamachana* symbiotic with zooxanthellae: Effect of jellyfish size and season. Marine Ecology Progress Series 168: 147–162.

Verdes, A., & M. Holford, 2018. Beach to Bench to Bedside: Marine Invertebrate Biochemical Adaptations and Their Applications in Biotechnology and Biomedicine. Results and Problems in Cell Differentiation 65: 359–376.

Verity, P. G., & V. Smetacek, 1996. Organism life cycles, predation, and the structure of marine pelagic ecosystems. Marine Ecology Progress Series 130: 277–293.

Vernon, H. M., 1895. The Respiratory Exchange of the Lower Marine Invertebrates. The Journal of Physiology 19: 18–70.

Vosjan, J. H., 1982. Respiratory Electron Transport System Activities in Marine Environments. Hydrobiological Bulletin 16: 61–68.

Wenner, A., 1977. Food Supply, Feeding Habits, and Egg Production in Pacific Mole Crabs (*Hippa pacifica* Dana). 31: 39–47.

Williams, T. A., P. G. Foster, C. J. Cox, & T. M. Embley, 2013. An archaeal origin of eukaryotes

supports only two primary domains of life. Nature Nature Publishing Group 504: 231–236, http://dx.doi.org/10.1038/nature12779.

Wilson, E. O., 1975. Sociobiology: The new synthesis. Harvard university Press.

Winkler, L. W., 1888. Die Bestimmung des im Wasser gelösten Sauerstoffes, Ber. Dtsch. Chem. Ges., 21, 2843–2854. .

Yahalomi, D., S. D. Atkinson, M. Neuhof, E. Sally Chang, H. Philippe, P. Cartwright, J. L. Bartholomew, & D. Huchon, 2020. A cnidarian parasite of salmon (Myxozoa: *Henneguya*) lacks a mitochondrial genome. Proceedings of the National Academy of Sciences of the United States of America 117: 5358–5363.

Yonge, C. M., 1937. Evolution and Adaptation in the Digestive System of the Metazoa. Biological Reviews 12: 87–114.

Young, G. A., & J. W. Hagadorn, 2010. The fossil record of cnidarian medusae. Palaeoworld 19: 212–221, http://dx.doi.org/10.1016/j.palwor.2010.09.014.

Zamponi, M., 2011. Dispersión de Cnidaria: comunidad marina pleuston y comunidad marina bentos. Revista Real Academia Galega de Ciencias 30: 91–112.

Zeman, S. M., M. Corrales-Ugalde, R. D. Brodeur, & K. R. Sutherland, 2018. Trophic ecology of the neustonic cnidarian *Velella velella* in the northern California Current during an extensive bloom year: insights from gut contents and stable isotope analysis. Marine Biology Springer Berlin Heidelberg 165: 1–13, https://doi.org/10.1007/s00227-018-3404-1.

Zimmer, M., 2009. GFP: from jellyfish to the Nobel prize and beyond. Chemical 38: 2823–2832.

Zou, D., K. Gao, J. Xia, Z. Xu, X. Zhang, & S. Liu, 2007. Responses of dark respiration in the light to desiccation and temperature in the intertidal macroalga, *Ulva lactuca* (Chorophyta) during emersion. Phycologia 46: 363–370.

CHAPTER 8

CONCLUSIONES GENERALES (en castellano)

Capítulo 2

1. Las técnicas de medida de consumo de oxígeno estudiadas en este trabajo han dado resultados comparables durante las incubaciones de organismos heterótrofos y autótrofos. Esto valida la aplicación de estas técnicas para la medida de tasas respiratorias similares. Es recomendable la realización de más comparaciones de este tipo para establecer de manera precisa el nivel de similitud entre dichas técnicas.

2. El estudio respiratorio de *Leptomysis lingvura* y *Ulva rigida* ha dado como resultado ratios R:Φ muy dispares; siendo de media 0.81 para el crustáceo y 0.17 para el alga verde. Estos resultados invitan a profundizar en el posible impacto de las enzimas del sistema de transporte fotosintético sobre los estudios del ETS mitocondrial.

Capítulo 3

3. Las condiciones óptimas para el análisis del ETS en medusas obtenidas de las pruebas en A. aurita son: utilizar una solución de fosfato tamponada de pH 8.5 y emplear sustratos en concentraciones 1mM en cubeta para NADH y NADPH. Se recomienda determinar el coeficiente de extinción de la solución de INT en lugar de usar valores de la literatura.

4. La energía de activación para la reacción enzimática del ETS deriva de las pruebas a diferentes temperaturas es 38.83 kJ mol^{-1}.

Capítulo 4

5. Los análisis del ETS constituyen una alternativa viable y útil para el estudio de la respiración en medusas. *Aurelia aurita* y *Pelagia noctiluca* respectivamente

presentaron una demanda de carbono media asociada a la respiración (*RCD*) de 0.53 y 0.58 mg C d^{-1} g masa húmeda^{-1}. Gracias a estos parámetros y basándonos en los estudios de poblaciones planctónicas determinadas, se puede estimar que una proliferación de *Pelagia noctiluca* de 20 g peso húmedo por m^3 podría ser devastador para la vida planctónica de las aguas de canarias.

6. Derivado de los estudios respiratorios, se pudo determinar que la transformación de energía asociada con el sistema de transporte de electrones respiratorio de las mitocondrias (*HET*) normalizado por masa húmeda de *A. aurita* y *P. noctiluca* es casi 1000 veces superior a la producción energética del sol por unidad de masa.

7. Si los rangos de *RCD* para *A. aurita* y *P. noctiluca* son extrapolados a nivel global, basándonos en estimaciones de las poblaciones de medusas globales, obtendríamos que las medusas oxidan casi un tercio del carbono oxidado por el mesozooplancton en los primeros 200 m de profundidad y entre 3.8 y 5.3% del carbono fijado por la producción primaria.

8. Al hacer la misma extrapolación a nivel global de los rangos de *HET* para *A. aurita* y *P. noctiluca* basándonos en las estimaciones de la población de medusas globales obtendríamos que el *HET* de las medusas es equivalente a entre 2 y 3% de la energía captada por los productores primarios.

Capítulo 5

9. En este trabajo se presentan algunos de los primeros valores de respiración y composición bioquímica de los integrantes del pleuston *Velella velella* y *Physalia physalis*. La incubación de estas dos especies flotantes tiene una serie de complicaciones que están ausentes cuando se emplean técnicas de medida enzimáticas.

10. El porcentaje de peso seco respecto al peso húmedo en estos integrantes del pleuston gelatinoso es mayor que en otros miembros del plancton gelatinoso llegando a ser de media 9% y 15% en *V. velella* y *P. physalis,* respectivamente. Seguramente debido a la presencia de estructuras quitinosas. Esto tiene importantes implicaciones en su contenido energético por peso húmedo comparado con otras medusas.

11. El contenido de proteínas, lípidos y carbohidratos es igual o superior a las concentraciones documentadas para otros organismos gelatinosos con la excepción del contenido en lípidos de algunas especies árticas. Esto apoya nuestra hipótesis de que *V. velella* y *P. physalis* deben tener un contenido nutricional similar a otras especies de plancton gelatinoso para explicar su consumo por varios depredadores.

12. La demanda de carbono asociada a la respiración de *P. physalis* y *V. velella* fue 0.72±0.38 mg C d^{-1} (g WM)$^{-1}$ y 0.69±0.33 mg C d^{-1} (g WM)$^{-1}$ respectivamente 10 veces inferior a la demanda potencial determinada por las medidas enzimáticas.

13. En el estudio aquí presente sobre *V. velella* y *P. physalis* se considera que la baja respiración normalizada por proteínas, el bajo ratio R:Φ (~0.1) resultante y la

baja respiración comparada con las cifras de ingestión de la bibliografía, apoyan la hipótesis de que estos organismos propulsados por el viento tienen una actividad respiratoria menor que aquellos que pulsan para desplazarse. Sin embargo, estos resultados no son concluyentes del todo al no explicar de forma adecuada el origen de una Φ tan elevada.

Capítulo 6

14. Se ha evidenciado los cambios que tienen lugar durante el desarrollo de *Aurelia aurita* de éfira a medusa a nivel morfológico, bioquímico y metabólico; estableciendo que el momento de transición de éfira a juvenil de medusa transcurre entre la segunda y tercera semana desde la liberación de la éfira de la estróbila.

15. Se calculó la tasa de crecimiento durante dicha transición de manera alternativa a estudios previos basado en una relación de la masa potencial en lugar de exponencial con el tiempo, observándose que el crecimiento es mucho mayor durante el periodo de éfira. Siendo especialmente elevado durante los primeros días de vida.

16. Las éfiras durante los primeros días de vida han presentado un mayor contenido en lípidos que en proteínas. Hemos considerado que estos sirven de fuente de alimento y flotabilidad mientras el perímetro de la campana desarrolla una continuidad completa. A medida que se acerca al estado juvenil el contenido de lípidos y proteína normalizado por peso húmedo disminuye a medida que aumenta el contenido en agua y finalmente el porcentaje de contenido en proteína termina siendo superior al de lípidos durante el momento de transición a juvenil.

17. La combinación de encoger en tamaño y disminuir el metabolismo respiratorio, permitieron la supervivencia de juveniles de *Aurelia aurita* hasta un máximo de 58 días en inanición.

ANEXOS

ANEXO I.

Symbols and Abbreviations

A	"Frequency factor"
AE	Assimilation efficiency
ATP	Adenosine triphosphate
C	carbon
CV	Container volume
DCC	Dry to carbon mass conversion
DM	Dry biomass
DOC	Dissolved organic carbon
E	Electrodes
Ea	Activation Energy
Ec	Energy content
ETS	Electron transport system
FA	Fatty acids
HET	Heterotrophic energy transformation
INT	2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride
K	Carbohydrate
K_M	Michaelis half-saturation constant
L	Lipid
MCR	Minimum weight-specific-carbon-requirements
NAD^+	Nicotinamide adenine dinucleotide oxidized
NADH	Nicotinamide adenine dinucleotide reduced
$NADP^+$	Nicotinamide adenine dinucleotide phosphate oxidized
NADPH	Nicotinamide adenine dinucleotide phosphate reduced
O	Optode
P	Protein
R	Aerobic respiration
R	C:CO_2 conversion
RCD	Respiratory carbon demand
Rg	universal gas constant
RQ	unitless ratio of CO_2 to O_2 during R
S	substrate
v	reaction rate
v_{max}	maximum reaction rate
W	Winkler
WDC	wet to dry mass conversion
WM	Wet biomass
Φ	Potential respiration
μ	specific growth rate from exponential growth
μ'	specific growth rate from potential growth

ANEXO II.

Figure List

3.2 Effect of pH on the rate of ETS enzyme activity in *A. aurita*. Results standardized by the maximum activity. n=3 **55**

3.3 Relationship between temperature and ETS enzyme activity in *A. aurita*. **A:** Effect of temperature on the reaction **B:** Linear regression based on the Arrhenius equation (Eqn 1.3) for the calculation of the activation energy Ea using the slope Ea= -slope - Rg =-(-4.67) - (8.314) = 38.68 kJ. **C:** Use of Ea determined in B and the Arrhenius equation to correct activities at the same temperature (20ºC) using Eqn 1.4. n=3 and the error bars are standard deviation. **56**

$$ETS_T = ETS_{T0} \cdot e^{\frac{Ea\left(\frac{1}{T0} - \frac{1}{T}\right)}{Rg}} \quad (1.4)$$

Where ETS_T is the corrected ETS activity at the desired temperature, ETS_{T0} is the original ETS activity, Ea is the activation energy in joules, T is the temperature to which you want to convert, T0 is the original temperature and Rg is the constant $8.314 \cdot 10^{-3}$ kJ mol^{-1} K^{-1}.

3.4 Relationship between activity and sample concentration of *A. aurita* **A:** enzyme activity at different enzyme concentration. 100% is equivalent to a concentration of 1 individual of approximately 350mg per 1 ml of buffer. **B:** ETS activity of the dilutions against their protein content. **56**

4.1 Physiological respiration (R) in *Aurelia aurita* (n=24 p<0.001). Regression analysis: (**A**) with wet mass (WM); (**B**) with potential respiration (Φ). Both plots' axes follow logarithmic scales. The power functions and coefficient of determinations (r2) are shown. The corresponding linear functions were (A) R = 8.93 WM + 16.64, r^2 = 0.89; (B) R = 0.98 Φ - 1.18, r^2 = 0.91. **65**

4.2 Physiological respiration (R) in *Pelagia noctiluca* (n=25 p<0.001). Regression analysis: (a) with wet mass (WM); (b) with potential respiration (Φ). Both plots' axes follow logarithmic scales. The power functions and coefficient of determinations (r2) are shown. The corresponding linear functions were (A) R = 20.47 WM - 12.93, r^2 = 0.92; (B) R = 0.29 Φ + 26.87, r^2 = 0.92. **66**

4.3 Physiological respiration (R) in both scyphozoans (n=49 p<0.001). Regression analysis: (a) with wet mass (WM); (b) with potential respiration (Φ). Both plots' axes follow logarithmic scales. The power functions and coefficient of determinations (r2) are shown. The corresponding linear functions were (A) R = 17.93WM + 1.18, r^2 = 0.86; (B) R = 0.29 Φ + 31.40, r^2 = 0.91. **67**

4.4 Protein (black circles) and lipid (gray squares) composition of *Aurelia aurita* (**A**) and *Pelagia noctiluca* (**B**) in the different sized samples. **68**

5.1 Examples of the physiological respiration incubations of the hydrozoans in this study. Optodes spots installed inside. Left: Discarded half-filled incubation for *P. physalis*. Right: Completely full incubations of *P. physalis* and *V. velella* in 600 mL Jars or 60 mL BOD bottles depending on the size of the organism. **87**

5.2 Compilation of gelatinous zooplankton biochemical composition measurements (mean±SD) from the literature with special attention to hydrozoan results. All values are normalized per dry mass (DM). Colours indicate the following gelatinous groups: Blue (Hydrozoan: Anthoathecata, Siphonophorae, Trachymedusae, Lepthothecata), red (Scyphozoan: Semaestomeae, Coronatae), yellow (Ctenophore), green (Thaliacean). Only articles containing protein (P), lipid (L) and carbohydrate (K) data were included. [1]This study's results, [2]Percy & Fife (1981) (Arctic species), [3]Arai et al. (1989), [4]Clarke et al. (1992), [5]Bailey et al. (1995), [6]Malej et al. (1993), [7]Lucas (1994), [8]Lucas (2009), [9]Dubischar et al. (2006), *Instead of mean±SD, these values represent the range (midpoint ± half the range). **93**

5.3 Compilation of gelatinous zooplankton respiration measurements from the literature with special attention on the hydrozoan respiratory measurements. Note the primary ordinate on the left is for physiological respiration (R) and the secondary ordinate on the right is for potential respiration (Φ). If the source of the data measured both R and Φ the species has a (R) or (Φ) to indicate so, otherwise measurements are R. Colours indicate the following gelatinous groups: Blue (Hydrozoan: Anthoathecata, Siphonophorae, Trachymedusae, Lepthothecata, Limnomedusae), orange (Scyphozoan: Semaestomeae, Coronatae), purple (Anthozoan), yellow (Ctenophore). Top: average respiratory values normalized by mg of protein (P). Bottom: average respiratory values normalized by mg of dry mass (DM). [1]This study's results, [10]Pardy & Dieckmann (1975), [11]Biggs (1977b), 12Purcell et al. (2019), 13Bondyale et al. unpublished, 14Larimer & Ashby (1962), 15Bailey et al. (1995), [16]Barangé et al. (1989), [17]Nival et al. (1972) *Instead of mean±SD, these values represent the range (midpoint ± half the range). **95**

6.1 Compilation of the average and standard deviation of the central disk diameter (D) and wet mass (WM). This results fitted to a power function follows the equation WM=0.49D2.36 with the R2 and the regression parameters. **111**

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7.2 Obtenida de Richardson et al. (2009) a su vez modificada de Parsons 1979. Esquematización de la evolución de los principales representantes de los niveles tróficos marinos. Se muestra la transición de los cnidarios como depredador mayoritario a compartir el nicho trófico con competidores y depredadores más complejos. Pone de manifiesto la supervivencia de las medusas y como la retirada de competidores y depredadores puede crear el escenario idóneo para su retorno al podio. **130**

7.3 Esquema simplificado de la respiración fisiológica (R) y su control a nivel molecular en el sistema de transporte de electrones (ETS). Arriba, medusa y la reacción respiratoria con el consumo de oxígeno correspondiente. Circulo superior, células formando el tejido de la medusa. Círculo inferior, ilustración de una mitocondria. Recuadro inferior, esquema del ETS presente en la membrana mitocondrial interna. Comienza con la producción de CO_2 y NADH en el ciclo de Krebs a partir del acetil-CoA, continúa con la oxidación de NADH y la generación del flujo de electrones y termina con la reducción de O_2 formando H2O. Durante el proceso se observa también el bombeo de protones (H^+) y la generación de ATP. **134**

ANEXO III.

Table List

ANEXO IV.

Contribuciones durante la tesis doctoral

- **PUBLICACIONES**

1. Bondyale-Juez, D. R., T. T. Packard, M. A. Viera-Rodríguez, & M. Gómez, 2017. Respiration: comparison of the Winkler technique, O2 electrodes, O2 optodes and the respiratory electron transport system assay. Marine Biology Springer Berlin Heidelberg 164: 1–11, https://doi.org/10.1007/s00227-017-3271-1.

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METHOD

Respiration: comparison of the Winkler technique, O_2 electrodes, O_2 optodes and the respiratory electron transport system assay

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Abstract
Aerobic respiration is a biological energy generation process that consumes organic carbon and oxygen. In the ocean, the balance between photosynthesis and respiration is recognized as critical to understanding the ocean's impact on the hydrospheric and atmospheric CO_2. Techniques to determine respiration can be based on inorganic chemistry, electrochemistry, photochemistry, and enzymology. Here, for method comparison, physiological respiration was simultaneously measured by the Winkler method (W), O_2 electrodes (E), and O_2 optodes (O). These techniques detected respiratory O_2 consumption (R), in situ, in dark incubation chambers. Respiratory electron transport system activity measurements detected potential respiration (Φ), biochemically. *Leptomysis lingvura*, a marine mysid, and *Ulva rigida*, a species of green algal sea lettuce, were the two organisms tested. Physiological respiration results from each technique were not statistically significantly different (multiple paired Student's t tests, p value > 0.05) and were inside the range of similar published measurements. The mean dry-mass-specific respiration in *L. lingvura* and *U. rigida* was 0.147 ± 0.037 and 0.023 ± 0.008 μmol O_2 h^{-1} (mg dry mass)$^{-1}$, $n = 9$, respectively. The R-to-Φ ratios were different in the two organisms. However, linear regression between R and Φ for *L. lingvura* and *U. rigida* was stronger ($r^2 = 0.814$ and 0.313) than the linear regression between R and dry biomass ($r^2 = 0.643$ and 0.213). The application of Passing–Bablok regression analysis evidenced the high correlation between the results, and the Bland–Altman analysis examined the average difference ("bias") and limits of agreement between the methods.

Introduction

Respiration is measured for many reasons: investigating physiology, quantifying the carbon cycle, determining primary and secondary production, calculating carbon flux, studying the biological pump, calculating CO_2 emission and O_2 depletion rates, and understanding oceanic metabolic balance, amongst others. Here, the respiration measurements by four different techniques were compared while working with two different types of organisms. The respiration methodologies used were the Winkler method (W), O_2 electrodes (E), O_2 optodes (O), and the kinetic assay for the respiratory electron transport system (ETS). The first three methods allow the determination of physiological respiration; the last, the determination of its enzymatic basis. The organisms chosen for these experiments were a coastal pericardian crustacean, the mysid, *Leptomysis lingvura* (G.O. Sars, 1866), and a littoral-zone macroalgae, the chlorophyte, *Ulva rigida* (C. Agardh, 1823).

Aerobic respiration is the focus of this research. It is the catabolic process by which eukaryotes and many prokaryotes obtain vital energy using a series of redox reactions that end with the reduction of O_2 as a final electron acceptor. Respiration has been extensively studied, but in the aquatic sciences, photosynthesis had long been considered more important for understanding oceanic metabolism. This assumption began to be challenged at the turn of the millennium when the relative roles of oceanic anabolism, catabolism, and their complex coupling started to be assessed (Dortch and Packard 1989; Arístegui and Harrison 2002; Williams and Del Giorgio 2005; Duarte et al. 2013; Ducklow and Doney 2013;

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2. Belcher, A., K. Cook, D. Bondyale-Juez, G. Stowasser, S. Fielding, R. A. Saunders, D. J. Mayor, & G. A. Tarling, 2020. Respiration of mesopelagic fish: a comparison of respiratory electron transport system (ETS) measurements and allometrically calculated rates in the Southern Ocean and Benguela Current. ICES Journal of Marine Science 77: 1672–1684.

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Respiration of mesopelagic fish: a comparison of respiratory electron transport system (ETS) measurements and allometrically calculated rates in the Southern Ocean and Benguela Current

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Belcher, A., Cook, K., Bondyale-Juez, D., Stowasser, G., Fielding, S., Saunders, R. A., Mayor, D. J., and Tarling, G. A. Respiration of mesopelagic fish: a comparison of respiratory electron transport system (ETS) measurements and allometrically calculated rates in the Southern Ocean and Benguela Current. – ICES Journal of Marine Science, doi:10.1093/icesjms/fsaa031.

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Mesopelagic fish are an important component of marine ecosystems, and their contribution to marine biogeochemical cycles is becoming increasingly recognized. However, major uncertainties remain in the rates at which they remineralize organic matter. We present respiration rate estimates of mesopelagic fish from two oceanographically contrasting regions: the Scotia Sea and the Benguela Current. Respiration rates were estimated by measuring the enzyme activities of the electron transport system. Regression analysis of respiration with wet mass highlights regional and inter-specific differences. The mean respiration rates of all mesopelagic fish sampled were 593.6 and 354.9 µl O_2 individual^{-1} h^{-1} in the Scotia Sea and Benguela Current, respectively. Global allometric models performed poorly in colder regions compared with our observations, underestimating respiratory flux in the Scotia Sea by 67–88%. This may reflect that most data used to fit such models are derived from temperate and subtropical regions. We recommend caution when applying globally derived allometric models to regional data, particularly in cold (<5°C) temperature environments where empirical data are limited. More mesopelagic fish respiration rate measurements are required, particularly in polar regions, to increase the accuracy with which we can assess their importance in marine biogeochemical cycles.

Keywords: allometric, Benguela Current, ETS, mesopelagic fish, respiration, respiratory flux, Scotia Sea, Southern Ocean.

Introduction

The uptake of carbon dioxide (CO_2) by the ocean through the biological carbon pump plays an important role in the partitioning of CO_2 between the atmosphere and ocean (Kwon *et al.*, 2009). Understanding and quantifying the processes controlling the efficiency of this pump are therefore vital for predictions of future climate. Carbon may be transported by passive sinking (the gravitational pump), physical mixing, or active transport through vertical migration of metazoans (Boyd *et al.*, 2019). Previously, the gravitational pump was thought to be the dominant mechanism for transferring organic carbon to the deep sea. However, the importance of additional mechanisms, in particular the role of the

3. Martínez, I., A. Herrera, M. Tames-espinosa, D. R. Bondyale-juez, V. Romero-kutzner, T. T. Packard, & M. Gómez, 2020. Protein in marine plankton: A comparison of spectrophotometric methods. Journal of Experimental Marine Biology and Ecology Elsevier 526: 151357.

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Protein in marine plankton: A comparison of spectrophotometric methods

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ABSTRACT

Measuring protein is critical to many investigations in oceanography and marine biology. Here, we compared seven colorimetric protein assays (Rutter, Rutter-SDS, Markwell, BCA, microBCA, Bradford and microBradford) for measuring protein in a mysid (*Leptomysis lingvura*), in a jellyfish (*Pelagia noctiluca*), and in three different size fractions of marine plankton (0.7–50, 50–200 and 200–2000 μm). Significant differences occurred in all of these samples. However, the mBCA method was the most accurate for all samples except the mysid, the Rutter method was the least accurate in all organisms, and the Bradford and microBradford methods consistently underestimated protein. The time-dependent behavior of the protein signal was most accurately determined if the analysis was carried out rapidly and consistently. In relation to the limit of detection (LOD), the most sensitive method for low protein levels was the microBCA assay (at 7 μg mL^{-1} protein). The most sensitive method at higher levels of protein was the Bradford method (at 472 μg mL^{-1} protein). The BCA method was the most linear and the microBCA method, the most sensitive method for detecting bovine serum albumin. We recommend the latter two methods for measuring protein under our assay conditions.

1. Introduction

Protein is vital to life. All the biochemical life-defining reactions of energy production, locomotion, reproduction, information transfer and storage, and all other processes are catalyzed by proteins, the enzymes. In addition all the structures, cell-walls, and membranes have protein components. Thus, it is a component of all marine organisms and its measurement, in the ocean, like that of organic carbon, is a defining parameter in studying nitrogen flow through marine ecosystems. However, there are many ways to measure protein and each one has advantages and disadvantages. Furthermore, the diversity of oceanography and marine biology investigations leads to a demand for different types of protein analysis. For example, physiological, biochemical (Moore et al., 2012a, 2012b), genetic, molecular biological, and ecological (Dortch and Packard, 1989) investigations, each may require protein to be measured in a specific manner. Finding an accurate assay to obtain the total protein content has been a challenge for more than 100 years (Kjeldahl, 1888) and yet, given the increase in marine research, it is even more important now. Protein analysis is needed continually in biochemical, physiological, and ecological research, from the perspective of energy content and flow, specific enzymatic activities, specific physiological rates, and nitrogen cycling. To achieve accuracy, sensitivity, low-cost, and a high data-acquisition rate, choosing a protein assay is critical for research success.

There are several methods for determining total protein content that are based on nitrogen analysis or on dye-based colorimetric measurements, among these are (1) individual amino-acid analysis (AOAC, 1984), (2) Kjeldahl (AOAC, 1984), (3) total ninhydrin protein (Marks et al., 1985; Starcher, 2001), (4) ultraviolet absorption (A_{280}; Waddell, 1956), (5) Biuret reaction (Ellman, 1962), (6) Lowry (Lowry et al., 1951), (7) Bradford (Bradford, 1976) and (8) Smith (Smith et al., 1985) methods. All of these techniques have advantages and disadvantages (Conklin-Brittain et al., 1999; Olson and Markwell, 2007; Sapan et al., 1999). The greatest disadvantages of the colorimetric methods are the interferences with buffers (Walker, 1996), their tendency to bind different amino acid compounds in different proportions, and a 2-h incubation period (Lowry et al., 1951). Using buffer solutions in biochemical assays is important because they stabilize the pH during the assay and most biological processes are pH-dependent. Any pH shift will change reaction rates during the analysis (Nelson and Cox, 2008). Commonly, biological buffers incorporate anionic detergents such as Triton X-100, with the objective of improving the permeability of the cells and the solubility and stability of enzymes and redox dyes (Lester and Smith, 1961; Miozzari et al., 1978; Owens and King, 1975; Packard,

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4. Purcell, J. E., D. R. Bondyale-Juez, V. Romero-Kutzner, I. Martínez, R. Caprioli, M. Tames-Espinosa, J. Almunia, E. Alonso, T. T. Packard, & M. Gómez, 2019. Food supply effects on the asexual reproduction and respiratory metabolism of Aurelia aurita polyps. Hydrobiologia 846: 135–146.

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PRIMARY RESEARCH PAPER

Food supply effects on the asexual reproduction and respiratory metabolism of *Aurelia aurita* polyps

Jennifer E. Purcell · Daniel R. Bondyale-Juez · Vanesa Romero-Kutzner · Ico Martínez · Rosa Caprioli · Mayte Tames-Espinosa · Javier Almunia · Ester Alonso · Theodore T. Packard · May Gómez

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Abstract Because *Aurelia* spp. blooms have important regional effects, it is urgent to determine factors that may affect their proliferation and their effects on food webs. The life cycle of most scyphozoans includes an attached stage (polyp or scyphistoma) that reproduces asexually. To test the effects of food availability (unfed, fed 1-, 2-, or 3-times weekly), we measured production rates, mass, and metabolism of *Aurelia aurita* polyps. Metabolic measurements were physiological respiratory O_2 consumption (R), potential O_2 consumption (Φ), and potential CO_2 production (ψ_{NADP}). Φ and ψ_{NADP} were calculated from enzymatic activities of the respiratory electron transport system (ETS) and from the CO_2-producing enzyme, $NADP^+$-dependent isocitrate dehydrogenase (NADP-IDH), respectively. The production of polyps dramatically increased from ~ 0.1 polyp day^{-1} (unfed) to ~ 0.65 polyp day^{-1} (fed 3-times $week^{-1}$) over 33 days. Mass and metabolism (R, Φ, ψ_{NADP}) per polyp were significantly lower in unfed polyps, but indistinguishable among fed polyps. Our results suggested that the polyps maintained low-metabolic rates, putting available energy into asexual reproduction. The polyps were adapted to survive and reproduce when unfed, confirming their contribution to population persistence and potential jellyfish blooms.

Keywords Jellyfish · Scyphistoma · Starvation · Respiration · Metabolism · ETS · IDH · CO_2 production

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5. Tames-Espinosa, M., I. Martínez, V. Romero-Kutzner, D. R. Bondyale-Juez, T. T. Packard, & M. Gomez, 2018. NADP+-dependent isocitrate dehydrogenase activity in marine plankton. Marine Chemistry Elsevier 204: 86–94.

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NADP$^+$-dependent isocitrate dehydrogenase activity in marine plankton

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ABSTRACT

NADP$^+$-isocitrate dehydrogenase (NADP-IDH), as one of the most active intracellular CO_2-producing enzymes, was measured in marine plankton by adapting an enzyme assay to the 0.7–50 μm, 50–200 μm, and 200–2000 μm size fraction of the Canary Islands coastal plankton community. The variability of NADP-IDH activity in relation to pH, temperature, dilution of the sample, centrifugation or substrates was measured. In our hands, the maximum NADP-IDH activity (V_{max}) in marine plankton samples was attained in 0.1 M phosphate buffer at pH 8.2 (pKa_1 = 7.6 and pKa_2 = 8.8), by adding 6 mM $MgCl_2$, 0.3 mM NADP$^+$ and 2 mM DL-trisodium isocitrate. The optimum temperature in these subtropic mesozooplankton samples was 28 °C, with an Arrhenius energy of activation (Ea) of 20.4 kcal mol^{-1} (85.4 J mol^{-1}), and an Arrhenius collision frecuency factor (A) of $2.9 \cdot 10^{11}$ mol NADPH s^{-1} (kg of protein)$^{-1}$. The apparent Michaelis-Menten Km values for the substrates in crude homogenates at pH 8.5 and 18 °C were 271 ± 63 μM for isocitrate and 18 ± 3 μM for NADP$^+$. This enzyme, in addition to its high CO_2-producing activity, is also important in regulating other CO_2-producing enzymes. Thus, it can be used to calculate: (1) respiration in marine samples; (2) carbon flux in the water column; (3) metabolic adaptations to environmental changes; (4) roles of the plankton components of the food chain; and (5) the reactive oxygen species (ROS) scavenging capacity of the marine plankton resources.

1. Introduction

Since the 1980s, the assessment and understanding of the processes that control the carbon fluxes between atmosphere, the marine surface waters, the deep ocean, and the benthos, have been the focus of international research programs (e.g., Joint Global Ocean Flux Study, JGOFS). One of the processes that impact on the carbon fluxes in the ocean is the CO_2 production related to respiration. However, in oceanography, there are constraints in determining CO_2 changes in seawater (Mayzaud et al., 2005), so respiratory rates have mainly been reported as O_2 consumption. As a result, respiratory quotients (RQs), ratios between CO_2 produced and O_2 consumed, are required to calculate respiratory CO_2 production rates from either direct O_2 consumption measurements (Berggren et al., 2012; Romero-Kutzner et al. 2015), from calculations of O_2 consumption from ETS activity measurements (Hernández-Leon and Gómez, 1996; Osma et al., 2014, 2016; Packard and Codispoti, 2007; Packard and Gómez, 2013), or from biomass (Steinberg et al., 2008). The fact that RQ is now known to be more variable than expected (Berggren et al., 2012; Romero-Kutzner et al., 2015) adds impetus to developing methodology to assess seawater CO_2 production rates more directly.

Here, we propose the first step of a further enzymatic assessment of this process. The biochemistry of respiratory CO_2 production is dominated by NAD(P)$^+$- dependent isocitrate dehydrogenase in the Krebs cycle (IDH_{KC} in Fig. 1) (Nelson and Cox, 2008). In cells, other biochemical reactions produce CO_2, but their production rates can be predicted from IDH_{KC} activity (Packard et al., 1996; Roy and Packard, 2001; Tames-Espinosa et al., subm.). Of those linked to respiration, pyruvate dehydrogenase (PDH) serves to bridge the metabolism of glucose (glycolysis) to the Krebs cycle. Then, inside this cycle of enzyme reactions, both IDH_{KC} and alpha-ketoglutarate dehydrogenase (KGDH in Fig. 1), produce CO_2, being the IDH_{KC} activity 1.1 times the KGDH activity (Walsh and Koshland, 1984, Fig. 1). Finally, linked to, but not in the Krebs cycle, are the malic enzyme (ME in Fig. 1) and phosphoenol pyruvate carboxykinase (PEPCK in Fig. 1). They also generate CO_2 but at much reduced rates. IDH_{KC} activity is 8.4 times greater than these activities.

IDH_{KC} encompass two isoenzymes that catalyse the conversion of isocitrate to α - ketoglutarate in a reaction summarized in Eq. (1) (Nelson and Cox, 2008).

$$Isocitrate + NAD(P)^+ \leftrightarrow \alpha - ketoglutarate + NAD(P)H + CO_2 \quad (1)$$

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- **COMUNICACIONES EN CONGRESOS COMO AUTOR PRINCIPAL**

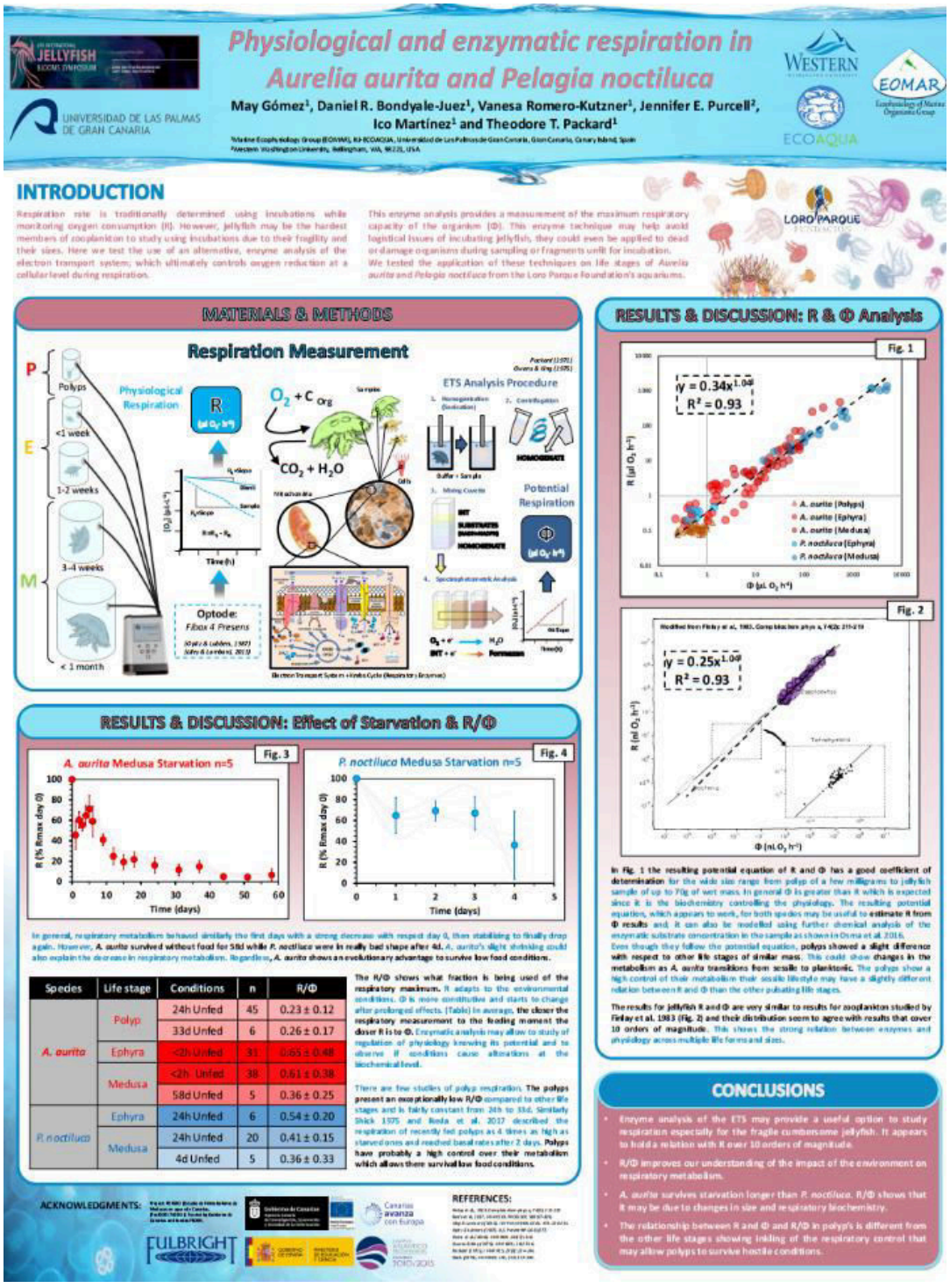
1. Presentaciones orales:

1. **AUTORES:** Daniel R. Bondyale Juez; Vanesa Romero Kutzner; Theodore T. Packard; Ico Martinez; May Gómez Cabrera.
   TÍTULO: Respiration, protein, lipid & carbohydrate composition in *Physalia physalis* and *Velella velella*. Predator and prey of the pleuston.
   CONGRESO: International Symposium of Marine Science (ISMS 2020)

2. **AUTORES:** Daniel R Bondyale Juez; Vanesa Romero Kutzner; Jennifer E. Purcell; Ico Martinez Sanchez; Theodore T. Packard; May Gómez Cabrera
   TÍTULO: Respiratory metabolism analysis in *Aurelia aurita* and *Pelagia noctiluca*
   CONGRESO: 6th International Jellyfish Blooms Symposium (2019)

2. Posters:

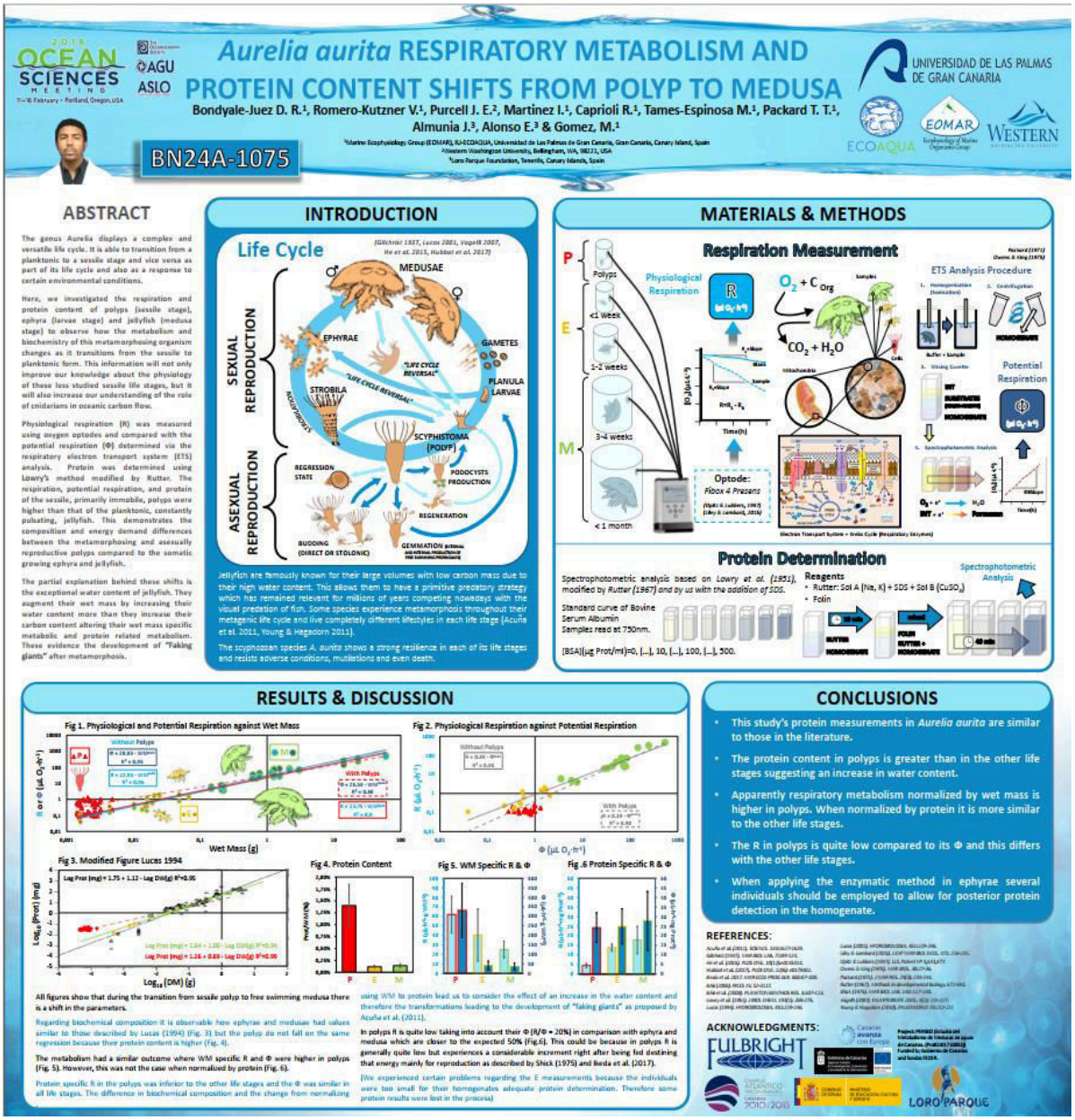
1. **AUTORES:** May Gómez Cabrera; Daniel R Bondyale Juez; Vanesa Romero Kutzner; Jennifer E. Purcell; Ico Martinez Sanchez; Theodore T. Packard.
TÍTULO: Physiological and Enzymatic Respiration in *Aurelia aurita* and *Pelagia noctiluca*
CONGRESO: 6th International Jellyfish Blooms Symposium (2019)



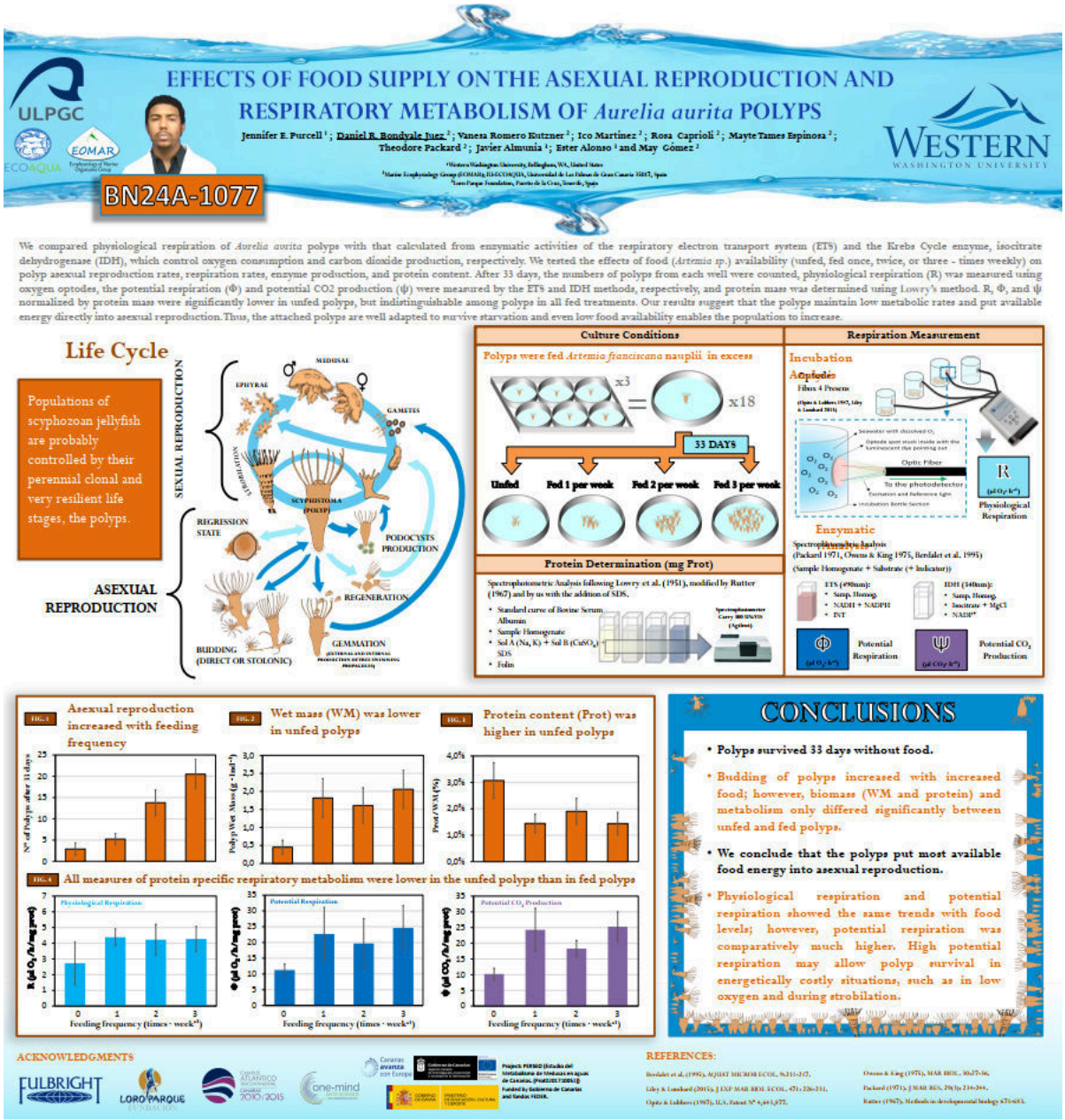
2. **AUTORES:** Daniel Rickue Bondyale Juez; Vanesa Romero Kutzner; Jeniffer E. Purcell; Ico Martínez; Rosa Caprioli; Mayte Tames Espinosa; Javier Almunia; Ester Alonso; Theodore Packard; May Gómez

TÍTULO: *Aurelia aurita* Respiratory Metabolism and Protein Content Shifts from Polyp to Medusa

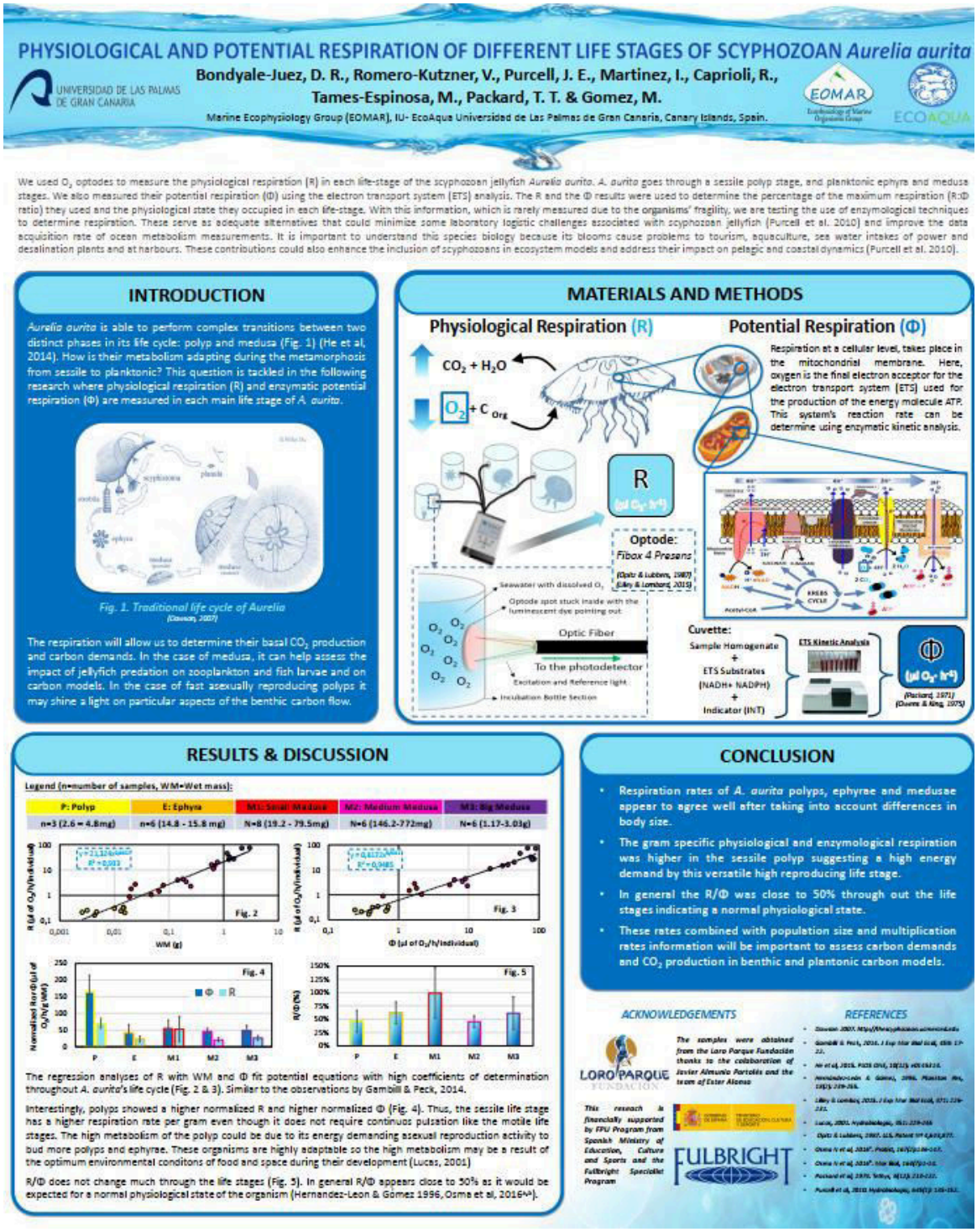
CONGRESO: Ocean Science Meeting (2018)



3. **AUTORES:** Jeniffer E. Purcell; Daniel Rickue Bondyale Juez; Vanesa Romero Kutzner; Ico Martínez; Rosa Caprioli; Mayte Tames Espinosa; Javier Almunia; Ester Alonso; Theodore Packard; May Gómez.
TÍTULO: Effects of Food Supply on the Asexual Reproduction and Respiratory Metabolism of *Aurelia aurita* Polyps
CONGRESO: Ocean Science Meeting (2018)



4. **AUTORES:** Daniel Rickue Bondyale Juez; Vanesa Romero Kutzner; Ico Martínez; Mayte Tames Espinosa; Theodore Packard; May Gómez.
TÍTULO: Physiological and potential respiration of different life stages of scyphozoan *Aurelia aurita*.
CONGRESO: Maritime Spatial Planning, Ecosystem Approach and Supporting Information System (MAPSIS)



DECLARATION

I herewith declare that I have produced this work without the prohibited assistance of third parties and without making use of aids other than specified; notions taken over directly or indirectly from other sources have been identified as such. This work has not previously been presented in identical or similar form to any examination board. The dissertation work was conducted from 2016-2020 under the supervision of Prof. María M. Gómez Cabrera and Dr. Theodore T. Packard at the Universidad de Las Palmas de Gran Canaria.

This dissertation was finished writing in Las Palmas de Gran Canaria on 14 of December 2020.

Daniel R. Bondyale Juez