On the Respiratory Metabolism of Marine Plankton: A Time-Course Study in Bacteria and Medusa

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Doctor of Philosophy

Universidad de Las Palmas de Gran Canaria

Las Palmas de Gran Canaria

December 2021

Doctoral Program:

Oceanography and Global Change

Agradecimientos

Porque el tiempo es vida, y la vida reside en el corazón.

Michael Ende, Momo (1973)

Empiezo la redacción de la tesis por los agradecimientos. *Gracias*,

May, por velar por los tuyos, por mí, y por permanecer despierta.

Ted, por sembrar semillas de curiosidad y eureka.

Dani, por tu atención y esfuerzo. Tú dedicación.

Ico, por ser siempre justa y omnipresente.

Mayte, por lo compartido.

Ali, por guía y maestra.

Nuestro viaje EOMAR, WestCoast2018: de Friday Harbor a CICESE.

Nat, e Igor, por ser compañeros, y estar cerca estando lejos.

Fede, por no abandonar el barco y dejar bajarme.

También *a*,

Xavier García (ICM-CSIC, Barcelona),

Holger Freund (ICBM, Wilhemshaven),

Juan Luis Gómez Pinchetti (ULPGC, CBM, Gran Canaria),

Angelo Santana del Pino (ULPGC),

Chris Hawes, Maike Kittelmann y Louise Hughes (Oxford Brooks, Oxford),

Ulf Riebesell (GEOMAR, Kiel),

Antonio Ricarte Sabater (UA, Alicante),

Jennifer Purcell (Bellingham, USA),

Cathy Lucas, Dan Mayor, Kathryn Cook, Alejandra Sánchez Franks (NOC, Southampton) y al JC191 Team.

Gracias,

A la familia
*T*o my Friends
A las gotitas de agua con sal

A mi abuela,

Felisa Mateo Hidalgo

Abstract

Respiration is an ancient process and a ubiquitous component of contemporary biospheric metabolism. The respiratory rate represents the activity of catabolic biochemical pathways. It is the process by which energy is transformed within an organism and, on much a larger scale, within the biosphere, itself. Stressors, such as those associated with anthropogenic climate change can impact the physiology of an organism. Bacterioplankton and gelatinous zooplankton are conspicuous components of marine ecosystems. However, their respiratory metabolism is widely approached by allometric scaling, and CO_2 production is estimated by using a constant, the respiratory quotient (RQ). These practices present restrictions. In this thesis, we use physiological respiration rates (O_2 consumption and CO_2 production) in addition to respiratory enzymatic rates, to study the physiology of marine bacteria and the medusozoan jellyfish, *A. aurita*. In addition, we explore the respiratory metabolic response of these organisms under stress conditions. In **Chapter 2**, two marine bacterial species were cultured from nutrient-sufficiency to nutrient-limitation under two different carbon sources (food type). In **Chapter 3,** *A. aurita* polyps were exposed to a simulated marine heatwave, and their changes in respiratory enzymology and physiology were documented. In **Chapter 4,** the metamorphosis of *A. aurita* was monitored from polyp to the medusa life stage. The studies here elucidate the metabolic response of marine bacteria in post-bloom conditions as one finds in an oligotrophic ocean. In parallel, these studies document the performance of the jellyfish *A. aurita*, and their potential proliferation in conditions predicted for our changing oceans.

Thesis preview

The present thesis entitled, *On the respiratory metabolism of marine plankton: A time-course study in bacteria and medusa* has been conducted within the Doctoral Program of Oceanography and Global Change from the University of Las Palmas de Gran Canaria and has been supervised by Dr. May Gómez and Dr. Ted Packard. It is comprised of three original research articles developed under the aegis of the projects BIOMBA (CTM 2012-32729), MERMAC (CEI 2017-10), and PERSEO (ProID201710051), all granted to May Gómez. In addition, the thesis benefitted from collaboration with the visiting researcher, Dr. Jennifer Purcell (within a FULBRIGHT grant to the University of Las Palmas de Gran Canaria). The thesis program was enriched by an 8-month research stay at the National Oceanography Center in Southampton (United Kingdom) and a month-long, trans-Atlantic, oceanographic expedition on the British research vessel, R/V James Cook (JC191).

This thesis is structured in two parts. The former is divided into several chapters that encompass a general introduction with the major objectives and hypothesis of the work, followed by original published and unpublished, scientific research articles describing laboratory experiments. These chapters are written entirely in English, thereby complying with the requirements for obtaining the International Mention of the Doctor Title (Real Decreto 99/2011 del 28 de enero por el que se regulan las enseñanzas oficiales del doctorado, Art. 15).

The second part of the thesis includes a summary in Spanish, with an introduction, thesis objectives, summaries of the investigations carried out, and a compilation of the most relevant conclusions. This format complies with the format of the thesis written in a language other than Spanish according to the regulations outlined by the Doctoral Studies of the University of Las Palmas from Gran Canaria (BOULPGC 9 de enero de 2013, modificado en el BOULPGC de 4 marzo de 2019 Capitulo III, Artículo 10).

The development of this thesis would not have been possible without the financial support received from the Government of the Canary Islands and Agencia Canaria de Investigación, Innovación y Sociedad de la Información) (TESIS2015 01 011) and the European Social Fund.

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CHAPTER

1

General Introduction

We are like dwarfs on the shoulders of giants, so that we can see more than they, and things at a greater distance, not by virtue of any sharpness of sight on our part, or any physical distinction, but because we are carried high and raised up their giant size.

Bernard of Chartres in Metalogicon of John of Salisbury, 1159

1.1. Origin of life and metabolism

The planet is 4.5 billion years old. From geological evidence, a hydrosphere on Earth dates back to 4.3 billion years ago (bya) (Mojzsis et al., 2001). The formation of prebiotic organic molecules from water and organic chemical compounds led to the emergence of life (Fleury et al., 2017). These prebiotic organic compounds were originated from subaqueous and subaerial volcanic activity. Ongoing investigations lead scientists to search for today's analogues to young planet Earth's prebiotic environments. Today's analogues include: Yellowstone National Park in Wyoming (USA), Hell's Gate near Rotorua (New Zealand), Kamchatka (Russia), Iceland, Dallol in the Danakil depression (Ethiopia), El Tatio (Chile), and oceanic hydrothermal vents (Deamer, 2021).

These prebiotic environments have several characteristics in common: the abundance of organic compounds, presence of minerals, high-temperature gradients, and, a redox potential, with reducing ambient. The origin and evolution of organic matter on Earth still trigger scientists' curiosity. In investigating these modern analogues, scientists have learned that oxygen is not essential for life. This is because oxygen was either absent or in low concentrations in Earth's early atmosphere (Kasting, 1993; Zimorski et al., 2019; Ozaki and Reinhard, 2021).

In contrast to oxygen, energy is critical for life; it enables existence, reproduction and survival. All organisms, autotrophs, and heterotrophs, have evolved biochemical pathways to collect, convert and store energy, principally via the synthesis, first of acetyl phosphate (AcP) (Whicher et al., 2018) and then of adenosine triphosphate (ATP) (Carlson et al., 2007) to sustain physiological functions. All cells have, basically, the same catabolic pathways, that break down organic matter and transform it into usable cellular energy.

These processes are ubiquitous (Lane, 2006). They use and control glycolysis, the citric acid cycle (TCA, or Krebs cycle), and the respiratory electron transport system (ETS). The before-mentioned metabolic pathways are the major steps that

comprise cellular respiration. In the cells, ATP production is controlled and powered by proton gradients, electron flux through the ETS, and associated with proton and electron donors, which transfer electrical currents through the ETS (Figure 1). Ochoa (1943) demonstrated that respiration is coupled to ATP production, but was not fully explained until Peter Mitchell (1961) proposed that ATP production was driven by the electromagnetic fields (EMFs) and pH gradients across the mitochondrial inner membrane (chemiosmosis). This led to Mitchell's Nobel Prize in 1977 for his chemiosmotic explanation for ATP production.

Electron donors (electropositive) and acceptors (electronegative) vary throughout the abiotic environment (Falkowski et al., 2008). For example, in anaerobic respiration, H_2 can act as an electron donor and CO_2 as an electron acceptor, fueling methanogenesis, simultaneously (Canfield et al., 2006). Whereas in aerobic respiration, oxygen acts as an electron acceptor. The most important difference between anaerobic and aerobic metabolism is the rate at which energy is transformed.

In addition, metabolism encompasses a vast web of interlocked enzymes that catalyse all biochemical reactions. How these interlocking enzyme systems emerged is largely unknown (Noda-Garcia et al., 2018; Becerra, 2021). Several enzymes that feed electrons to the respiratory ETS have been traced to the last universal common ancestor (LUCA), these include: NADH dehydrogenase, succinate dehydrogenase, and hydrogenase (Castresana et al., 1999). LUCA, despite its anaerobic metabolism, is thought to have used trace amounts of oxygen to respire (Weiss et al., 2016). Knowledge of LUCA's metabolism provides evidence that respiratory pathways, linked to an ETS and ATP synthesis evolved early in evolution, around 3.5 bya (Castresana et al., 1999; Noda-Garcia et al., 2018).

Evolutionary biochemistry considers that single enzymes to entire metabolic networks, evolved from geochemically-catalysed reactions to meet specific physiological and environmental conditions. In this sense, metabolism is not only a molecular, cellular, and ecological phenomenon, it is a planetary phenomenon, that evolved from geochemical activity on Earth (Goldford and Segrè, 2018).

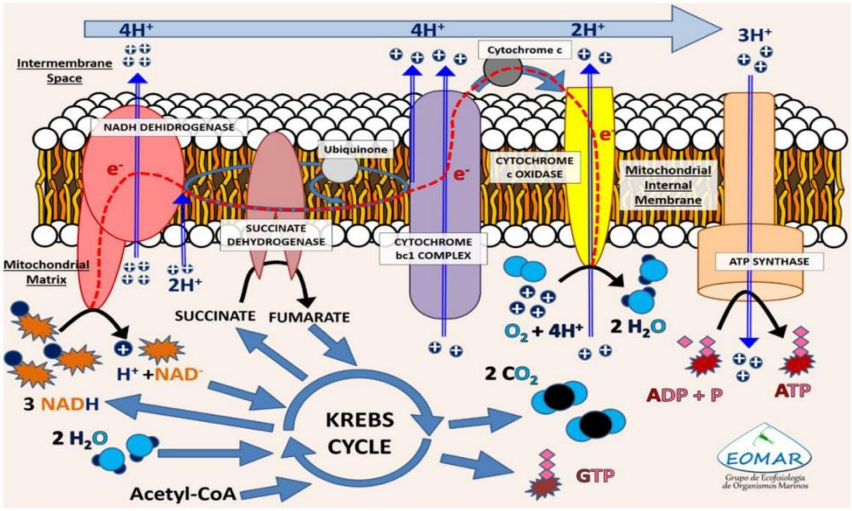


Figure 1. Representation of the main biochemical pathways involved in aerobic respiration located in the mitochondria: The tricarboxylic carbon cycle (TCA, or Krebs Cycle), Electron Transport System, and ATP-synthesis. The Krebs Cycle is linked to the final product of glycolysis, pyruvic acid. This pyruvic acid combines with coenzyme A (CoA) to form acetyl coenzyme A (Acetyl CoA) which then enters the Krebs cycle. For each acetyl-CoA, the Krebs cycle liberates, 3 molecules of NADH, 1 molecule of reduced flavin adenine dinucleotide ($FADH_2$), CO_2, and guanine triphosphate (GTP). Respiratory electron transport system enzyme-complexes then oxidise NADH and $FADH_2$, thereby releasing electrons that are transported through the mitochondrial inner membrane. Concurrently, this electron flux causes a cross-membrane pumping of protons (H^+), from the mitochondrial matrix side of the inner mitochondrial membrane, to the inner membrane space on the outer side of the inner mitochondrial membrane. The outer mitochondrial membrane that would be at the top of the figure is not shown. In complex IV, cytochrome oxidase, the electrons reduce oxygen to form H_2O in the presence of protons (H^+). In other words, a pH gradient is generated by proton pumping through the membrane and proton consumption

in the matrix to form water. This pH gradient across the inner mitochondrial membrane is a store of free energy since the flow of protons back through the membrane (to the matrix) drives the synthesis of ATP. This chemiosmotic coupling of electron transfer and proton pumping with ATP synthesis was the *magnum opus* of Peter Mitchell (1961) in his chemiosmotic hypothesis. The appearance of mitochondria coincides with the first protozoans 2,5 bya, and it took another 2 bya for the evolution of multicellular life. Figure illustration by D.R. Bondyale-Juez.

1.2. Evolution of eukaryotic aerobic metabolism

Inconceivably, photoautotrophic cyanobacteria by producing O_2 modified Earth's atmospheric chemistry, from a reducing to an oxidizing one. They used water as their reducing agent and liberated molecular oxygen. This change forced the biggest revolution in the history of life, the oxygenation of the planet, also called the Great Oxidation Event (GOE) (Vernadsky, 1926; Olejarz et al., 2021). Oxygen concentrations started to rise with oxygenic photosynthesis around 2.5 bya (Planavsky et al., 2014; Krause et al., 2018), fueling aerobic metabolism (Wignall and Twitchett 1996; Vaquer-Sunyer and Duarte, 2008) (Figure 2). This oxygen was originally toxic for early cells that were unaccustomed to it (Margulis and Sagan, 1986). A recent study by Canfield et al. (2021), finds that oxygen, although toxic to the older aero-intolerant prokaryotes, did not impede the development of early eukaryote ecosystems. For example; melatonin is believed to be an ancient antioxidant that drove oxygen to be metabolically tolerable (Manchester et al., 2015).

The closest relatives to eukaryotes are thought to be the recently discovered, mostly anaerobic, Asgard archea[1] (Spang et al., 2015; Zaremba-Niedzwiedzka et al., 2017; MacLeod et al., 2019; Cai et al., 2020; Williams et al., 2020; Liu et al., 2021). Comparative analysis suggests that the last Asgard archaea common ancestor was an amino-acid-degrading anaerobe that produced H_2 and fatty acids as by-products. It acquired ATP from substrate-level phosphorylation by catabolizing 2-oxoacid intermediates (Imachi et al., 2020). In other words, respiratory pathways, such as ETS and TCA can also be traced to the last common ancestor of Asgard archaea.

However, it is unclear how the first eukaryotic cell emerged. The most widely accepted evolutionary model defends symbiogenesis, in which an archaeon host cell and a proteobacterial endosymbiont unified to become the first eukaryotic cell (Mereschkowsky, 1910; Margulis, 1967; Koonin, 2015; López-García and

[1] Deep-Sea Archaeal Group or Marine Benthic Group-B

Moreira, 2015; Martin et al., 2015; Eme et al., 2017; Williams et al., 2020, 2013). The proteobacterium would evolve into mitochondria, and the archeon, as the host cell, would develop the nucleus (Margulis, 1967; Ettema, 2016; Imachi et al., 2020). Therefore, the origin of complex cells is inseparable from the origin of mitochondria.

However, aerobic eukaryotes would not arise for almost another 1 bya after the start of the GOE (Figure 2). The earliest evidence of their evolution occurred from about 1.7-2 bya, when atmospheric oxygen rose to a little more than 1% of its current level (Figure 2) (López-García and Moreira, 2020). Aerobic respiration accounts as a physiological adaption to the environmental abiotic conditions. Despite being a slow process, these physiological adaptions started before GOE. In the same way that LUCA already could use trace amounts of oxygen to respire (Weiss et al., 2016), a key enzyme of aerobic metabolism, cytochrome oxidase, was present in the respiratory pathways of the archaeal and bacterial common ancestor (Castresana et al., 1994, 1999).

Cytochrome oxidase is a terminal enzyme of the respiratory ETS, catalysing the reduction of oxygen to water. It also acts as a redox-linked proton pump. The common ancestor of archaea and bacteria must have been anaerobic because oxygen concentration was still low (Xavier et al., 2021). This means that cytochrome oxidase is older than the GOE (Castresana et al., 1994, 1999).

Once adapted to oxygen, respiration uses oxygen as the terminal electron acceptor. The organisms could now break down organic matter into water and carbon dioxide, in a step-wise manner. In that sense, aerobic respiration released much more energy than anaerobic respiration. This new way of energy transformation, a product of the archaea-bacteria symbiogenesis, allowed eukaryotes to “inherit the world”, to quote a phrase used by Nick Lane (Lane, 2006). Then, eukaryote ecosystems evolved with nutrient availability (Brocks et al., 2017; Eckford-Soper and Canfield, 2020), development in feeding, predation,

multicellularity, and cellular organization (Knoll et al., 2011). The first animals, according to fossils and biomarkers, consisted mainly of sponges of the phylum Porifera and jellyfish of the phylum Cnidaria (Cloud and Glaessner, 1982; Narbonne, 2005). Particularly, colony-forming, benthic, and sessile Cnidaria were abundant (Narbonne, 2005). This dates back to 550 million years ago (mya), at the time when the metazoans inherited the world (Figure 2).

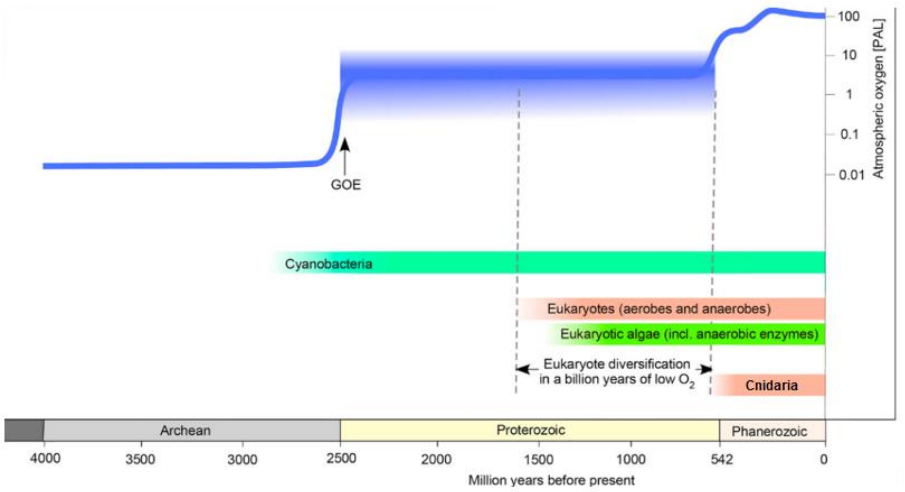


Figure 2. Summary of oxygen accumulation in Earth history and life evolution. PAL: percent of current atmospheric oxygen level. GOE: great oxidation event. (Modified from Zimorski et al. 2019)

1.3. Medusozoans, early metazoans

From anaerobic LUCA to the emergence of the first O_2-respiring animals on Earth, 3.5 bya passed. The diversification of Cnidaria was one of the oldest evolutionary events (Cartwright et al., 2007; Han et al., 2016). This phylum has a simple nervous and muscular system, that is thought to represent the most ancient form of existent neurons and muscles (Steinmetz et al., 2012; Marlow and Arendt 2014; Moroz, 2021). Phylogenetic analysis argues that Cnidarians are a sister clade to Bilateria, which comprises 99% of extant animals (Whelan et al., 2017). This same analysis also revealed that the split in the phylum Cnidaria, into Anthozoa and Medusozoa, occurred in the Precambrian, about 642-532 mya (Dos Reis et al., 2015). Medusozoans, which include Scyphozoa, Cubozoa, and Hydrozoa, have a unique characteristic mitochondrial genome structure (Bridge et al., 1992). Cnidarians do not have a vascular system or excretory organs. Their only transport system is a passive one, which depends on diffusion.

Oxygen is diffused through the cellular layers exposed to the environment, supplying the oxygen for respiration (Graham, 1988). The bell of medusozoans, has two layers, an ectoderm (epidermis) and an inner endoderm (gastrodermis). These two layers are separated by an acellular mesoglea (Holstein et al., 2011). The mesoglea can retain high concentrations of oxygen, a reserve for respiratory pathways that can serve under unfavourable environmental conditions (e.g. hypoxia tolerance) (Thuesen, 2005). Almost all medusozoans incorporate, a benthic, sessile polyp, and/or a planktonic medusa body plan (Gold et al., 2019) (Figure 3). Polyps can incorporate dissolved O_2 from the seawater through their body tissue, by diffusion, and also by the action of ciliary currents (Han et al., 2016).

The mobility characterized by Cnidarian medusa-forms, is considered to be a later biological innovation and took place during the Precambrian-Cambrian transition, about 550 mya (Collins, 2002; Marques and Collins, 2005; Narbonne, 2005). Gold et al. (2018) hypothesise that medusozoans evolved their complex medusa life stage by re-organising genetic pathways already present in the last common ancestor of Cnidarians. Shifts in life stages are considered to respond to new ecological niches (Gold et al., 2018).

Medusozoans metagenesis is one of the most ancient complex life cycles in metazoans (Marques and Collins, 2005; Kraus et al., 2015), and can include several life stages (medusa, planula larva, polyp, strobila, and ephyra) (Figure 3). This may have contributed to jellyfish success over the last 500 mya (Pitt et al., 2013). These life stages can proliferate, forming in high densities. The proliferation of polyp populations by asexual reproduction (budding) and success of ephyrae production (strobilation) determine population recruitment of planktonic medusae.

According to fossil records, medusozoans' morphogenesis has been stable over time, resulting in the continuity of shapes and forms from ancient to modern medusa. With anthropogenic climate change, it is expected that medusozoans, which have survived for about 600 mya, while facing major environmental changes, could regain the role of a monopolistic pelagic predator (Parson and Lalli, 2002; Richardson et al., 2009).

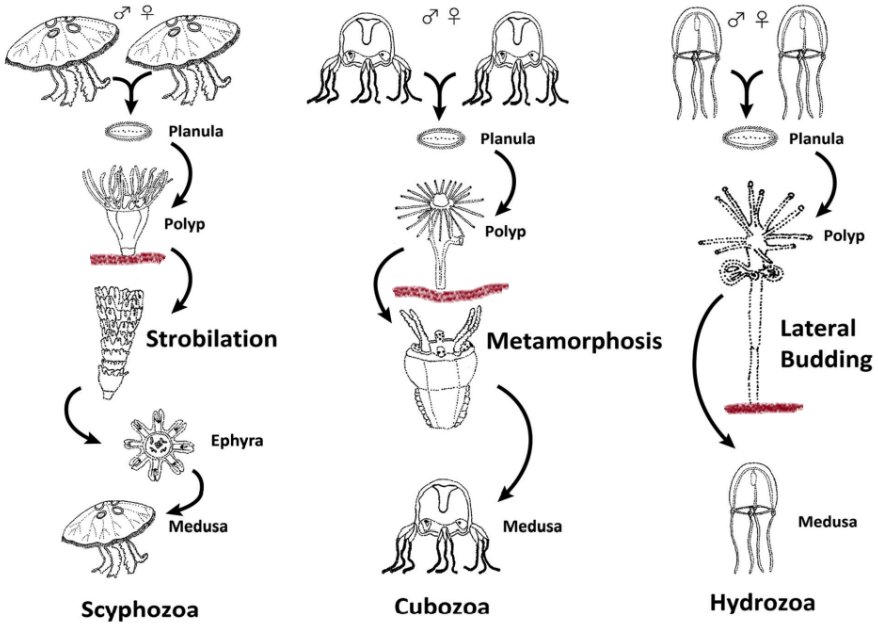


Figure 3. Main life cycle stages in Medusozoa (Scyphozoa, Cubozoa, and Hydrozoa). The polyp life stage is sessile. Budding is a type of asexual reproduction, forming new polyps from a parent body plan. (Modified from Boero et al. 2016)

1.4. The role of bacterioplankton and medusozoans in modern oceans

Energy and metabolism enabled the evolution of life. The main input of energy in the marine ecosystem is derived from the sun. The marine biota plays a key role in the composition of seawater (Sverdrup et al., 1942; Harvey, 1955; Redfield et al., 1963). All known life is based on organic compounds, which circulate through abiotic and biotic compartments. In other words, solar energy is fixed by primary producers (autotrophs) and is then transferred *via* predator-prey interactions to higher trophic levels. As mentioned before, all heterotrophs can oxidise ingested organic matter. They transform it into cellular energy through respiration and regulate their metabolism for growth and reproduction.

Microorganisms were the first inhabitants of the planet, whose role in the evolution of life has been explained in the last sections. Heterotrophic microbes can transform dissolved organic matter (DOM/DOC-dissolved organic carbon). One way is transforming energy into particulate organic carbon (POM/POC-particulate organic carbon) as biomass producers. Another way is through microbial respiration, DOM/DOC is converted to carbon dioxide (DIC) (Azam et al., 1983; Williams, 1984; Ducklow et al., 1986; Robinson, 2019). This way associated fluxes of materials are stimulated and biological energy is generated. It was Pomeroy in 1974, who first proposed that bacterioplankton play a key role in the marine food web, suggesting that most of the energy made from primary production is consumed by them (Pomeroy, 1974). This way, in modern trophic ocean systems, heterotrophic bacteria are responsible for up to >59% of the community respiration (Robinson and Williams, 2005) and are key in the balance between organic carbon storage and DIC.

Enlarging the scale to metazoans, and medusozoans, our jellyfish, were early to inhabit the oceans. Today, they are increasingly recognised as influential organisms in the marine environment. Medusozoan trophic interactions are extensive (Pauly et al., 2009; Riascos et al., 2012; Takao et al., 2014; Ates, 2017;

Choy et al., 2017). Jellyfish can occur in blooms, are a food source for many marine species (Lamb et al., 2017), and also play a relevant role as predators, being competitors of fish. They prey heavily on fish larvae and mesozooplankton (Behrends and Schneider, 1995; Lynam et al., 2005).

Jellyfish are also considered to be an important agent of carbon transport through the water column (Jelly-C, Figure 4). Periodically, they drop out of the water column, as POM/POC, a phenomenon known as jelly-falls (Billet et al., 2006; Lebrato et al., 2012, 2013, 2019). Jellyfish, alive or dead, can also release DOM/DOC in the water column (Condon et al., 2011). That means if jellyfish occur in blooms they can limit the bioavailable carbon to higher trophic levels and promote the microbial loop, generating alternations in the marine ecosystem (Condon et al., 2011). However, the organic matter produced and excreted by jellyfish is unknown (Tinta et al., 2021) and depends on the physiology and ambient conditions of medusozoans life stages (Pitt et al., 2009).

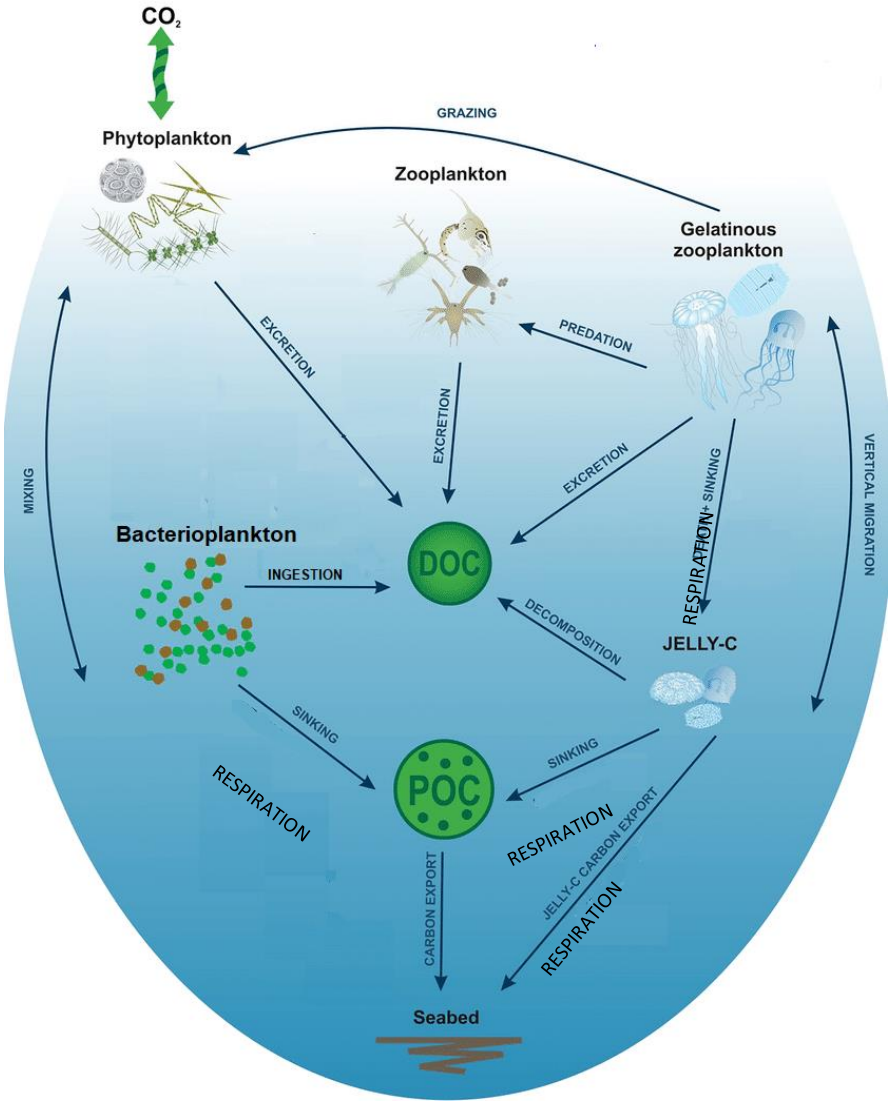


Figure 4. Jelly and microbial-pump and the physical (sinking, decomposition) or physiological processes (respiration, excretion) that transport biogenic particles (carbon export) from the upper ocean to the seabed, the benthic ecosystem (modified from Lebrato et al. 2019).

1.5. Metabolism, the ocean, and climate change

The fluxes of organic matter (or energy fluxes) in the sea, are in large part controlled by the marine biota (Figure 5). Physiology, as the biochemical response of an organism to its environment, can shed light on these organic processes. Nearly 60 years ago, Redfield et al. (1963), incorporating the ideas and findings of Harvey (1954), noted that studying the metabolism of marine organisms can illuminate the chemical and physical description of the sea. Today we know that the regulation of O_2 and CO_2 in the atmosphere is affected partially by oceanic DOM/DOC, the largest pool of reduced carbon above the Earth's crust (Fakhraee et al., 2021).

The estimation of organic matter in the ocean is routinely accomplished using sediment traps. These are collectors that harvest sinking particles falling towards the seafloor. However, all imports and export of material vary in time and space. In addition, no ecosystem is closed, and water bodies contain geographical, hydrographical subregions (Figure 5) that affect particle collection (Longhurst, 2010).

Measuring respiratory metabolism is an alternative to estimating organic matter flow in the oceans. The respiration in the ocean's interior is nourished by the energy transformation (autotrophic) in the waters above. This biological energy, as POC, continually rains down through the water column, until, much diminished by biological degradation, it reaches the seabed (Sverdrup et al., 1942; Riley 1951; Richards, 1957; Redfield et al., 1963).

Although the variability in metabolism, associated with different organisms and species, can reflect alternate energy requirements and energy transformation rates, the statistical scaling effect over large bodies of water results in some regularity (Redfield et al., 1963). Therefore, respiration is considered to be a good index of organic matter flow in the ocean, as it is the response to available energy-rich organic matter.

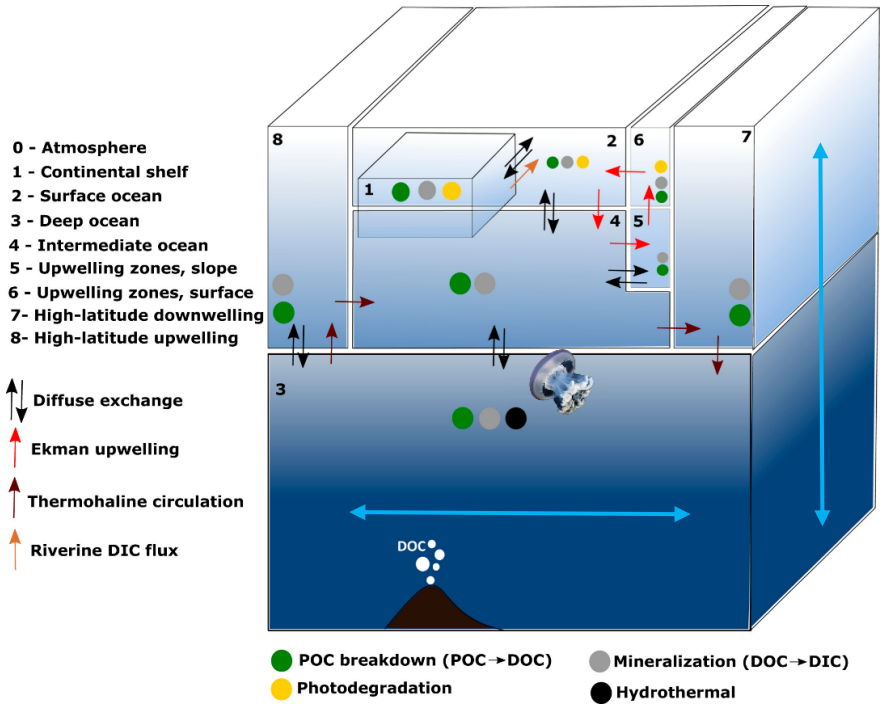


Figure 5. Box model for carbon transport from differentiated water masses according to their geographical and hydrographical origin. POC breakdown (green dot) and mineralization (grey dot) are both processes controlled by respiration. The presence of organic matter in each box is the result of physical transport mechanisms (diffusion, upwelling, thermohaline circulation, and river flux). Bacterioplankton and jellyfish are distributed throughout all water mass boxes (blue arrow). (Modified from Fakhraee et al. 2021)

Metabolism research has been one of the main areas of interest in Oceanography since Harvey (1928, 1957). Currently, it is of interest to investigate the role the ocean plays as a sink of CO_2 during anthropogenic climate change (Falkowski and Wilson, 1992). For this reason, the metabolic state of the ocean needs to be addressed (Del Giorgio and Duarte, 2002; Del Giorgio and Williams, 2005; Ducklow et al., 2013; Duarte et al., 2013). As global ocean parameters vary in time and space, marine organisms can buffer environmental changes (Pincebourde et al., 2009). However, it is largely unknown how anthropogenic climate change impacts marine ecosystems (Hoegh-Guldberg and Bruno, 2010).

In addition, quantification and anticipation of climate change effects are impossible, if the metabolic response, from individuals to ecosystems, towards the environment, in all its possible facets, is not clear. A metabolic insight is needed to make predictions of how climate change affects species, communities, populations, and ecosystems, respectively. One of the most important impacts of contemporary global climate change for many species is ocean warming (Hughes et al., 2017; Smale et al., 2019; Scanes et al., 2020a, 2020b). This is because all metabolic rates are affected by temperature. It increases the velocity of metabolic reactions, that are constrained by species-specific-temperature tolerance windows.

This way, marine heatwaves (MHW), prolonged extreme warm water events, and a rising phenomenon in the global oceans are leading to severe effects on the marine biota (Suryan et al., 2021). Under an MHW scenario, individuals need to adjust physiologically to the thermal stress (Leung et al., 2019). Changes in growth, reproduction (Shanks et al., 2020; Gall et al., 2021), feeding behavior (Mitterwallner et al., 2021), and survival are expected (Pérez et al., 2000).

During MHWs, because of highly stratified water columns and associated nutrient limitation, weaker primary production is expected (Hayashida et al., 2020). This can lead to energy-poor environments. Nevertheless, according to McCauley et al. (2015), some species will benefit from current or future ocean changes. Bacterioplankton and medusozoans are potentially major role-players in our future ocean, as they have been for millions of years. In this way, studying their metabolism is of major interest.

1.5.1. Future Challenges

The United Nations Ocean Decade (year 2021-2030), envisions research toward a sustainable and healthy future ocean. Research on marine ecosystems plays a pivotal role in achieving this purpose. Baltar et al. (2019) proposed the combination of evolution, metabolism, and climate change studies to enable predictions. The rationale behind this proposal is: that environmental changes stimulate evolution (e.g. aerobic metabolism) and evolution, in turn, stimulates changes in the environment (e.g. GOE).

Thus, understanding metabolic responses to environmental changes is key. However, in ecology, respiratory metabolism is commonly addressed by the Metabolic Theory of Ecology (MTE) (Brown et al., 2004). It is based on Kleiber's law relating respiration and biomass. In other words, this theory proposes biomass as an important factor that controls respiration. Respiration and biomass do correlate (Kleiber, 1932, 1961), because biomass packages mitochondria and the respiratory enzymes (Packard and Gómez, 2008; Martinez et al., 2010).

Mitochondria have been known since the 1950s to be the power stations of respiration (Siekevitz, 1957). However, the relationship between respiration and mitochondrial enzymes can be obscured. This relationship can be affected by e.g life stage, age, sex, reproductive state, nutritional state, etc. (Martínez et al., 2010). The conclusion is, biomass alone can not predict respiration, because of these fluctuations.

Accordingly, non-linearity between biomass and respiration has been found across metazoan taxa (Kolokotrones et al., 2010): bacteria (Aguiar-Gonzaléz et al., 2012), plants (Reich et al., 2006; Mori et al., 2010) and marine zooplankton (Packard and Gómez, 2008; Martínez et al., 2010). Furthermore, Lane (2006, pages 156-177), in examining the MTE, finds many inherent flaws, so it can hardly be relied upon.

Nevertheless, many ecologically scientists accept and apply the MTE to their studies, not knowing its defects from a biochemical and physicochemical point of view. For example, the MTE has been applied to calculate total plankton respiration in the ocean (López-Urrutia et al., 2006) and jellyfish-derived organic matter flows (Luo et al., 2020).

Also, Baltar et al. (2019) advocate for the integration of metabolism in the marine ecosystem using the MTE. For practical purposes, the application of the MTE to calculate metabolism seems convenient, e.g., it helps to avoid manipulating the fragile jellyfish for ecophysiological studies. However, metabolism is not being directly measured.

In contrast to the MTE, modern biochemistry, since the early work of David Keilin in the 1920s with respiration and the electron transporting cytochromes (Keilin, 1966), and since the kinetic work on respiration, and electron transport by Chance and Williams (1954,1956), the respiratory ETS has been known to control respiration. Respiration reflects the ETS rate at which electrons are used by the enzyme cytochrome oxidase to reduce oxygen into water.

As we have seen in this chapter, metabolic pathways and networks are ancient, all existing life evolved from metabolism to meet specific physiological and environmental conditions. Thus, respiratory biochemistry can yield more understanding of respiratory metabolism than biomass alone (Keilin, 1966; Mahler and Cordes, 1971; Nelson and Cox, 2005).

1.6. Ecophysiology of marine organisms: Enzymatic and physiological assays

Biochemical methods on marine organisms involve the determination of the enzymatic activity linked to metabolic pathways. As stated above, these pathways mainly include: Electron Transport System (ETS) and the Tricarboxylic acid cycle (TCA). Biochemical reactions, in these pathways, are controlled by enzymes. Enzymes are proteins that catalyse reactions by binding to specific substrates (e.g. NAD and NADP, Savenkoff et al., 1995). Enzymatic assays are therefore important tools for monitoring zooplankton metabolism.

Our research group (EOMAR) is specialized in the study of marine organism metabolism based on enzymatic approaches. Different methodologies have been developed in the last decades. These *in vitro* methodologies include: electron transport system assay (ETS) (potential O_2 consumption, Φ) (Packard, 1971; Gómez et al., 1996; Osma et al., 2016; Bondyale et al., 2017), isocitrate dehydrogenase (potential CO_2 production, IDH) (Tames-Espinosa et al., 2018) and glutamate dehydrogenase (GDH) (as an ammonia index) (Fernández-Urruzola et al., 2011). These enzymatic assays require the addition of substrates in saturated concentration according to their maximum velocity (V_{max}) in the kinetic model proposed by Michaelis and Menten (1913). This way the reproducibility of the reaction is assured (Maldonado et al., 2012). The oxidation of the substrates is identical in all living cells (Vosjan, 1982).

Packard et al. (1971) proposed an enzymatic method based on the ETS as a proxy for respiration for all living organisms at any depth in the ocean. Besides the oceanographic application of respiratory ETS, it has been applied to cultured organisms in the laboratory, to study their respiratory metabolism. Experimental organisms vary from bacteria (Christensen et al., 1980; Romero-Kutzner et al., 2015), zooplankton (Herrera et al., 2014) to jellyfish (Purcell et al., 2019). These ETS assays follow the modified procedure proposed by Owens and King (1975). In this method, the transferred electrons through the ETS are collected by an

artificial chromogenic electron acceptor, the tetrazolium salt INT (which is reduced to a red formazan dye). The rate of INT-formazan production theoretically reveals the oxygen consumption capacity of an organism, or potential respiration (Φ). Stoichiometrically, the INT-formazan production rate (µmol) is related by a factor of 2 to the ETS activity (µmol e^-) and 0.5 to Φ (Packard, 1985).

As an index for respiratory CO_2 production Tames-Espinosa et al. (2018) developed the isocitrate dehydrogenase (IDH) assay for marine plankton. Respiratory potential CO_2 production can be calculated by multiplying IDH activity by the factor 3 (Roy and Packard, 2001). This is because IDH is only one of the three major enzymes producing CO_2 associated with the Krebs Cycle, the other two are pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase (Walsh and Koshland, 1984; Holms, 1986; Packard et al., 1996).

In nature, cells might be substrate limited; therefore this in vitro measurement might not reflect *in vivo* metabolic rates. This way, physiological rates are also measured for a better understanding of how these *in vitro*/*in vivo* rates are related under different conditions, enabling future predictions of the resultant ratio for different scenarios. However, to date, no affordable and feasible methodology for respiratory CO_2 production has been developed. This way respiratory CO_2 production are routinely converted from O_2 consumption rates assuming a constant respiratory quotient (RQ) of 1 (González et al., 2003; Bühring et al., 2006). The RQ represents the ratio of respiratory CO_2 production to the O_2 consumption of an organism.

In addition, the ratio of physiological-to-enzymatic rates can vary (Bämstedt, 1979; Hernández-León and Gómez, 1996; Herrera et al., 2011). Nevertheless, the variability is associated with physiological rates that are more sensitive to abiotic factors (Finlay et al., 1983) (e.g. temperature, pH, salinity, pollution), and biological factors (e.g. body mass, sex, food quality, and quantity). This

variability adds an additional value to enzymatic approaches that utilize saturated substrate in their assays, as it sets a comparable baseline of metabolism.

However, on a large scale, variability in respiratory ratios (physiological over enzymatic rates) is minor (Finlay et al., 1983; Arístegui and Montero 1995). In Figure 6 data of jellyfish respiratory ratios, measured in our laboratory, hold a relationship over 10 orders of magnitude and fit into the Figure of Finlay et al. (1983), where the relationship between the respiratory rate and the ETS activity is represented for different groups of organisms.

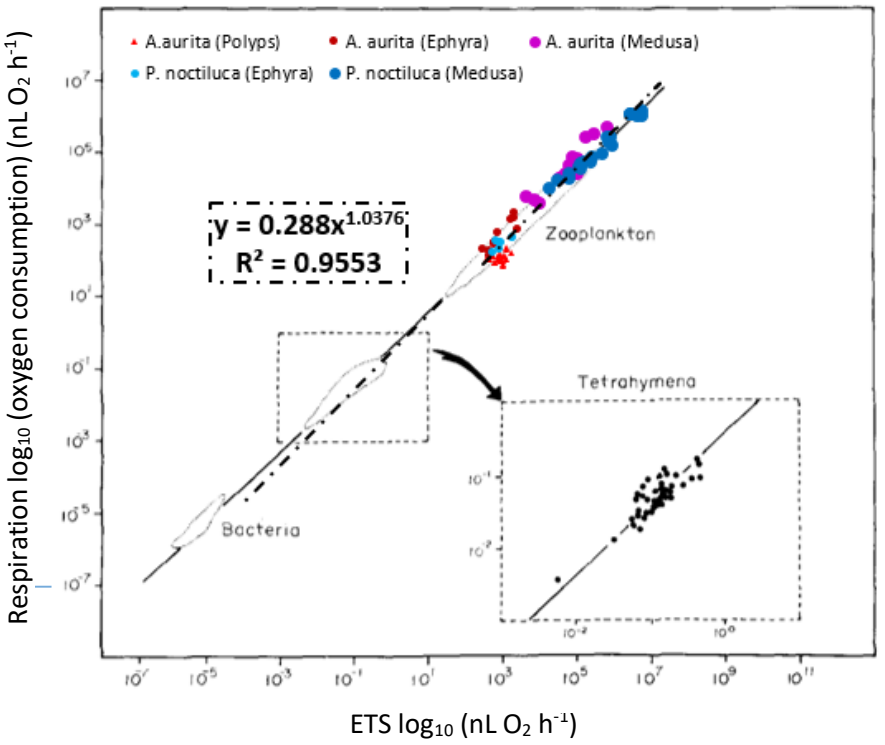


Figure 6. Relationship between respiratory rates, physiological (respiration), and enzymatic (ETS) in different groups of organisms. Data of bacteria were obtained from Christensen et al. (1980), zooplankton data from King and Packard (1975), and protozoa data from Finlay et al. (1983). Jellyfish data were obtained from EOMAR (*Perseo project*). (Modified from Finlay et al. 1983)

1.7. Thesis perspectives, objectives, and hypotheses

Probably we will never know, for certain, how life started on planet Earth, but respiratory metabolism was certainly an essential feature of its early startup. We know that respiration is an ancient process, and its aerobic form was a ubiquitous component of primordial biospheric metabolism. In that sense, studying respiratory metabolism provides valuable insight into how the biota responds to environmental changes.

Since immemorial times, bacterioplankton and gelatinous zooplankton have been a conspicuous component of marine ecosystems. However, their respiratory metabolism is widely approached by allometric scaling, and CO_2 production is estimated through a constant, the respiratory quotient (RQ). These practices present restrictions. In this thesis we use physiological respiration rates (O_2 consumption and CO_2 production), in addition to respiratory enzymatic rates (activities), to study the physiology of marine bacteria and the medusozoan *Aurelia aurita*.

The study of metabolism in these marine organisms has been addressed from different perspectives and different objectives. This research makes a step in defining biochemical and physiological responses in bacterioplankton and medusozoan respiration to biotic (metamorphosis) and abiotic factors (food availability and MHW). It will facilitate the interpretation of how these organisms respond to intrinsic (metamorphosis) and external conditions (food availability and MHW). The hypotheses of this research are based on these different perspectives and objectives.

Chapter 2

Perspective: Bacterial respiratory metabolism has been followed from well-fed conditions to starvation, and RQs have been calculated from respiratory O_2 and respiratory CO_2 measurements.

Hypothesis: A constant RQ can-not define respiratory physiological rates because the RQ changes from nutrient sufficient to nutrient-limitation conditions.

Objective: Compare the respiratory metabolism of two marine bacteria, under two carbon substrates and two experimental durations, short term, and long term, from well-fed conditions to starvation.

Chapter 3

Perspective: The metabolism of polyps from the common jellyfish *A. aurita* has been followed under a simulated marine heatwave (MHW).

Hypothesis: *A. aurita* polyps increase their physiological capacity in response to the MHW, but show the capacity to recover in the post-heatwave period (recovery period).

Objective: Compare the respiratory metabolism of *A. aurita* polyps under two different marine heatwave temperatures, over three different heatwave durations, and their recovery periods.

Chapter 4

Perspective: Respiratory and excretory metabolism has been investigated from metamorphosing *A. aurita*, transitioning from polyp to medusa life stages.

Hypothesis: *A. aurita* would show changes in respiratory metabolic and enzyme activities, evincing shifts in biochemical composition during life stage transitions.

Objective: Monitor the metabolism of metamorphosing *A. aurita* as it shifts from polyp to medusa life stages, and comparison of the biochemical composition of each life stage.

1.8. References

Aguiar-González, B., Packard, T. T., Berdalet, E., Roy, S., and Gómez, M., 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, 412, 1-12.

Arístegui, J., and Montero, M. F., 1995. The relationship between community respiration and ETS activity in the ocean. Journal of plankton research, 17(7), 1563-1571.

Ates, R.M.L., 2017. Benthic scavengers and predators of jellyfish, material for a review. Plankt. Benthos Res. 12, 71–77. https://doi.org/10.3800/PBR.12.71.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., and Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Marine ecology progress series, 257-263.

Baltar, F., Bayer, B., Bednarsek, N., Deppeler, S., Escribano, R., Gonzalez, C. E., Hansman, R., Mishra, R. K., Moran, M. A., Repeta, D. J., Robinson, C., Sintes, E., Tamburini, C., Valentin, L. E and Herndl, G. J., 2019. Towards integrating evolution, metabolism, and climate change studies of marine ecosystems. Trends in ecology and evolution, 34(11), 1022-1033.

Båmstedt, U., 1979. Seasonal variation in the respiratory rate and ETS activity of deep-water zooplankton from the Swedish west coast. In Cyclic phenomena in marine plants and animals (pp. 267-274). Pergamon

Becerra, A., 2021. The Semi-Enzymatic Origin of Metabolic Pathways: Inferring a Very Early Stage of the Evolution of Life. J. Mol. Evol. 2021 893 89, 183–188. https://doi.org/10.1007/S00239-021-09994-0.

Behrends, G., and Schneider, G., 1995. Impact of *Aurelia aurita* medusae (Cnidaria, Scyphozoa) on the standing stock and community composition of mesozooplankton in the Kiel Bight (western Baltic Sea). Marine Ecology Progress Series, 127, 39-45.

Billett, D. S. M., Bett, B. J., Jacobs, C. L., Rouse, I. P., and Wigham, B. D., 2006. Mass deposition of jellyfish in the deep Arabian Sea. Limnology and Oceanography, 51(5), 2077–2083. https://doi.org/10.4319/lo.2006.51.5.2077.

Boero, F., Brotz, L., Gibbons, M. J., Piraino, S., and Zampardi, S., 2016. 3.10 Impacts and effects of ocean warming on jellyfish. Explaining Ocean Warming: Causes, Scale, Effects and Consequences. Gland: IUCN, 213-237.

Bondyale-Juez, D. R., Packard, T. T., Viera-Rodríguez, M. A., and Gómez, M., 2017. Respiration: comparison of the Winkler technique, O_2 electrodes, O_2 optodes and the respiratory electron transport system assay. Marine Biology, 164(12), 1-11.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., and West, G. B., 2004. Toward a metabolic theory of ecology. Ecology, 85(7), 1771-1789.

Bühring SI, Lampadariou N, Moodley L, Tselepides A, Witte U., 2006. Benthic microbial and whole-community responses to different amounts of ^{13}C-enriched algae:*in situ* experiments in the deep Cretan sea (eastern Mediterranean). Limnol Oceanogr 51: 157−165.

Bridge, D., Cunningham, C. W., Schierwater, B., DeSalle, R., Buss, L. W., 1992. Class-level relationships in the phylum Cnidaria: evidence from mitochondrial genome structure. Natl. Acad Sci. 89, 8750–8753.

Brocks, J.J., Jarrett, A.J.M., Sirantoine, E., Hallmann, C., Hoshino, Y., Liyanage, T., 2017. The rise of algae in Cryogenian oceans and the emergence of animals. Nat. 2017 5487669 548, 578–581. https://doi.org/10.1038/nature23457.

Cai, M., Liu, Y., Yin, X., Zhou, Z., Friedrich, M.W., Richter-Heitmann, T., Nimzyk, R., Kulkarni, A., Wang, X., Li, W., Pan, J., Yang, Y., Gu, J.D., Li, M., 2020. Diverse Asgard archaea including the novel phylum Gerdarchaeota participate in organic matter degradation. Sci. China Life Sci. 63, 886–897. https://doi.org/10.1007/S11427-020-1679-1.

Canfield, D.E., Rosing, M.T., Bjerrum, C., 2006. Early anaerobic metabolisms. https://doi.org/10.1098/rstb.2006.1906.

Canfield, D.E., Zuilen, M.A. van, Nabhan, S., Bjerrum, C.J., Zhang, S., Wang, H., Wang, X., 2021. Petrographic carbon in ancient sediments constrains Proterozoic Era atmospheric oxygen levels. Proc. Natl. Acad. Sci. 118. https://doi.org/10.1073/PNAS.2101544118.

Carlson, C. A., Del Giorgio, P. A., and Herndl, G. J., 2007. Microbes and the dissipation of energy and respiration: from cells to ecosystems. Oceanography, 20(2), 89-100.

Cartwright, P., Halgedahl, S. L., Hendricks, J. R., Jarrard, R. D., Marques, A. C., Collins, A. G., and Lieberman, B. S., 2007. Exceptionally preserved jellyfishes from the Middle Cambrian. PloS one, 2(10), e1121.

Castresana, J., Evolution, D.M.-J. of M., 1999. Respiratory chains in the last common ancestor of living organisms. Springer.

Castresana, J., Lübben, M., Saraste, M., Higgins, D.G., 1994. Evolution of cytochrome oxidase, an enzyme older than atmospheric oxygen. EMBO J. 13, 2516–2525. https://doi.org/10.1002/J.1460-2075.1994.TB06541.X.

Chance, B., and Williams, G. R., 1954. Kinetics of cytochrome b5 in rat liver microsomes. Journal of Biological Chemistry, 209(2), 945-951.

Chance, B., and Williams, G. R., 1956. The respiratory chain and oxidative phosphorylation. Adv Enzymol Relat Areas Mol Biol, 17, 65-134.

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

Chi, X., Dierking, J., Hoving, H.-J., Lüskow, F., Denda, A., Christiansen, B., Sommer, U., Hansen, T., Javidpour, J., 2021. Tackling the jelly web: Trophic ecology of gelatinous zooplankton in oceanic food webs of the eastern tropical Atlantic assessed by stable isotope analysis. Limnol. Oceanogr. 66, 289–305. https://doi.org/10.1002/LNO.11605.

Choy, C.A., Haddock, S.H.D., Robison, B.H., 2017. Deep pelagic food web structure as revealed by in situ feeding observations. Proceedings. Biol. Sci. 284. https://doi.org/10.1098/rspb.2017.2116.

Christensen, J. P., Owens, T. G., Devol, A. H., and Packard, T. T., 1980. Respiration and physiological state in marine bacteria. Marine Biology, 55(4), 267-276.

Cloud, P., Glaessner, M.F., 1982. The Ediacarian Period and System: Metazoa Inherit the Earth, science.sciencemag.org.
Condon, R.H., Steinberg, D.K., Del Giorgio, P.A., Bouvier, T.C., Bronk, D.A., Graham, W.M., Ducklow, H.W., Karl, D.M., Analyzed, H.W.D., 2011. Jellyfish blooms result in a major microbial respiratory sink of carbon in marine systems. Natl. Acad Sci. 108, 10225–10230. https://doi.org/10.1073/pnas.1015782108.
Deamer, D., 2021. Where Did Life Begin? Testing Ideas in Prebiotic Analogue Conditions. Life 2021, Vol. 11, Page 134 11, 134. https://doi.org/10.3390/LIFE11020134.
Del Giorgio, P., and Williams, P. (Eds.). 2005. Respiration in aquatic ecosystems. OUP Oxford.
Del Giorgio, P. A., and Duarte, C. M., 2002. Respiration in the open ocean. Nature, 420(6914), 379-384.
Dos Reis, M., Thawornwattana, Y., Angelis, K., Telford, M. J., Donoghue, P. C., and Yang, Z., 2015. Uncertainty in the timing of origin of animals and the limits of precision in molecular timescales. Current biology, 25(22), 2939-2950.
Duarte, C. M., Regaudie-de-Gioux, A., Arrieta, J. M., Delgado-Huertas, A., and Agusti, S., 2013. The oligotrophic ocean is heterotrophic. Annual Review of Marine Science, 5, 551-569.
Ducklow, H. W., Purdie, D. A., Williams, P. J. L., and Davies, J. M., 1986. Bacterioplankton: a sink for carbon in a coastal marine plankton community. Science, 232(4752), 865-867.
Ducklow, H. W., and Doney, S. C., 2013. What is the metabolic state of the oligotrophic ocean? A debate. Annual Review of Marine Science, 5, 525-533.
Eckford-Soper, L.K., Canfield, D.E., 2020. The global explosion of eukaryotic algae: The potential role of phosphorus? PLoS One 15, e0234372. https://doi.org/10.1371/JOURNAL.PONE.0234372.
Eme, L., Spang, A., Lombard, J., Stairs, C. W., Ettema, T. J. G., 2017. Archaea and the origin of eukaryotes. nature.com. https://doi.org/10.1038/nrmicro.2017.133.
Ettema, T.J.G., 2016. Mitochondria in the second act. Nat. 2016 5317592 531, 39–40. https://doi.org/10.1038/nature16876
Falkowski, P. G., Fenchel, T., and Delong, E. F., 2008. The microbial engines that drive Earth's biogeochemical cycles. science, 320(5879), 1034-1039.
Falkowski, P. G., and Wilson, C., 1992. Phytoplankton productivity in the North Pacific ocean since 1900 and implications for absorption of anthropogenic CO 2. Nature, 358(6389), 741-743.
Fernández-Urruzola, I., Packard, T. T., and Gómez, M., 2011. GDH activity and ammonium excretion in the marine mysid, *Leptomysis lingvura*: effects of age and starvation. Journal of experimental marine biology and ecology, 409(1-2), 21-29.
Finlay, B. J., Span, A., and Ochsenbein-Gattlen, C., 1983. Influence of physiological state on indices of respiration rate in protozoa. Comparative Biochemistry and Physiology Part A: Physiology, 74(2), 211-219.
Fleury, B., Carrasco, N., Millan, M., Vettier, L., Szopa, C., 2017. Organic chemistry in a CO_2 rich early Earth atmosphere. Earth Planet. Sci. Lett. 479, 34–42. https://doi.org/10.1016/J.EPSL.2017.09.026.
Gall, M.L., Holmes, S.P., Campbell, H., Byrne, M., 2021. Effects of marine heatwave conditions across the metamorphic transition to the juvenile sea urchin (*Heliocidaris erythrogramma*). Mar. Pollut. Bull. 163, 111914. https://doi.org/10.1016/J.MARPOLBUL.2020.111914.
Goldford, J.E., Segrè, D., 2018. Modern views of ancient metabolic networks. Curr. Opin. Syst. Biol. 8, 117–124. https://doi.org/10.1016/J.COISB.2018.01.004.
González N, Anadón R, Viesca L., 2003. Carbon flux through the microbial community in a temperate sea during summer:role of bacterial metabolism. Aquat Microb Ecol 33: 117−126.
Graham, J. B., 1988. Ecological and evolutionary aspects of integumentary respiration: body size, diffusion, and the Invertebrata. American Zoologist, 28(3), 1031-1045.
Gold, D. A., Katsuki, T., Li, Y., Yan, X., Regulski, M., Ibberson, D., Holstein, T., Steele, R. E., Jacobs, D. K. and Greenspan, R. J., 2019. The genome of the jellyfish *Aurelia* and the evolution of animal complexity. Nature ecology and evolution, 3(1), 96-104.
Gómez, M., Torres, S., and Hernández-León, S., 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. South African Journal of Marine Science, 17(1), 15-20.
Han, J., Zhang, X., Komiya, T., 2016. Integrated Evolution of Cnidarians and Oceanic Geochemistry Before and During the Cambrian Explosion. Cnidaria, past, Present Futur. World Medusa her Sisters 15–29. https://doi.org/10.1007/978-3-319-31305-4_2.
Harvey, H. W., 1956. The Chemistry and Fertility of Sea Waters, HW Harvey, Sc. D., FRS Cambridge: Cambridge University Press, 1955. Journal of the Marine Biological Association of the United Kingdom, 35(1), 289-289.
Harvey, H.W., 1928. Biological Chemistry and Physics of Sea Water. Cambridge University Press.
Hernández-León, S., and Gómez, M., 1996. Factors affecting the respiration/ETS ratio in marine zooplankton. Journal of Plankton Research, 18(2), 239-255.
Herrera, A., Packard, T., Santana, A., and Gómez, M., 2011. Effect of starvation and feeding on respiratory metabolism in *Leptomysis lingvura* (GO Sars, 1866). Journal of experimental marine biology and ecology, 409(1-2), 154-159.
Herrera, A., Gómez, M., Packard, T. T., and de Puelles, M. F., 2014. Zooplankton biomass and electron transport system activity around the Balearic Islands (western Mediterranean). Journal of Marine Systems, 131, 54-62.
Hayashida, H., Matear, R. J., and Strutton, P. G., 2020. Background nutrient concentration determines phytoplankton bloom response to marine heatwaves. Global change biology, 26(9), 4800-4811.
Hoegh-Guldberg, O., Bruno, J.F., 2010. The impact of climate change on the world's marine ecosystems. Science (80-.). https://doi.org/10.1126/science.1189930.
Holms, W. H., 1986. The central metabolic pathways of Escherichia coli: relationship between flux and control at a branch point, efficiency of conversion to biomass, and excretion of acetate. Current topics in cellular regulation, 28, 69-105.

Holstein, T. W., Watanabe, H., and Özbek, S., 2011. Signaling pathways and axis formation in the lower metazoa. Current topics in developmental biology, *97*, 137-177.

Hughes, T.P., Kerry, J.T., Álvarez-Noriega, M., Álvarez-Romero, J.G., Anderson, K.D., Baird, A.H., Babcock, R.C., Beger, M., Bellwood, D.R., Berkelmans, R., Bridge, T.C., Butler, I.R., Byrne, M., Cantin, N.E., Comeau, S., Connolly, S.R., Cumming, G.S., Dalton, S.J., Diaz-Pulido, G., Eakin, C.M., Figueira, W.F., Gilmour, J.P., Harrison, H.B., Heron, S.F., Hoey, A.S., Hobbs, J.-P.A., Hoogenboom, M.O., Kennedy, E. V., Kuo, C., Lough, J.M., Lowe, R.J., Liu, G., McCulloch, M.T., Malcolm, H.A., McWilliam, M.J., Pandolfi, J.M., Pears, R.J., Pratchett, M.S., Schoepf, V., Simpson, T., Skirving, W.J., Sommer, B., Torda, G., Wachenfeld, D.R., Willis, B.L., Wilson, S.K., 2017. Global warming and recurrent mass bleaching of corals. Nat. 2017 5437645 543, 373–377. https://doi.org/10.1038/nature21707.

Imachi, H., Nobu, M. K., Nakahara, N., Morono, Y., Ogawara, M., Takaki, Y., Takano, Y., Uematsu, K., Ikutua, T., Ito, M., Mtsui, Y., Miyazaki, M., Murata, K., Saito, Y., Sakai, S., Song, C., Tasumi, E., Yamanaka, Y., Yamaguchi, T., Kamagata, Y., Tamaki, H., and Takai, K., 2020. Isolation of an archaeon at the prokaryote–eukaryote interface. Nature, 577(7791), 519-525.

Kasting, J.F., 1993. Earth's early atmosphere. Science (80-.). 259, 920–926. https://doi.org/10.1126/SCIENCE.11536547

Keilin, D., 1966. The history of cell respiration and cytochrome. CUP Archive.

King, F. D., and Packard, T. T., 1975. Respiration and the activity of the respiratory electron transport system in marine zooplankton 1. Limnology and Oceanography, 20(5), 849-854.

Kleiber, M., 1961. The fire of life. An introduction to animal energetics. The fire of life. An introduction to animal energetics.

Kleiber, M., 1932. Body size and metabolism. Hilgardia, 6(11), 315-353.

Knoll, A. H., 2011. The multiple origins of complex multicellularity. Annual Review of Earth and Planetary Sciences, 39, 217-239.

Kolokotrones, T., Savage, V., Deeds, E. J., and Fontana, W., 2010. Curvature in metabolic scaling. Nature, 464(7289), 753-756.

Koonin, E. V., 2015. Origin of eukaryotes from within archaea, archaeal eukaryome and bursts of gene gain: eukaryogenesis just made easier? Philos. Trans. R. Soc. B Biol. Sci. 370. https://doi.org/10.1098/RSTB.2014.0333.

Kraus, J.E., Fredman, D., Wang, W., Khalturin, K., Technau, U., 2015. Adoption of conserved developmental genes in development and origin of the medusa body plan. Evodevo 6, 23. https://doi.org/10.1186/s13227-015-0017-3.

Krause, A.J., Mills, B.J.W., Zhang, S., Planavsky, N.J., Lenton, T.M., Poulton, S.W., 2018. Stepwise oxygenation of the Paleozoic atmosphere. Nat. Commun. 2018 91 9, 1–10. https://doi.org/10.1038/s41467-018-06383-y.

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

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

Lebrato, M., Molinero, J.C., Cartes, J.E., Lloris, D., Mélin, F., Beni-Casadella, L., 2013. Sinking jelly-carbon unveils potential environmental variability along a continental margin. PLoS One 8. https://doi.org/10.1371/journal.pone.0082070.

Lebrato, M., Pitt, K.A., Sweetman, A.K., Jones, D.O.B., Cartes, J.E., Oschlies, A., Condon, R.H., Molinero, J.C., Adler, L., Gaillard, C., Lloris, D., Billett, D.S.M., 2012. Jelly-falls historic and recent observations: A review to drive future research directions. Hydrobiologia. https://doi.org/10.1007/s10750-012-1046-8.

Lebrato, M., Pahlow, M., Frost, J. R., Küter, M., de Jesus Mendes, P., Molinero, J. C., and Oschlies, A., 2019. Sinking of gelatinous zooplankton biomass increases deep carbon transfer efficiency globally. Global Biogeochemical Cycles, 33(12), 1764-1783.

Leung, J.Y.S., Russell, B.D., Connell, S.D., 2019. Adaptive Responses of Marine Gastropods to Heatwaves. One Earth 1, 374–381. https://doi.org/10.1016/J.ONEEAR.2019.10.025.

Liu, Y., Makarova, K.S., Huang, W.-C., Wolf, Y.I., Nikolskaya, A.N., Zhang, X., Cai, M., Zhang, C.-J., Xu, W., Luo, Z., Cheng, L., Koonin, E. V., Li, M., 2021. Expanded diversity of Asgard archaea and their relationships with eukaryotes. Nat. 2021 5937860 593, 553–557. https://doi.org/10.1038/s41586-021-03494-3.

López-García, P., Moreira, D., 2020. Cultured Asgard Archaea Shed Light on Eukaryogenesis. Cell 181, 232–235. https://doi.org/10.1016/J.CELL.2020.03.058.

López-Urrutia, Á., San Martin, E., Harris, R. P., and Irigoien, X., 2006. Scaling the metabolic balance of the oceans. Proceedings of the National Academy of Sciences, 103(23), 8739-8744.

Luo, J. Y., Condon, R. H., Stock, C. A., Duarte, C. M., Lucas, C. H., Pitt, K. A., and Cowen, R. K., 2020. Gelatinous zooplankton-mediated carbon flows in the global oceans: a data-driven modeling study. Global Biogeochemical Cycles, 34(9), e2020GB006704.

Longhurst, A. R., 2010. Ecological geography of the sea. Elsevier.

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

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

Martínez, I., Gómez, M., and Packard, T. T., 2010. Potential respiration is a better respiratory predictor than biomass in young *Artemia salina*. Journal of experimental marine biology and ecology, 390(2), 78-83.

Mereschkowsky, C., 1910. Theorie der zwei Plasmaarten als Grundlage der Symbiogenesis, einer neuen Lehre von der Entstehung der Organismen. Biologisches Centralblatt, 30, 278-288.

MacLeod, F., Kindler, G., Wong, H., Chen, R., Burns, B.P., 2019. Asgard archaea: Diversity, function, and evolutionary implications in a range of microbiomes. ncbi.nlm.nih.gov.

Mahler, H. R., and Cordes, E. H., 1971. Biological chemistry.

Manchester, L.C., Coto-Montes, A., Boga, J.A., Andersen, L.P.H., Zhou, Z., Galano, A., Vriend, J., Tan, D.-X., Reiter, R.J., 2015. Melatonin: an ancient molecule that makes oxygen metabolically tolerable. J. Pineal Res. 59, 403–419. https://doi.org/10.1111/JPI.12267.

Margulis, L., and Sagan, D., 1986. Microcosmos. University of California Press.

Margulis, L., 1967. On the origin of mitosing cells. Journal of theoretical biology, 14(3), 225-IN6.

Marlow, H., and Arendt, D., 2014. Evolution: ctenophore genomes and the origin of neurons. Current Biology, 24(16), R757-R761.

Marques, A.C., Collins, A.G., 2005. Cladistic analysis of Medusozoa and cnidarian evolution. Invertebr. Biol. 123, 23–42. https://doi.org/10.1111/j.1744-7410.2004.tb00139.x.

Martin, W., Garg, S., Zimorski, V., 2015. Endosymbiotic theories for eukaryote origin. royalsocietypublishing.org 370, 20140330. https://doi.org/10.1098/rstb.2014.0330.

McCauley, D.J., Pinsky, M.L., Palumbi, S.R., Estes, J.A., Joyce, F.H., Warner, R.R., 2015. Marine defaunation: Animal loss in the global ocean. Science (80-.). https://doi.org/10.1126/science.1255641.

Michaelis, L., and Menten, M. L., 1913. Die Kinetik der Invertinwirkung. Biochem. z, 49(333-369), 352.

Mitchell, P., 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature, 191(4784), 144-148.

Mitterwallner, V., Suci, A.N.N., Zamani, N., Lenz, M., 2021. Exposure to moderately elevated temperatures changes food preferences in the tropical marine herbivore *Haliotis squamata*. Mar. Biol. 2021 1687 168, 1–12. https://doi.org/10.1007/S00227-021-03922-Y.

Mojzsis, S.J., Harrison, T.M., Pidgeon, R.T., 2001. Oxygen-isotope evidence from ancient zircons for liquid water at the Earth's surface 4,300 Myr ago. Nat. 2001 4096817 409, 178–181. https://doi.org/10.1038/35051557.

Mori, S., Yamaji, K., Ishida, A., Prokushkin, S. G., Masyagina, O. V., Hagihara, A., and Umari, M., 2010. Mixed-power scaling of whole-plant respiration from seedlings to giant trees. Proceedings of the National Academy of Sciences, 107(4), 1447-1451.

Moroz, L. L., 2021. Multiple origins of neurons from secretory cells. Frontiers in Cell and Developmental Biology, 9.

Narbonne, G.M., 2005. The Ediacara biota: Neoproterozoic origin of animals and their ecosystems. Annu. Rev. Earth Planet. Sci. 33, 421–442. https://doi.org/10.1146/ANNUREV.EARTH.33.092203.122519.

Nelson, D.L., Cox, M.M., 2008. Lehninger principles of biochemistry, Fifth Edition. W.H. Freeman and Company.

Noda-Garcia, L., Liebermeister, W., Tawfik, D.S., 2018. Metabolite-Enzyme Coevolution: From Single Enzymes to Metabolic Pathways and Networks. Annu. Rev. Biochem. 87, 187–216. https://doi.org/10.1146/ANNUREV-BIOCHEM-062917-012023.

Ochoa, S.,1943. Efficiency of aerobic phosphorylation in cell-free heart extracts. Journal of Biological Chemistry, 151(2), 493-505.

Olejarz, J., Iwasa, Y., Knoll, A.H., Nowak, M.A., 2021. The Great Oxygenation Event as a consequence of ecological dynamics modulated by planetary change. Nat. Commun. 2021 121 12, 1–9. https://doi.org/10.1038/s41467-021-23286-7.

Osma, N., Fernández-Urruzola, I., Gómez, M., Montesdeoca-Esponda, S., and Packard, T. T., 2016. Predicting in vivo oxygen consumption rate from ETS activity and bisubstrate enzyme kinetics in cultured marine zooplankton. Marine Biology, 163(7), 1-14.

Owens, T. G., and King, F. D., 1975. The measurement of respiratory electron-transport-system activity in marine zooplankton. Marine Biology, 30(1), 27-36.

Ozaki, K., Reinhard, C.T., 2021. The future lifespan of Earth's oxygenated atmosphere. https://doi.org/10.1038/s41561-021-00693-5.

Packard, T. T., 1985. Measurement of electron transport activity of microplankton. Advances in aquatic microbiology, 3, 207-261.

Packard, T. T., and Gómez, M., 2008. Exploring a first-principles-based model for zooplankton respiration. ICES Journal of Marine Science, 65(3), 371-378.

Packard, T. T., 1971. The Measurement of Respiratory Electron-transport Activity in Marine Phytoplankton. J. Mar. Res. 29 (3), 235–244.

Packard, T., Berdalet, E., Blasco, D., Roy, S. O., St-Amand, L., Lagacé, B.,Lee, K., and Gagne, J. P., 1996. CO_2 production predicted from isocitrate dehydrogenase activity and bisubstrate enzyme kinetics in the marine bacterium *Pseudomonas nautica*. Aquatic Microbial Ecology, 11(1), 11-19.

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

Pauly, D., Graham, W., Libralato, S., Morissette, L., Deng Palomares, M.L., 2009. Jellyfish in ecosystems, online databases, and ecosystem models. Hydrobiologia 616, 67–85. https://doi.org/10.1007/s10750-008-9583-x.

Perez, T., Garrabou, J., Sartoretto, S., Harmelin, J. G., Francour, P., and Vacelet, J., 2000. Massive mortality of marine invertebrates: an unprecedented event in northwestern Mediterranean. Comptes rendus de l'Academie des sciences. Serie III, Sciences de la vie, 323(10), 853-865.

Pincebourde, S., Sanford, E., Helmuth, B., 2009. An intertidal sea star adjusts thermal inertia to avoid extreme body temperatures. Am. Nat. 174, 890–897. https://doi.org/10.1086/648065.

Pitt, K.A., Duarte, C.M., Lucas, C.H., Sutherland, K.R., Condon, R.H., Mianzan, H., Purcell, J.E., Robinson, K.L., Uye, S.I., 2013. Jellyfish Body Plans Provide Allometric Advantages beyond Low Carbon Content. PLoS One 8, 1–10. https://doi.org/10.1371/journal.pone.0072683.

Pitt, K. A., Welsh, D. T., and Condon, R. H., 2009. Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production. Hydrobiologia, 616(1), 133-149.

Planavsky, N.J., Asael, D., Hofmann, A., Reinhard, C.T., Lalonde, S. V., Knudsen, A., Wang, X., Ossa Ossa, F., Pecoits, E., Smith, A.J.B., Beukes, N.J., Bekker, A., Johnson, T.M., Konhauser, K.O., Lyons, T.W., Rouxel, O.J., 2014.

Evidence for oxygenic photosynthesis half a billion years before the Great Oxidation Event. Nat. Geosci. 2014 74 7, 283–286. https://doi.org/10.1038/ngeo2122.

Planavsky, N. J., Crowe, S. A., Fakhraee, M., Beaty, B., Reinhard, C. T., Mills, B. J., Holstege, C. and Konhauser, K. O., 2021. Evolution of the structure and impact of Earth's biosphere. Nature Reviews Earth and Environment, 2(2), 123-139.

Pomeroy, L. R., 1974. The ocean's food web, a changing paradigm. Bioscience, 24(9), 499-504.

Purcell, J. E., Bondyale-Juez, D. R., Romero-Kutzner, V., Martínez, I., Caprioli, R., Tames-Espinosa, M., Almunia, J., Alonso, E., Packard, T.T., and Gómez, M., 2019. Food supply effects on the asexual reproduction and respiratory metabolism of *Aurelia aurita* polyps. Hydrobiologia, 846(1), 135-146.

Redfield, A. C., Ketchum, B. H., and Richards, F. A., 1963. The influence of organisms on the composition of seawater. The sea, 2, 26-77.

Reich, P. B., Hungate, B. A., and Luo, Y., 2006. Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. Annu. Rev. Ecol. Evol. Syst., 37, 611-636.

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

Richards, F. A., 1957. Oxygen in the ocean. Treatise on Marine Ecology and Paleoecology, edited by: Hedgpeth, JW, 1, 185-238.

Richardson, A.J., Bakun, A., Hays, G.C., Gibbons, M.J., 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2009.01.010.

Riley, G. A., 1951. Oxygen, phosphate, and nitrate in the Atlantic Ocean. Bull. Bingham Oceanogr. Coll., 13(1), 1-124.

Robinson, C., and Williams, P. L. B., 2005. Respiration and its measurement in surface marine waters. Respiration in aquatic ecosystems, 147-180.

Robinson, C., 2019. Microbial Respiration, the Engine of Ocean Deoxygenation. Front. Mar. Sci. 0, 533. https://doi.org/10.3389/FMARS.2018.00533.

Romero-Kutzner, V., Packard, T. T., Berdalet, E., Roy, S. O., Gagné, J. P., and Gómez, M., 2015. Respiration quotient variability: bacterial evidence. Marine Ecology Progress Series, 519, 47-59.

Roy, S., and Packard, T., 2001. CO_2 production rate predicted from isocitrate dehydrogenase activity, intracellular substrate concentrations and kinetic constants in the marine bacterium *Pseudomonas nautica*. Marine Biology, 138(6), 1251-1258.

Savenkoff, C., Packard, T. T., Rodier, M., Gérinno, M., Lefèvre, D., and Denis, M., 1995. Relative confribution of dehydrogenases to overall respiratory ETh activity in some marine organisms. Journal of plankton research, 17(8), 1593-1604.

Scanes, E., Parker, L., O'Connor, W., Dove, M., Ross, P. M., 2020. Heatwaves alter survival of the Sydney rock oyster, Saccostrea glomerata. Marine pollution bulletin, 158, 111389.

Scanes, E., Scanes, P., communications, Ross, P.M., 2020. Climate change rapidly warms and acidifies Australian estuaries. Nature communications, 11(1), 1-11.

Shanks, A.L., Rasmuson, L.K., Valley, J.R., Jarvis, M.A., Salant, C., Sutherland, D.A., Lamont, E.I., Hainey, M.A.H., Emlet, R.B., 2020. Marine heat waves, climate change, and failed spawning by coastal invertebrates. Limnol. Oceanogr. 65, 627–636. https://doi.org/10.1002/LNO.11331.

Siekevitz, P., 1957. Powerhouse of the cell. Scientific American, 197(1), 131-144.

Smale, D.A., Wernberg, T., Oliver, E.C.J., Thomsen, M., Harvey, B.P., Straub, S.C., Burrows, M.T., Alexander, L. V., Benthuysen, J.A., Donat, M.G., Feng, M., Hobday, A.J., Holbrook, N.J., Perkins-Kirkpatrick, S.E., Scannell, H.A., Sen Gupta, A., Payne, B.L., Moore, P.J., 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat. Clim. Chang. 1. https://doi.org/10.1038/s41558-019-0412-1.

Spang, A., Saw, J. H., Jørgensen, S. L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A. E., van Ejik, R., Schepler, C., Guy, L., and Ettema, T. J., 2015. Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature, 521(7551), 173-179.

Steinmetz, P. R., Kraus, J. E., Larroux, C., Hammel, J. U., Amon-Hassenzahl, A., Houliston, E., Wörheide, G., Nickel, M., Degnan, Bernard and Technau, U. (2012). Independent evolution of striated muscles in cnidarians and bilaterians. Nature, 487(7406), 231-234.

Suryan, R.M., Arimitsu, M.L., Coletti, H.A., Hopcroft, R.R., Lindeberg, M.R., Barbeaux, S.J., Batten, S.D., Burt, W.J., Bishop, M.A., Bodkin, J.L., Brenner, R., Campbell, R.W., Cushing, D.A., Danielson, S.L., Dorn, M.W., Drummond, B., Esler, D., Gelatt, T., Hanselman, D.H., Hatch, S.A., Haught, S., Holderied, K., Iken, K., Irons, D.B., Kettle, A.B., Kimmel, D.G., Konar, B., Kuletz, K.J., Laurel, B.J., Maniscalco, J.M., Matkin, C., McKinstry, C.A.E., Monson, D.H., Moran, J.R., Olsen, D., Palsson, W.A., Pegau, W.S., Piatt, J.F., Rogers, L.A., Rojek, N.A., Schaefer, A., Spies, I.B., Straley, J.M., Strom, S.L., Sweeney, K.L., Szymkowiak, M., Weitzman, B.P., Yasumiishi, E.M., Zador, S.G., 2021. Ecosystem response persists after a prolonged marine heatwave. Sci. Reports 2021 111 11, 1–17. https://doi.org/10.1038/s41598-021-83818-5.

Sverdrup, H. U., Johnson, M. W., and Fleming, R. H., 1942. The Oceans: Their physics, chemistry, and general biology (Vol. 1087). New York: Prentice-Hall.

Takao, M., Okawachi, H., Uye, S.I., Okawachi, H., 2014. Natural predators of polyps of *Aurelia aurita* s.l. (Cnidaria: Scyphozoa: Semaeostomeae) and their predation rates. Plankt. Benthos Res. 9, 105–113. https://doi.org/10.3800/pbr.9.105.

Tames-Espinosa, M., Martínez, I., Romero-Kutzner, V., Bondyale-Juez, D. R., Packard, T. T., and Gomez, M., 2018. $NADP^+$-dependent isocitrate dehydrogenase activity in marine plankton. Marine Chemistry, 204, 86-94.

Thuesen, E. V., 2005. Intragel oxygen promotes hypoxia tolerance of scyphomedusae. J. Exp. Biol. 208, 2475–2482. https://doi.org/10.1242/jeb.01655.

Tinta, T., Klun, K., and Herndl, G. J., 2021. The importance of jellyfish–microbe interactions for biogeochemical cycles in the ocean. Limnology and Oceanography, 66(5), 2011-2032.
Weiss, M., Sousa, F., Mrnjavac, N., Neukirchen, S., Roettger, M., Nelson-Sathi, S., William, F. M., 2016. The physiology and habitat of the last universal common ancestor. nature.com. https://doi.org/10.1038/NMICROBIOL.2016.116.
Williams, P. J. L., Flows of Energy and Materials in Marine Ecosystems: 271, 1984.
Vosjan, J. H.,1982. Respiratory electron transport system activities in marine environments. Hydrobiological Bulletin, 16(1), 61-68.
Vaquer-Sunyer, R., and Duarte, C. M., 2008. Thresholds of hypoxia for marine biodiversity. Proceedings of the National Academy of Sciences, 105(40), 15452-15457.
Vernadsky V., 1926. The Biosphere. Trans. Langmuir, D McMenamin, Springer, New York.
Walsh, K., and Koshland, D. E., 1984. Determination of flux through the branch point of two metabolic cycles. The tricarboxylic acid cycle and the glyoxylate shunt. Journal of Biological Chemistry, 259(15), 9646-9654.
Whelan, N. V., Kocot, K. M., Moroz, T. P., Mukherjee, K., Williams, P., Paulay, G., Moroz, L. and Halanych, K. M., 2017. Ctenophore relationships and their placement as the sister group to all other animals. Nature ecology and evolution, 1(11), 1737-1746.
Whicher, A., Camprubi, E., Pinna, S., Herschy, B., Lane, N., 2018. Acetyl Phosphate as a Primordial Energy Currency at the Origin of Life. Orig. Life Evol. Biosph. 2018 482 48, 159–179. https://doi.org/10.1007/S11084-018-9555-8.
Wignall, P. B., and Twitchett, R. J., 1996. Oceanic anoxia and the end Permian mass extinction. Science, 272(5265), 1155-1158.
Williams, T., Cox, C., Foster, P., Szöllósi, G. J., 2020. Phylogenomics provides robust support for a two-domains tree of life. nature.com.
Williams, T.A., Foster, P.G., Cox, C.J., Embley, T.M., 2013. An archaeal origin of eukaryotes supports only two primary domains of life. Nat. 2013 5047479 504, 231–236. https://doi.org/10.1038/nature12779.
Xavier, J. C., Gerhards, R. E., Wimmer, J. L., Brueckner, J., Tria, F. D., and Martin, W. F., 2021. The metabolic network of the last bacterial common ancestor. Communications biology, 4(1), 1-10.
Zaremba-Niedzwiedzka, K., Caceres, E. F., Saw, J. H., Bäckström, D., Juzokaite, L., Vancaester, E., Seitz, K. W., Anantharaman, K., Starnawski, P., Kjeldsen, K. U., Stott, M. B., Nunoura, T., Banfield, J. F., Schramm, A., Baker, B. J., Spang, A., and Ettema, T. J., 2017. Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature, *541*(7637), 353-358.
Zimorski, V., Mentel, M., Tielens, A.G.M., Martin, W.F., 2019. Energy metabolism in anaerobic eukaryotes and Earth's late oxygenation. Free Radic. Biol. Med. 140, 279–294. https://doi.org/10.1016/J.FREERADBIOMED.2019.03.030

CHAPTER

2

Respiration in Marine Bacteria

In nature, nothing exists alone.

Rachel Carlson, 1962

(This manuscript has been previously published as: Romero-Kutzner, V., Packard, T. T., Berdalet, E., Roy, S. O., Gagné, J. P., and Gómez, M. (2015). Respiration quotient variability: bacterial evidence. *Marine Ecology Progress Series, 519,* 47-59)

2.1. Abstract

Respiratory metabolism was compared between 2 different physiological states of acetate- and pyruvate-grown cultures of *Pseudomonas nautica* and *Vibrio natriegens*. Here, we analyse 35 h and 520 h experiments in which time-courses of protein, pyruvate, acetate, respiratory CO_2 production (RCO_2), respiratory O_2 consumption (RO_2), isocitrate dehydrogenase (IDH) activity, and potential respiration (Φ) were measured. Respiratory quotients (RQs) were calculated as the ratio of the respiration rates (RCO_2/RO_2). Such RQs are widely used in ocean ecosystem models, in calculations of carbon flux, and evaluations of the ocean's metabolic balance. In all the cultures, the RQ tended to increase. In the case of *P. nautica* on acetate, the RQ rose nearly an order of magnitude from values below 1 during carbon-substrate sufficiency to values close to 10 during carbon-substrate deficiency. In all cultures, the respiration rates during the growth period paralleled the biomass increase, but after the substrates were exhausted, the respiration rates fell. In contrast, through this same transition period, the IDH activity and the Φ remained relatively high for the first 10 h of carbon-substrate deprivation, and then, these enzyme activities fell slowly, along with the biomass, as the carbon-substrate deprivation continued. The nutritional state of the bacteria affected the RQ, rendering the RQ variable for physiological and ecological purposes. These results argue that ecosystem models, oceanographic calculations of carbon flux, and evaluations of the ocean's metabolic balance that are influenced by bacterial metabolism need to be reconsidered in light of RQ variability.

2.2. Introduction

The respiration quotient (RQ), the ratio of the CO_2 produced to the O_2 consumed in respiration, is an old concept, dating back to the 1860s. Armsby and Moulton (1925) and Lusk (1928) discuss its use in animal husbandry and human physiology, presenting research of Pettenkofer and Voit (1866), Warburg (1926), and others who were measuring RQ much earlier. RQ is a dimensionless ratio, calculated mole per mole, and is an index of the type of organic matter being oxidised in respiration. From the shift in RQ from 0.7 to 1.0, an investigator can differentiate lipid-based biological oxidation (metabolism) from carbohydrate-based metabolism.

The majority of studies of respiration in aquatic ecosystems are based on RQ ranging from 0.7 to 1.2 (Berggren et al., 2012), but in many ecological calculations of microbial respiration, an RQ of 1 is assumed (González et al., 2003; Bühring et al., 2006). This practice occurs even though it is known that the RQ varies from 0.65 to 1.4 in individual ecological microbial communities (del Giorgio et al., 2006). Amado et al. (2013) argue that such an assumption is the only feasible option given the dearth of physiological studies that document the RQ variability in bacterial respiration and they conclude that more investigations of RQ variability are needed.

A recent study of respiration in yeast found an RQ range of 0.4 to 1.4 (Slavov et al., 2014). When an organism is oxidizing carbohydrates, its CO_2 production rate and its O_2 consumption rate are equal, i.e. the RQ = 1. When an organism is oxidizing protein, the RQ is around 0.8, and when oxidizing lipids, it is close to 0.7 (Cantarow and Schepartz, 1967; Guyton, 1971; Hoar, 1975; Gnaiger, 1983; Stanier and Forsling, 1990).

When burning organic compounds richer in O_2 than carbohydrates, such as carboxylic acids, and when converting carbohydrates to fat, RQ values can be >1. When metabolism consumes oxygen-rich oxalic acid ($C_2H_2O_4$), RQ can rise to 4 (Dilly, 2001). At the other end of the scale, gluconeogenesis (glucose synthesis) occurs when RQ falls below 0.7 (Cantarow and Schepartz, 1967). In this way, RQ variations are generally explained by the fact that the amounts of CO_2 and O_2 produced are dependent on the oxidation state of the substrate and the pathways by which the substrate is metabolized (Burton, 1982; Kader, 1987).

Berggren et al. (2012) argue from their field studies of Quebec lakes that variability in RQ indicates shifts in bacterial physiology and carbon consumption that cannot be deduced from other measurements. However, in oceanography, only a handful of RQ measurements have been made (Oviatt et al., 1986; Robinson et al., 2002). Nevertheless, when the effort is made to determine the RQ for each situation, as did Obernosterer et al. (2008) in their Southern Ocean iron-fertilization experiment, an RCO_2 production or a carbon oxidation calculation is more accurate.

Whether in the laboratory or the field, an RQ is essential in calculating either organic carbon consumption or CO_2 production from RO_2 consumption measurements (Boucher et al., 1994; Bergström, 2011; Giering et al., 2014). In this way, RQ becomes an influential factor in ocean carbon-cycle studies, for calculating carbon flux from plankton metabolism (Packard and Christensen, 2004; Steinberg et al., 2008; Packard and Gómez, 2013; Osma et al., 2014), and in investigating whether the ocean is autotrophic or heterotrophic (Ducklow and Doney, 2013).

Here, we measure the RQ in cultures of the marine bacteria *Pseudomonas nautica* and *Vibrio natriegens* as they pass from carbon substrate sufficiency to carbon substrate limitation.

P. nautica is an oil-degrading bacterium from the Gulf of Fos, France (Bonin et al., 1987a,b), and was used to advance studies of oil-spill bio-remediation (Swannell et al., 1996). *V. natriegens* is a well-studied non-pathogenic marine bacterium discovered by Payne et al. (1961) in a Georgia salt marsh (Lee and Levy, 1987; Lee 1995).

Carbon-substrate limitation conditions are common in the ocean as marine microbial communities pass from bloom to post-bloom conditions (Liu et al., 2013), and are likely dominant in oligotrophic ecosystems where bacterial respiration accounts for up to 59% of plankton respiration (Robinson and Williams, 2005). Here, we show that in our cultures, the RQ can range higher than the upper values reported above.

To investigate this wide range, we examine the time-courses of the physiological respiration rates (RO_2 and RCO_2) and the enzymatic activities of the respiratory electron transport system (ETS) and isocitrate dehydrogenase (IDH). ETS and IDH activities are the biochemical enzyme activities that largely control the RO_2 consumption and RCO_2 production (Packard et al., 1996a; Nelson and Cox, 2005; Gnaiger, 2009). Via aerobic respiration, marine organisms obtain the energy to live from a wide range of compounds that are reduced in different, but well-coordinated biochemical pathways. Two of these key pathways are the Krebs or tricarboxylic acid (TCA) cycle and ETS. IDH is proposed to be a future analytical method for CO_2 calculations in the ocean (Packard et al., 1996a; Roy and Packard, 2001; Tames-Espinosa et al., 2018), but it needs further investigation, and improvement (Robinson and Williams, 2005).

In our study, we work with 2 substrates: acetate and pyruvate. Acetate, a 2-carbon molecule, is transformed into acetyl-CoA at the entry of the TCA and, via IDH and alpha-ketoglutarate dehydrogenase, loses both carbons as CO_2 and produces 8 reducing equivalents (e^-) that in turn, lead to the consumption

of 2 molecules of O_2 ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$) at cytochrome oxidase in the ETS. Accordingly, the potential RQ for acetate would be 1.0. When pyruvate (a 3-carbon molecule) is cycled through the TCA, it produces 10 reducing equivalents and 3 molecules of CO_2 and it consumes 2.5 O_2 molecules. This would result in an RQ of 1.2. Thus, the use of these different carbon substrates becomes a tool to generate different values of RQ. In *P. nautica*, we found that acetate-grown cultures showed an RQ increase to 10 during carbon-limitation, whereas RQ in pyruvate-grown cultures remained near 1.0 (Roy et al., 1999). However, because of the continued lack of published physiological information about RQ, we wanted to investigate this topic with another species and over longer periods of carbon-limitation. We expected that different levels of carbon limitation and different durations of starvation would generate a wider range of RQs.

2.3. Material and Methods

2.3.1. Experimental Design

To investigate the RQ in different bacterial growth stages, time-course experiments were run on batch cultures at 22˚C, maintained on pyruvate or acetate as described by Berdalet et al. (1995) and Packard et al. (1996b). Short-term experiments were run for a maximum of 35 h, and a (different) set of long-term experiments were run for 2 to 3 weeks (wk). The bacteria cultures were grown in 25 cotton-plugged 500 ml Erlenmeyer flasks containing 100 ml of media kept slowly rotating on an ‘orbital shaker table’. At intervals over the different time courses, 2 flasks were chosen randomly, 25 ml of culture were transferred to the airtight respirometer flasks, and the respiration was measured. Then, samples of the remaining culture in the chosen flasks were taken in duplicate for growth (optical density at 550 nm

[OD550]), protein, pyruvate, acetate, RCO_2, RO_2, IDH activity, and potential respiration (Φ) as the cultures grew exponentially, reached steady state, and passed into senescence.

2.3.2. Bacterial cultures

V. natriegens (ATCC 33788) and *P. nautica* (Strain 617 from Dr. P. Bonin, Université de la Méditerranée, Marseille, France) were used for these cultures. Before any experiment, the bacteria had been adapted for a minimum of 15 generations to the experimental media (pyruvate or acetate). To inoculate the cultures, a sample from a mother culture in exponential or early stationary phase was used. Cultures were continuously agitated on an orbital shaker at 100 rpm at 22ºC and growth was followed spectrophotometrically at 550 nm (OD550). The OD550 had an initial value after inoculation of 0.1 absorbance units.

2.3.3. Culture Media

The general culture procedures have been described by Berdalet et al. (1995) and Roy et al. (1999). More specifically, *P. nautica* was cultured according to Packard et al. (1996a) and the medium for *V. natriegens* was developed from the media of Niven et al. (1977), Baumann and Baumann (1981), King and Berman (1984) and Nissen et al. (1987). The optimal conditions for growth of *V. natriegens* were experimentally established. They were grown on: 400 mM NaCl, 10 mM $MgSO_4 \cdot 7H_20$, 10 mM $CaCl_2 \cdot 2H_20$, 10 mM KCl, 25 mM NH_4Cl, 0.33 mM phosphate buffer, 0.01 mM $FeSO_4 \cdot 7H_20$. Initial concentration of the carbon source in culture media was 30 mM sodium acetate or 20 mM pyruvate. (Note that these concentrations would provide the same amount of organic carbon at the start of an experiment). Reagents for the culture media were obtained from Sigma-Aldrich Co. All components were dissolved in 0.22 µm filtered deionized water (except $FeSO_4 \cdot 7\ H_20$ and

phosphate buffer) and pH was adjusted to 7.5 with 1 N NaOH. To remove particles, the medium was filtered through a GF/F glass fiber filter. Later it was autoclaved for 45 min at 121˚C. Then, to avoid precipitation during the autoclaving, the phosphate buffer (0.67 M, pH 7.5) and the iron sulphate solution ($FeSO_4 \cdot 7H_2O$, 0.1 mM) were prepared separately. The PO_4 buffer was sterilized by autoclaving and $FeSO_4$ solution was filtered through 0.22 µm acrodiscs. Finally, both solutions were kept frozen and were added to the culture medium just before use.

2.3.4. Protein measurements

For every flask, protein samples were taken in duplicate. Five to 10 ml of culture, depending on the level of biomass, were centrifuged at 10000 x *g* at 4˚C for 15 min and then frozen in liquid nitrogen (Ahmed et al., 1976). Later, the bacterial pellets were defrosted, mixed well with 2 to 4 ml 1 N NaOH (at 22˚C), and analysed for protein in aliquots of 0.5 ml by the Lowry Method (Lowry et al., 1951) according to Berdalet et al. (1995). If the absorbance at 750 nm of the samples exceeded 0.4, the homogenates were diluted and analysed again. For standardization, duplicate measurements of Bovine Serum Albumin (BSA) from Sigma Chemical Company were used.

2.3.5. Biochemical parameters

For acetate and/or pyruvate and enzyme activity (IDH and ETS), samples were also taken in duplicate from every experimental flask and prepared as for the protein samples (Berdalet et al., 1995). The supernatant fluid was collected in an acid-rinsed Corex tube and then stored in liquid nitrogen for acetate or pyruvate analysis after centrifugation. Samples were thawed and adjusted to pH 2 by adding concentrated phosphoric acid and analysed by high performance liquid chromatography.

Pellets for IDH extraction were resuspended in 2 ml of buffer at 0 to 4˚C and activity was determined spectrophotometrically at 340 nm following the NADPH production (Reeves et al., 1971, 1972; Holms and Bennett, 1971; Berdalet et al., 1995) after $NADP^+$ addition. IDH catalyzes the reaction producing CO_2 during isocitrate oxidation.

Results are given as µmol CO_2 min^{-1} (liter of culture)$^{-1}$. The IDH activity was calculated from the regression line of OD340 versus time. NADPH was used as the standard converting OD340 to CO_2 (µmol) because, from the equation above, NADPH production is stoichiometrically equal to CO_2 production (1:1).

Samples for ETS were resuspended at 0 to 4˚C in 2 ml of homogenizing buffer, and measured kinetically for ETS activity, at 490nm, with a modification of the Packard and Williams (1981) method as described in Packard and Christensen (2004). To calculate Φ, ETS in µmol e^-min^{-1} L^{-1} is divided by 4 ($4e^- + 4H^+ + O_2 \rightarrow 2H_2O$) to give µmol O_2 min^{-1} L^{-1} of culture.

2.3.6. Respiration measurements

RCO_2 and RO_2 were simultaneously measured by a Micro-Oxymax system (Columbus Instruments International Corporation, Columbus, OH, USA). The Micro-Oxymax is a computer-controlled closed-circuit respirometer. The system monitors gas concentrations in the headspace above the culture (www.colinst.com) via 2 well-sealed tubes. The culture itself was sampled by syringe through a septum in each experimental flask.

Calculations of incremental and accumulated values for consumption of O_2 and production of CO_2 were possible because of periodic sensing of the gas concentration. Oxygen measurements are carried out with an oxygen detector based on the principle of a PbO_2 fuel cell. CO_2 measurements were made with an infrared CO_2 detector (sensitive to the 2000 µm absorption peak of CO_2).

Both were part of the Micro-Oxymax system. The detection limit was 10 ppm of O_2 and CO_2. This is equivalent to 0.44 µmol L^{-1}. The maximum sensitivity for the two rates was 8.9 nmols h^{-1}. The instrument had a multiple sample chamber (for up to 20 channels), a reference chamber and a computerized data acquisition and analysis system.

The Micro-Oxymax maintained aerobic conditions because it periodically refreshed (replenished) the air in the headspace if the O_2 level fell below 19.3 % to avoid oxygen limitation. Cultures were maintained at 22˚C and continuously shaken (to optimize gas exchange). Respiration was given as µmol O_2 min^{-1} L^{-1} and µmol CO_2 min^{-1} L^{-1}. Note that here, RCO_2 refers to CO_2 generated by intact bacteria suspended in their growth medium and RO_2 refers to O_2 consumed under the same conditions.

A normal measurement took about 30 min. Measurements over 30 min intervals were referenced against a baseline, a control was made in duplicate. The calibration of the oxygen detector was done with high precision gas standards. The respirometry for all the original measurements was described by Berdalet et al. (1995) and Packard et al. (1995).

2.3.7. Statistical analysis

Data were analysed using the program R from the R Development Core Team 2010 (R Foundation for Statistical Computing, Vienna, Austria). Relationships between RCO_2/RO_2 over different time scales (short and long-term experiments) were obtained from the regression equations, using confidence limits of 95% and the Pearson correlation coefficients. ANCOVA was applied to determine statistical differences between slopes and ordinates in the regression lines. Normality of residuals was confirmed by Shapiro-Wilk test.

2.4. Results

Data summary

Two types of experiments were conducted. Short-term experiments that ran up to 35 h and long-term (2 to 3 wk) experiments that ran for 330 and 520 h. In all these experiments, we used the 2 bacterial species *P. nautica* and *V. natriegens* growing on either acetate or pyruvate. In Figure 1, measurements of substrate (carbon source), protein, enzyme activity (IDH and Φ), RCO_2, and RO_2 are shown. Figure 2 presents comparable long-term experiments in which we made the same measurements. Data for both Figures are represented on the same scale so that all the time-courses could be shown in the same graph. However, this required the use of multipliers, as explained in the legends for Figures 1 and 2.

2.4.1. Enzyme activity: IDH and Φ

Short-term experiments. Enzymatic activity is present even during starvation (Figure 1). In general, the IDH activity and Φ rise during the hours of substrate sufficiency. After substrate limitation sets in, both parameters in the *P. nautica* cultures stay relatively constant with Φ higher than IDH. This can be seen when either enzyme activity is normalized by biomass because both the enzyme activities and the biomass trend in parallel with culture age (Figure 1). During the same conditions of substrate limitation, IDH in the *V. natriegens* cultures retains similarly high activity levels.

Long-term experiments. Enzymatic processes in the long-term experiments (Figure 2) after 24 h varied little. In all experiments, enzymatic activity was observable even during carbon substrate limitation. Almost all IDH and Φ values were similar; IDH ranged from 0.5 to 66 µmol CO_2 min^{-1} l^{-1} of culture, while Φ ranged from 1.3 to 284 µmol O_2 min^{-1} l^{-1} of culture. In *P. nautica* on acetate (Figure 2A), IDH rose with carbon substrate limitation and then decreased with Φ until ~350 h. In *V. natriegens* growing on acetate, both IDH and Φ dropped after carbon substrate limitation to levels lower than 4.7 µmol CO_2 min^{-1} l^{-1} and 6.2 µmol O2 min^{-1} l^{-1} (Figure 2B), respectively. In experiments with *P. nautica* growing on pyruvate, Φ and IDH remained almost constant for 350 h after carbon substrate limitation (Figure 2C). In experiments with *V. natriegens* growing on pyruvate, Φ maintained activity around 72 µmol O_2 min^{-1} l^{-1} of culture for the first 100 h and then decreased to barely detectable levels of activity after 500 h. IDH activities decreased more rapidly in the first 100 h to 13 µmol CO_2 min^{-1} l^{-1} of culture and then more slowly in the next 400 h to 4.5 µmol CO_2 min^{-1} l^{-1} of culture (Figure 2D). Substrate deficiency characterized most of the culture's life. Note that both IDH and Φ tended to decrease with time as the bacterial cultures starved.

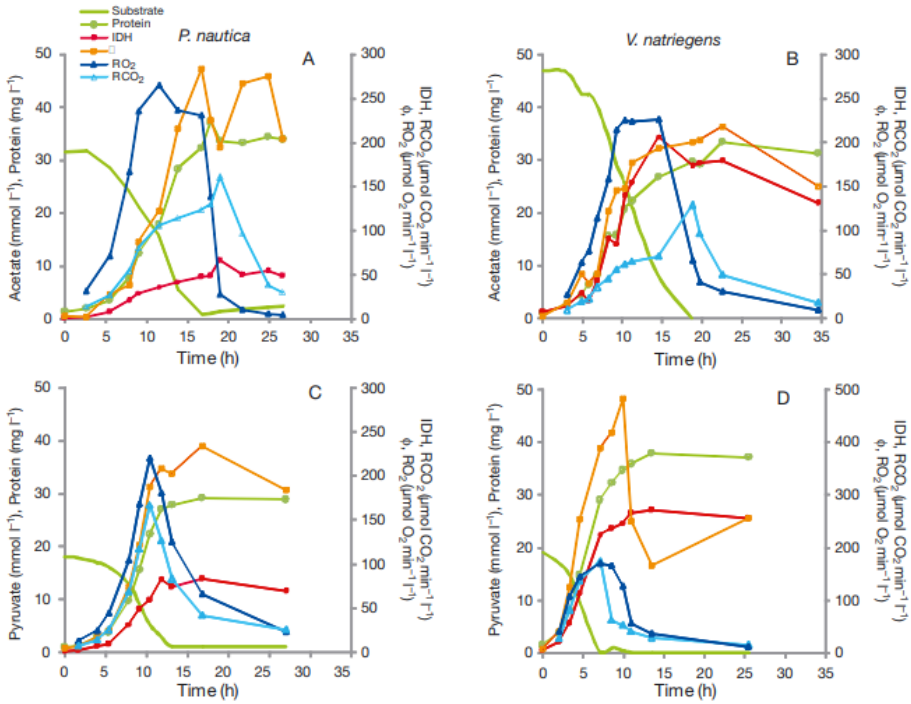


Figure 1. Time courses of respiration, enzyme activity, biomass, and carbon source in the short-term experiments. The key in panel **A** explains the curves. **A.** *P. nautica* in acetate. **B.** *V. natriegens* in acetate. **C.** *P. nautica* in pyruvate. **D.** *V. natriegens* in pyruvate. Note that, depending on the experiment, the cultures are substrate-sufficient in the first 5-15 h and substrate-limited (starved) afterwards. For display clarity, the protein data have been divided per 10 and RCO_2 and RO_2 have been multiplied by 5. IDH: isocitrate dehydrogenase, Φ: potential respiration, RO_2: O_2 consumed, RCO_2: CO_2 generated.

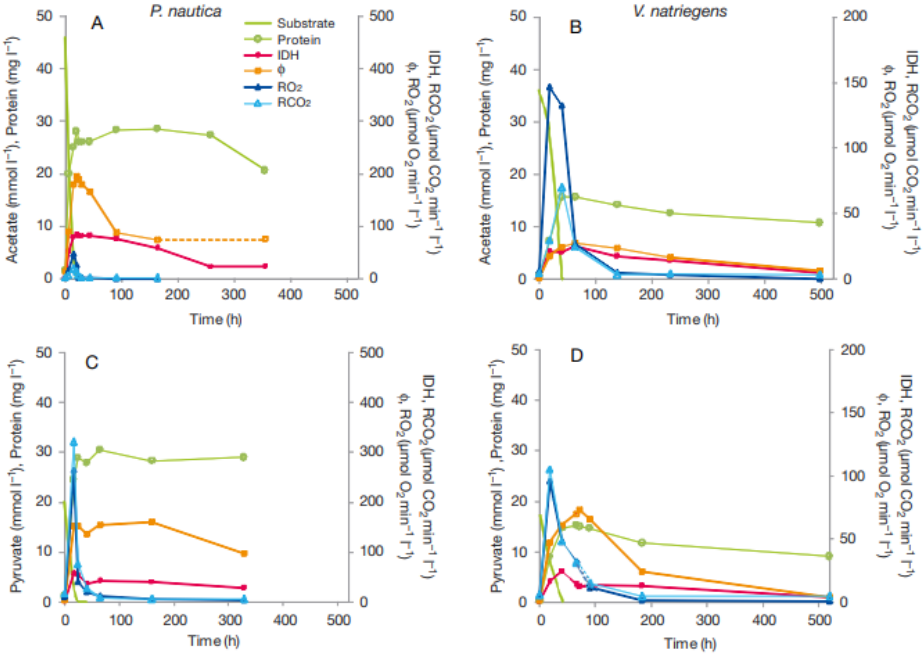


Figure. 2. Time courses of respiration, enzyme activity, biomass, and carbon source in the long-term experiments. Note that except for the first 30-35 h, the cultures are continually starved for carbon. They are substrate-limited. During this period the biomass and the enzyme activities decline while the respiration ($RO_2$and RCO_2) is barely measurable. To fit the data on this graph, the protein data has been divided by 10, while the respiration (RO_2 and RCO_2) has been multiplied by 10. Dotted lines symbolize absent data. IDH: isocitrate dehydrogenase, Φ: potential respiration, RO_2: O_2 consumed, RCO_2: CO_2 generated.

2.4.2. Physiological measurements: RCO_2 and RO_2

Short-term experiments. In all short-term experiments, RO_2 was higher than RCO_2 before starvation was reached (Figure 1). Experimental results with *P. nautica* and *V. natriegens* on acetate were similar, but the 2 curves were out of phase. The peaks were displaced in time. In these experiments, RO_2 rose rapidly, but as starvation began, around 15 h, it fell. During this RO_2 fall, RCO_2 peaked and then rapidly declined (Figure 1A, B). In the 2 pyruvate-based experiments, pyruvate decreased from an initial level of 20 mmol l^{-1} to nearly zero after ~10 h (Figure 1C, D). In the *P. nautica* culture, both RCO_2 and RO_2 rose and fell in parallel (Figure 1C). The decrease in respiration occurred before carbon substrate depletion. In the comparable *V. natriegens* pyruvate culture (Figure 1D), the respiration signals were out of phase; however, in both of the acetate-based cultures, they were even more out of phase.

In comparing the RCO_2 to RO_2 in the short-term experiments (Figure 1 and 3), the relationship between RCO_2 and RO_2 differs with each experiment. High variability is clear (Figure 3). However, note that in the *P. nautica* in pyruvate (pnpy) culture (Figure 1C and 3), the data from both the carbon-sufficient and the carbon-limited phases follows the same correlation ($RCO_2 = 0.7295\ RO_2 - 0.5969$, $n = 10$, $r^2 = 0.9882$, $p < 0.05$). For all the experiments, if the substrate-starved and the substrate-sufficient data are considered separately, 2 different relationships appear (Figure 4). From the equations in Table 1, it is clear that the starved acetate-based cultures have different RQs (slopes) than the starved pyruvate-based cultures.

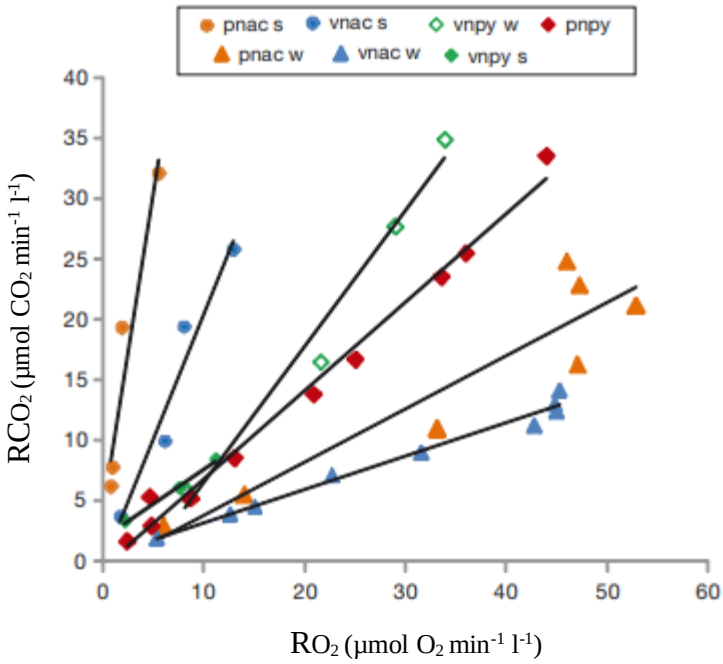


Figure 3. RCO_2 production versus RO_2 consumption in four short-term experiments, w is for well-fed and s is for starvation conditions. pnac = *P. nautica* in acetate, pnpy = *P. nautica* in pyruvate, vnac = *V. natriegens* in acetate, vnpy = *V. natriegens* in pyruvate. For pnpy, the well-fed and the starved conditions had the same slope.

Table 1. Minimum, maximum and mean RQ from exponential phase (w=well fed) and stationary phase (s=starvation) for short-term and long-term experiments. (Note that in all experiments the transition points between the well-fed and starved conditions were excluded). Regression equations corresponds to $RCO_2 = mRO_2 + b$. pnac= *P. nautica* on acetate, vnac= *V. natriegens* on acetate, pnpy= *P. nautica* on pyruvate, vnpy= *V. natriegens* on pyruvate.

			RQ						
	Bact-eria	Cond-ition	MIN	MAX	Mean	SD ±	Regression* y=	r^2	(n)
Short-term experiments	pnac	w	0.3	0.5	0.4	0.06	0,4054x -0,4045	0.92	6
		s	5.7	9.59	7.2	1.66	5,2962x +3,4891	0.92	4
	vnac	w	0.3	0.3	0.3	0.02	0,2769x +0,3332	0.98	9
		s	1.6	2.4	2.0	0.32	2,0564x -0,4225	0.95	4
	pnpy	w	0.6	0.8	0.6	0.07	0,7598x -1,2327	0.99	5
		s	0.6	1.1	0.9	0.3			2
	vnpy	w	0.7	1.0	0.9	0.16	1,126x -5,0411	0.97	4
		s	0.7	1.3	1.0	0.34	0,5737x +1,7177	0.99	3
Long-term experiments	pnac	w	0.3	0.8	0.5	0.25	0,383x +0,1797	0.99	3
		s	2.4	7.8	4.7	2.23	4,2758x -0,1633	0.76	5
	vnac	w	0.2	0.7	0.4	0.33			2
		s	0.6	1.2	0.9	0.29	0,928x -0,0242	0.99	3
	pnpy	w	1.2	1.9	1.6	0.34	1,1533x +1,5905	0.99	3
		s	0.6	1.2	0.9	0.30	0,3266x +0,3467	0.98	3
	vnpy	w	0.8	1.1	1.0	0.18			2
		s	1.0	4.5	2.4	1.64	0,86x +0,3836	0.99	4

*****Regression lines: p-values <0.05 in all cases

Long-term experiments. RCO_2 and RO_2 decreased to low values within the first 150 h of the experiment (Figure 2). After that time, RCO_2, although low, steadily increased in relation to RO_2. This trend contrasted greatly with both the biomass and the enzyme activity data, all of which maintained relatively high values throughout substrate limitation. All the slopes between the 2 respiration measurements (RCO_2 and RO_2) for the long-term experiments were compared (Figure 4) and displayed a good correlation in all cases except in *V. natriegens* on acetate. The slope of these functions was the overall RQ. This is another way to estimate RQ, but requires sufficient samples collected during the same culture condition. ANCOVA analysis between cultures that were growing on the same carbon substrate (i.e. *P. nautica* or *V. natriegens* on acetate) shows that the slopes were not significantly different (Figure 4; $p > 0.05$). In contrast, for the same bacterial species but for different substrates (i.e. *P. nautica* on acetate or pyruvate), the slopes appeared to be significantly different ($p < 0.05$). All the slopes (RQs) during starvation conditions were significantly different ($p < 0.05$).

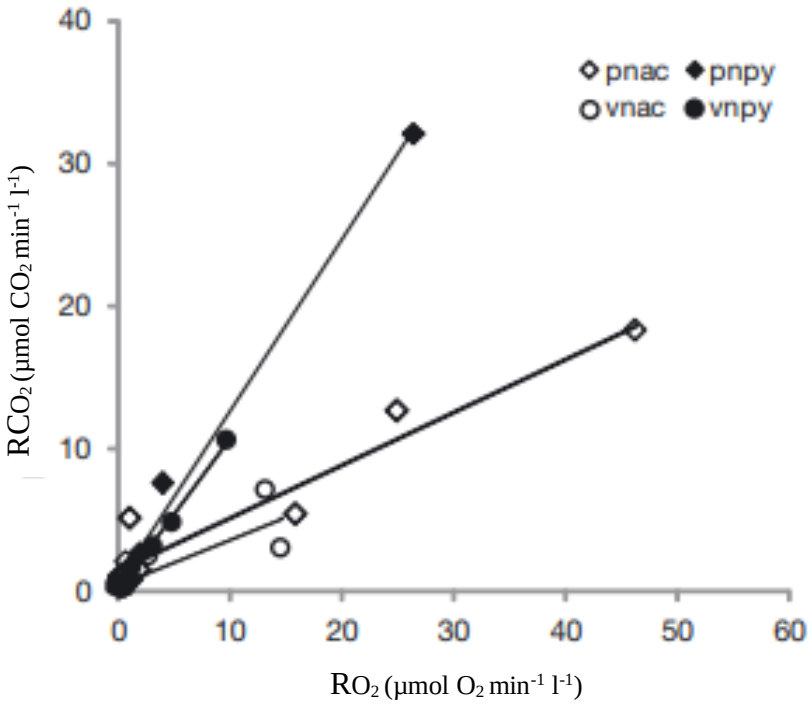


Figure 4. Long term experiments with regression equations that include all data points. pnac = *P. nautica* in acetate (RCO_2=0,3681 RO_2 + 1,5454; r^2=0,93; n=9; p<0.05) pnpy = *P. nautica* in pyruvate (RCO_2=1,204 RO_2 + 0,3718; r^2=0,99; n=7; p<0.05), vnac = *V. natriegens* in acetate (RCO_2=0,3103 RO_2 + 0,5962; r^2=0,65; n=6; p=0.05), vnpy = *V. natriegens* in pyruvate (RCO_2=1,0624 RO_2 + 0,0701; r^2=0,99; n=7; p<0.05). Note that the carbon sources dictate the parallelism of the curves, not the bacterial species. In addition, note that the RQ (slope) is higher for the three-carbon substrate (pyruvate) than for the two carbon one (acetate). This difference can not be seen in the short-term experiments (Figure 3).

2.4.3. Respiration quotient

Short-term experiments. RQ measurements in the short-term experiments are shown in Figure 5. When the cultures were substrate-sufficient, RQs were low, but when carbon substrate limitation started in the acetate cultures of both *P. nautica* and *V. natriegens*, the RQ values rose dramatically (Table 1, Figure 5A, B). In *P. nautica* on pyruvate, the RQ rose only at the end of the experiment (Figure 5C). In contrast to the constant pre-starvation RQ values in the first 3 experiments, in the *V. natriegens* culture growing on pyruvate (Figure 5D), the signal displayed noticeable noise between hours 7 and 12 of the experiment. Afterwards, at 25 h, it rose to an RQ of 1.4. In summary, these short-term experiments show a distinct shift in the RQ when either bacteria species was growing on acetate (Figure 5A, B). In contrast, when growing on pyruvate, both species displayed an RQ shift, but it was less pronounced than in Figure 5C, and D.

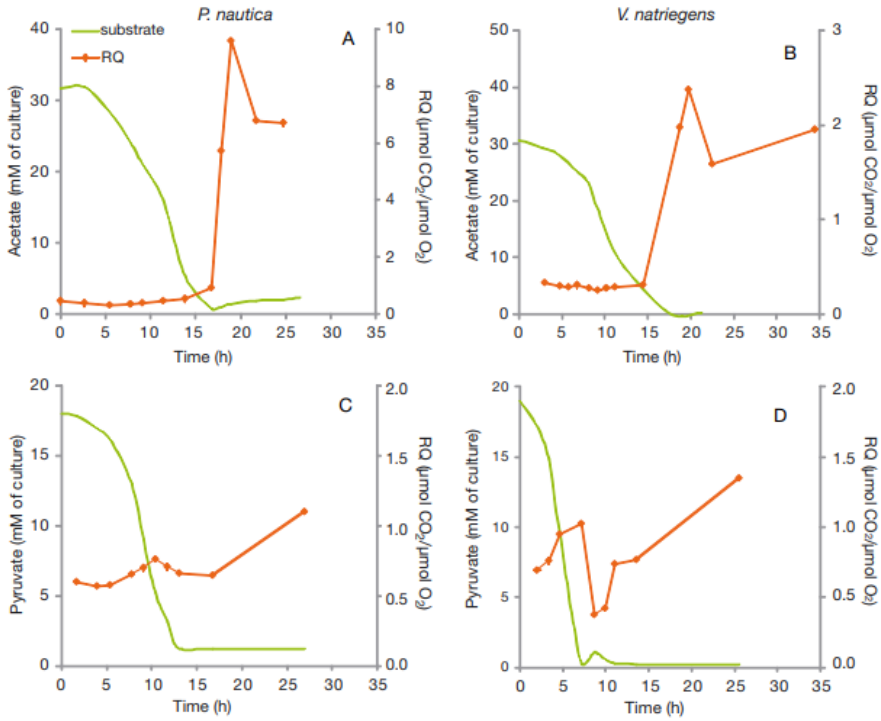


Figure 5. Short term experiments. RQ and substrate (carbon source) versus time. Note the carbon substrate sufficiency on the left and carbon substrate depletion (starvation) on the right. The top panels are acetate-based cultures; the bottom panels are pyruvate-based cultures. Left panels are *P. nautica.* Right panels are *V. natriegens.* In all experiments RQ rises during starvation.

Long-term experiments. The time-course signature of the long-term experiments (Figure 6) is comparable to that of the short-term experiments (Figure 5). Of the 4 experiments, *P. nautica* on acetate (Figure 6A) behaved most similarly to the short-term experiments. In all long-term experiments, carbon substrate limitation began within the first 50 h. We see an RQ jump when carbon substrate limitation was reached, with a subsequent rise to the end of the experiment (Figure 6A). When *P. nautica* was growing on pyruvate (Figure 6C) and carbon substrate limitation set in after 65 h, the RQ rose. However, it rose at a lower rate than in the second experiment (*V. natriegens*) on pyruvate in which RQ rose to 4.5 at the experiment end (Figure 6D). In *V. natriegens* growing on acetate (Figure 6B), RQ rose to 1.2 after acetate exhaustion; however, this RQ was lower than the high value (8.0) found in the *P. nautica* culture growing on acetate (Figure 6A, Table 1).

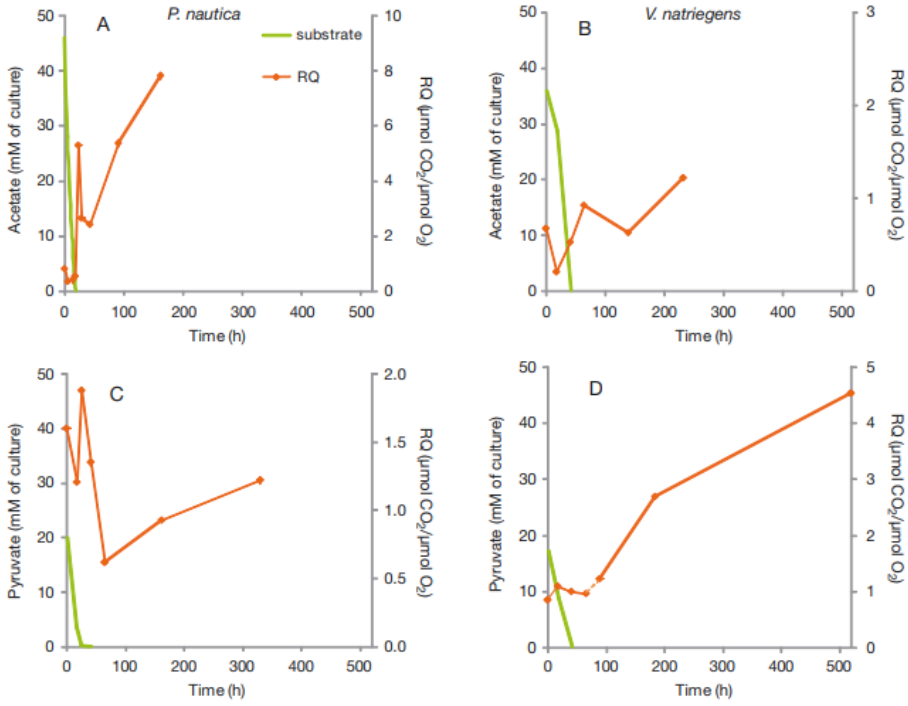


Figure 6. Long term experiments. RQ and substrate versus time. **A.** *P. nautica* in acetate. **B** *V. natriegens* in acetate. **C.** *P. nautica* in pyruvate. **D.** *V. natriegens* in pyruvate. In all panels RQ increases during the late starvation period.

2.5. Discussion

2.5.1. RQ and enzyme activity

Physiological RQ and enzyme activity RQ shifts can result from the variable activities of different biochemical pathways. In the Krebs cycle, RCO_2 is the collective activity of 3 enzymes: isocitrate dehydrogenase (IDH, EC 1.1.1.42), alpha-ketoglutarate dehydrogenase (α-KGDH, EC 1.2.7.3), and pyruvate dehydrogenase (PDH, EC 1.2.2.2) (Walsh and Koshland, 1984; Holms, 1986a,b). RO_2, although largely controlled by the ETS, is also influenced by all the oxidases, oxygenases, and hydroxylases in a cell. In Figures 1 and 2, we see that in starvation, IDH and Φ activities are still present at relatively high levels, and an IDH dominance is not detectable. Consequently, the RQ elevation (Figure 5 and 6) cannot be attributed to the relative activity trends of IDH and ETS. In nature, microorganisms are able to react quickly to environmental changes, adapting their metabolic profiles to organic inputs (Martinez et al., 1996; Mudryk and Donderski, 1997; Sala and Güde, 2004; Mudryk and Skórczewski, 2006). Still, such a mechanism requires time-consuming enzyme induction or activation. Here, the maintenance of IDH and Φ levels after starvation suggests that *V. natriegens* and *P. nautica* are prepared to bypass this time-consuming step to be ready to react immediately via Michaelis-Menten kinetics to any fortuitous availability of substrates. Thus, the differential impact of declining substrates ($NADP^+$, isocitrate, NADH, and succinate) induced by substrate-limitation could provide the explanation for the declining physiological rates.

2.5.2. RQ variability and carbon limitation

In this study, we observed a rising RQ after nutrient depletion in cultures of 2 marine bacteria. The RQ ranged from a low of 0.3 during exponential growth to a high of 9.6 during senescence and nutrient depletion (Figure 5A). In comparison, the RQs in fresh waters of Quebec ranged from 0.25 to 2.26 (Berggren et al., 2012). Those authors argue that the common assumptions of 0.8 to 1.0 RQ values in the literature are not justified. Furthermore, they argue that the use of a constant RQ depresses estimations of the metabolic balance between respiration and photosynthesis, i.e. the P/R ratio. They maintain that high RQs have a physiological explanation based on the different biochemical pathways of DOC degradation. However, it is not obvious how RQ could rise to 10 (Figure 5A) by a biochemical mechanism. This would mean that O_2 consumption is severely repressed or that CO_2 production is highly stimulated. It is difficult to see how starvation could cause those shifts. Possibly, vegetative cells are being transformed into spores, but what reactions are involved is unknown. Here, the 2 physiological signals of the respiration do appear to shift in the right direction for the RQ to rise. As starvation sets in, RO_2 decreases before RCO_2, causing the RQ rise (Figure 1A, B). Why this uncoupling occurs is not obvious. Recent research with nematodes has shown that starvation decreases RO_2 without decreasing the activity of ETS (Chin et al., 2014). This impact was traced to increases in the concentration of alpha-ketoglutarate, the product of IDH activity. Since CO_2 is also a product of IDH activity, perhaps RCO_2 is also stimulated, resulting in a rise in the RQ. Here, we saw the decrease in RO_2 and stability in the ETS but not an increase in the IDH activity and RCO_2. Nevertheless, could starvation have caused an increase in the alpha-ketoglutarate by another mechanism in our cultures? Additional experimentation is required to address this question. Another observed trend in our results was that the temporal signatures of the RQs

differed between the acetate- and the pyruvate-grown cultures, independent of the species. This difference occurred in short-term experiments but not in the long-term ones (Figures 5 and 6). A comparison of the slopes in the RCO_2-RO_2 regressions (Figure 4) showed that the biochemistry, not the bacterial species, determined the RQ. An ANCOVA test confirmed it by showing that there was no significant difference in the slopes of *P. nautica* and *V. natriegens* growing on the same substrate (Figure 4). Because acetate and pyruvate are products of lipid and carbohydrate metabolism, respectively, the short-term experimental results (Table 1) confirmed that carbohydrate consumption leads to higher RQs (0.7 to 1) than does lipid consumption (0.4 to 0.3). This is consistent with the historical use of the RQ.

2.6. Conclusions

(1) RQ changes with nutritional impact on the physiological state. Bacteria under substrate-limitation have RQs higher than when they are growing under substrate-sufficient conditions.

(2) RQs rise by nearly a factor of 10 (Figure 5A) during substrate-deficiency (senescence). This suggests that the nutritional conditions of bacteria should be considered, in the future, when selecting an RQ for calculation purposes.

(3) Should this level of RQ variability (Figure 3) be found in phytoplankton, macroalgae, zooplankton, and benthic invertebrates, then ecosystem models, ocean carbon flux calculations, and predictions of the balance between ocean autotrophy and heterotrophy could be impacted.

(4) Respiration under starved and well-fed conditions of acetate- and pyruvate-based cultures of *P. nautica* and *V. natriegens* show that rates

of O_2 consumption and CO_2 production during substrate-sufficiency parallels the biomass increase (Figure 1), but that after nutrients are exhausted, both respiration rates fall rapidly. This drop in the respiration rates occurs much sooner and more rapidly than the slow decline in the enzyme activities.

(5) IDH activity and potential respiration (ETS activity expressed in terms of oxygen) can maintain high levels after nutrients are depleted (Figure 1 and 2), but after 10 h of starvation, these values and the cell protein begin to slowly decline as substrate-deficiency persists. The decline in the enzyme activities is much slower after the point of nutrient exhaustion than is the rapid decline in the physiological respiration rates.

2.7 References

Ahmed, S. I., Kenner, R.,A., King, F. D., 1976. Preservation of enzymatic activity in marine plankton by low-temperature freezing. Mar Chem, 4(2):133-139.

Amado, A. M., Meirelles-Pereira, F., Vidal, L. O., Sarmento, H., Suhett, A. L., Farjalla, V. F., 2013. Tropical freshwater ecosystems have lower bacterial growth efficiency than temperate ones. Frontiers in Microbiology; 4.

Arsmby, H. P., Moulton, C. R., 1925. The animal as a converter of matter of energy: A study role of livestock in food production. American Chemical Society Monograph Series. Chemical Catalog Co, Inc. New York 236.

Baumann, P., Baumann, L., 1981. The Marine Gram-negative Eubacteria: Genus Photobacterium, Beneckea, Alteromonas, Pseudomonas and Alcaligenes, In: Starr S, Trüper H G, Balows A, Schelegel (eds). The prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, applications. Springer-Verlag, New York: 1352-1394.

Berdalet, E., Packard, T., Legacé, B., Roy, S., St-Amand, L., Gagne, J.P., 1995. CO_2 production, O_2 consumption and isocitrate dehydrogenase in the marine bacterium *Vibrio natriegens*. Aqua Micro Ecol 9(3): 211-217.

Berggren, M., Lapierre, J., Del Giorgio, P. A., 2012. Magnitude and regulation of bacterioplankton respiratory quotient across freshwater environmental gradients. ISME Journal 6(5): 984-993.

Bergström, I., 2011. Carbon gas fluxes from boreal aquatic sediments. Monographs of the Boreal Environment Research. Finnish Environment Institute (38).

Bonin, P., Gilewicz, M., Bertrand, J. C., 1987. Denitrification by a marine bacterium *Pseudomonas nautica* strain 617. Annales De l'Institut Pasteur Microbiology, 138(3): 371-383.

Bonin, P., Barbotin, J. N., Dhulster, P., Bertrand, J., 1987. Nitrate reduction in simulated microniches by a denitrifying marine bacterium. Canadian Journal of Microbiology, 33(3): 276-279.

Boucher, G., Clavier, J., Garrigue, C., 1994. Oxygen and carbon dioxide fluxes at the water-sediment interface of a tropical lagoon. Mar Ecol Prog Ser 107:185-193.

Burton, W. G., 1982. Post-Harvest Physiology of Food Crops. Longman, New York: 399.

Bühring, S. I., Lampadariou, N., Moodley, L., Tselepides, A., Witte, U., 2006. Benthic microbial and whole-community responses to different amounts of 13C-enriched algae: In situ experiments in the deep Cretan sea (eastern Mediterranean). Limnology and Oceanography, 51(1 I): 157-165.

Cantarow, A., Schepartz, B., 1967. Biochemistry, 4th edn, W. B. Saunders Co., Philadelphia.

Chin, R. M., Fu, X., Pai, M. Y., Vergnes, L., Hwang, H., Deng, G., Diep, S., Lomenick. B., Meli, V.S., Monsalve, G. C., Hu, E., Whelan, S. A., Wang, J. X., Jung, G., Solis, G. M., Fazlollhi, F., Kaweeteerawat, C., Quach, A., Nili. M., Krall, A. S., Godwin, H. A., Chang, H. R., Faull, F., Guo, F., Jiang, M., 2014. The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. Nature 510:397-401.

Del Giorgio, P. A., Pace, M.L., Fischer, D., 2006. Relationship of bacterial growth efficiency to spatial variation in bacterial activity in the Hudson River. Aquatic Microbial Ecology, 45(1): 55-67.

Dilly, O., 2001. Microbial respiratory quotient during basal metabolism and after glucose amendment in soils and litter. Soil Biol Biochem 33(1): 117-27.

Ducklow, H. W., Doney, S. C., 2013. What is the metabolic state of the oligotrophic ocean? A debate. Ann Rev Mar Sci 5:525-533.

Ferguson, S.J., 2010. ATP synthase: From sequence to ring size to the P/O ratio. Proceedings of the National Academy of Sciences of the United States of America, 107(39): 16755-16756.

Giering, S. L. C, Sanders, R., Lampitt, R. S., Anderson, T. R., Tamburini, C., Boutrif, M., Zubkov, M.V., 2014. Reconciliation of the Carbon Budget in the Ocean's Twilight Zone. Nature 507 (7493): 480-483.

Gnaiger, E., 1983. Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. Polarographic oxygen sensors. Springer Verlag 337-345.

Gnaiger, E., 2009. Mitochondrial Pathways and Respiratory Control. Gnaiger E (Ed.), 2nd ed. OROBOROS MiPNet Publications, Innsbruck.

González, N., Anadón, R., Viesca, L., 2003. Carbon flux through the microbial community in a temperate sea during summer: Role of bacterial metabolism. Aquatic Microbial Ecology, 33(2): 117-126.

Guyton, A. C., 1971. Textbook of medical Physiology. 4th edn, W. B. Saunders Co., Philadelphia.

Hoar, W. S., 1975. General and Comparative Physiology, 2nd edn, Prentice Hall, Upper Saddle River, N.J.

Holms, W. H., Bennett, P. M., 1971. Regulation of isocitrate dehydrogenase activity in *Escherichia coli* on adaptation to acetate. J of general microbiology 65(1): 57-68.

Holms, W. H., 1986a. The central metabolic pathways of *Escherichia coli*: relationship between flux and control at a branch point, efficiency of conversion to biomass, and excretion of acetate. Current topics in cellular regulation 28:69-105.

Holms, W. H., 1986b. Evolution of the glyoxylate bypass in *Escherichia coli* - An hypothesis which suggests an alternative to the Krebs cycle. FEMS microbiology letters 34(2): 123-127.

Kader, A. A., 1987. Respiration and gas exchange of vegetables. Weichmann J (ed), Postharvest Physiology of Vegetables. Marcel Dekker, New York 25–43.

King, G. M., Berman, T., 1984. Potential effects of isotope dilution on apparent respiration in ^{14}C heterotrophy experiments. Mar Ecol Prog Ser 19:175-180.

Lee, K., 1995. Bioremediation studies in low-energy shoreline environments. Proceedings of the 2nd International Oil Spill Research and Development Forum. International Maritime Organization, May 23-26 London, United Kingdom.

Lee, K., Levy, E. M., 1987. Enhanced biodegradation of a light crude oil in sandy beaches. Proceedings of the 1987 Oil Spill Conference. American Petroleum Institute, Washington, D.C. 411-416.

Liu, M., Xiao, T., Sun, J., Wei, H., Wu, Y., Zhao, Y., Zhang. W., 2013. Bacterial community structures associated with a natural spring phytoplankton bloom in the yellow sea, china. Deep-Sea Research Part II: Topical Studies in Oceanography 97:85-92.

Lowry, O.H., Rosebrough, N. J., Farr, A. L., Randall, R. J., 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193(1): 265-275.

Lusk, G., 1928. The elements of the science of nutrition. W. B. Saunders Philadelphia 4th edition: 402.

Martinez, J., Smith, D. C., Steward, G. F., Azam, F., 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aqua Micro Ecol 10(3): 223-230.

Mudryk, Z., Donderski, W., 1997. The occurrence of heterotrophic bacteria decomposing some macromolecular compounds in shallow estuarine lakes. Hydrobiol 342-343:71-78.

Mudryk, Z. J., Skórczewski, P., 2006. Enzymatic activity and degradation of organic macromolecules by neustonic and planktonic bacteria in an estuarine lake. Polish J of Ecol 54(1): 3-14.

Nelson, D. L., Cox, M. M., 2005. Lehninger Principles of Biochemistry. W H Freeman and Company, New York, 1216.

Nissen, H., Heldal, M., Norland, S., 1987. Growth, elemental composition, and formation of polyphosphate bodies in *Vibrio natriegens* cultures shifted from phosphate-limited to phosphate-pulsed media. Canadian J Microb 33(7): 583–588.

Niven, D. F., Collins, P. A., Knowles, C. J., 1977. Adenylate energy charge during batch culture of *Beneckea natriegens*. J General Microbiol 98(1): 95-108.

Obernosterer, I., Christaki, U., Lefèvre, D., Catala, P., Van Wambeke, F., Lebaron, P., 2008. Rapid bacterial mineralization of organic carbon produced during a phytoplankton bloom induced by natural iron fertilization in the southern ocean. Deep-Sea Research Part II: Topical Studies in Oceanography, 55(5-7): 777-789.

Osma, N., Fernández-Urruzola, I., Packard, T. T., Postel, L., Gómez, M., Pollehne, F., 2014. Short-term patterns of vertical particle flux in northern Benguela: A comparison between sinking POC and respiratory carbon consumption. J Mar Syst. 2014.

Oviatt, C. A., Rudnick, D. T., Keller, A. A., Sampou, P. A., Almquist. G. T., 1986. A comparison of system (O_2 and CO_2) and C^{-14} measurements of metabolism in estuarine mesocosms, Marine Ecology- Progress Series (28): 57-67.

Packard, T. T., Williams, P. J. L., 1981. Rates of respiratory oxygen consumption and electron transport in surface seawater from the northwest Atlantic. Oceanol Acta 4(3): 351-358.

Packard, T. T., Berdalet, E., Blasco, D., Roy, S. O., St-Amand, L., Lagacé, B., Lee, K., Gagné, J. P., 1996a. CO_2 production predicted from isocitrate dehydrogenase activity and bisubstrate enzyme kinetics in the marine bacterium *Pseudomonas nautica*. Aquatic Microbial Ecol 11(1): 11-19.

Packard, T. T., Berdalet, E., Blasco, D., Roy, S. O., St-Amand, L., Lagacé. B., Lee, K., Gagné, J.P., 1996b. Oxygen consumption in the marine bacterium *Pseudomonas nautica* predicted from ETS activity and bisubstrate enzyme kinetics. J Plankton Res 18(10): 1819-1835.

Packard, T.T., Christensen, J.P., 2004. Respiration and vertical carbon flux in the Gulf of Maine water column. J Mar Res 62(1): 93-115.

Packard, T.T., Gómez, M., 2013. Modeling vertical carbon flux from zooplankton respiration. Prog Oceanogr 110:59-68.

Payne, W.J., Eagon, R.G., Williams, A.K., 1961. Some Observations on the Physiology of *Pseudomonas Natriegens* Nov. Spec. Antoine Van Leeuwenhoek 27 (1): 121-128.

Pettenkofer, M. J., Voit, K., 1866. Untersuchungen über den Stoffverbrauch des normalen Menschen. Zeitschrift für Biologie 2:458-573.

R Development Core Team., 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.r-project.org.

Reeves, H. C., Rabin, R., Wegener, W.S., Ajl, S. J., 1971. Assays of enzymes of the tricarboxylic acid and glyoxylate cycles .In J.R. Norris and D.W. Ribbons (eds), Methods in microbiology volume 6A, Academic Press, New York: 425-462.

Reeves, H. C., Daumy, G. O., Lin, C. C., Houston, M., 1972. $NADP^+$-specific isocitrate dehydrogenase of *Escherichia coli*. I. Purification and characterization. BBA - Enzymology 258(1): 27-39.

Riebesell, U., Gattuso, J., Thingstad, T. F., Middelburg, J. J., 2013. Preface arctic ocean acidification: Pelagic ecosystem and biogeochemical responses during a mesocosm study. Biogeosciences, 10(8): 5619-5626.

Robinson, C., Williams, P. J. B., 2005. Respiration and its measurement in surface marine waters. Respiration in Aquatic Ecosystems (Eds. P.A. del Giorgio and P J le B Williams) OUP, Oxford: 147-180.

Robinson, C., Serret, P., Tilstone, G., Teira, E., Zubkov, M.V., Rees, A.P., Woodward, E. M., 2002. Plankton respiration in the Eastern Atlantic Ocean. Deep-Sea Research Part I: Oceanographic Research Papers, 49(5): 787-813.

Roy, S. O., Packard, T. T., Berdalet, E., St-Amand, L., 1999. Impact of acetate, pyruvate, and physiological state on respiration and respiratory quotients in *Pseudomonas nautica*. Aquatic Microbial Ecology 17(1): 105-110.

Roy, S.O., Packard, T. T., 2001. CO_2 production rate predicted from isocitrate dehydrogenase activity, intracellular substrate concentrations and kinetic constants in the marine bacterium *Pseudomonas nautica*. Mar Biol 138(6): 1251-1258.

Sala, M. M., Güde, H., 2004. Ectoenzymatic activities and heterotrophic bacteria decomposing detritus. Archiv fur Hydrobiol 160(3): 289-303.

Slavov, N., Budnik, B. A., Schwab, D., Airoldi, E. M., van Oudenaarden, A., 2014. Constant Growth Rate Can Be Supported by Decreasing Energy Flux and Increasing Aerobic Glycolysis. Cell Reports 7(3): 705-714.

Stanier, M., Forsling, M., 1990. Physiological Processes, McGraw-Hill, London.

Steinberg, D. K., Van Mooy, B. A. S., Buesseler, K. O., Boyd, P.W., Kobari, T., Karl, D. M., 2008. Bacterial vs. zooplankton control of sinking particle flux in the ocean's twilight zone. Limnol Oceanogr 53(4): 1327-1338.

Swannell, R. P. J., Lee, K., McDonagh, M., 1996. Field evaluations of marine oil spill bioremediation. Microbiol Rev 60(2): 342-65.

Tames-Espinosa, M., Martínez, I., Romero-Kutzner, V., Bondyale-Juez, D. R., Packard, T. T., and Gomez, M., 2018. $NADP^+$-dependent isocitrate dehydrogenase activity in marine plankton. Marine Chemistry, 204, 86-94.
Walsh, K., Koshland, J. D., 1984. Determination of flux through the branch point of two metabolic cycles. The tricarboxylic acid cycle and the glyoxylate shunt. J Biol Chem 259(15): 9646-9654.
Warburg, O., 1926. Über die Wirkung des Kohlenoxyds auf den Stoffwechsel der Hefe. Biochemische Zeitschrift 177: 471–486.

CHAPTER

3

A. aurita polyp response to simulated Marine Heatwaves

Mystery creates wonder and wonder is the basis of human's desire to understand.

Neil Armstrong, 1969

3.1. Abstract

The increase of jellyfish blooms has been associated, among other factors, with anthropogenic climate change. Global warming, as part of climate change, generates marine heatwaves (MHWs), and those have been observed in recent decades in ocean seawater. MHWs are periods of anomalous warm sea surface temperature which can last multiple days or even months. Marine ecosystems and fisheries are highly vulnerable to MHWs. Mortality, local extinctions, shifts in community structure, and depleted biodiversity are some major consequences for marine biota. Unfortunately, MHWs are expected to become more frequent, longer, and more extreme.

Here, we present the response of the polyps of *Aurelia aurita*, a cosmopolitan jellyfish, to simulated MHWs. These MHWs had three different durations (6, 16, and 20 days) and were carried out under a severe and extreme scenario, followed by a recovery period. We measured respiratory metabolic rates, growth, reproduction, and survival. The respiratory metabolic rates were determined by measuring physiological respiration with optodes and enzymatic respiratory electron transport system activity via tetrazolium reduction. Polyps of *A. aurita* decreased in size but represented greater asexual reproduction than the control group. All polyps survived in the extreme 20 days MHW scenario. Respiration rates, high during the MHWs, returned to control conditions after the recovery period. Our results suggest that *A. aurita* polyps can resist extreme MHWs for up to 20 days.

3.2. Introduction

Marine heatwaves (MHWs), a term first used a decade ago (Pearce et al., 2011), are periods of anomalous warm seawater temperatures (upper determined threshold, 90th percentile relative to the local long-term climatology), which can last from at least 5 days to months (Hobday et al., 2016). There has been an increase of MHWs in the global oceans, from 30% in 2012 to nearly 70% in 2016 (Suryan et al., 2021). MHWs are categorised into four groups according to their intensity: I (moderate), II (strong), III (severe), and IV (extreme). These categories are consistent with the 90th percentile definition of an MHW (Hobday et al., 2016), but based on multiples of the value represented by local climatological mean variations (Hobday et al., 2018). However, MHWs are still a developing research area (Oliver et al., 2021). The fact is that MHWs are increasing in duration and frequency, as a consequence of climate change and long-term ocean warming (Frölicher et al., 2018; Oliver et al., 2018; Oliver, 2019). Their ecological and socioeconomic impacts are driving research on MHWs (Oliver et al., 2021).

Ocean warming appears to be one of the most important contemporary global ocean change stressors for many species (Hughes et al., 2017; Smale et al., 2019; Scanes et al., 2020 a,b). Entire ecosystems can be affected by MHWs, e.g. the northeast Pacific marine heatwave (2014-2016) in the Gulf of Alaska was the longest registered MHW over the past decade and caused abrupt ecological changes across trophic levels. The ecosystem there has still not recovered today (Suryan et al., 2021; Weitzman et al., 2021). Loss in zooplankton abundance during this warm-water anomaly has been observed (McKinstry and Campbell, 2018). However, if thermal tolerance is exceeded, all trophic levels can be affected, causing mortality, species range shift, and community changes (Pörtner et al., 2007; Garrabou et al., 2009; Batten et al., 2018; Hughes et al., 2018; Rendina et al., 2019; Waal and Litchman, 2020;

Hayashida et al., 2020; Piatt et al., 2020). Hayashida et al. (2020) showed with model simulations, and satellite observations, that weaker primary production during MHWs is expected, due to enhanced stratification of seawater columns and nutrient limitation.

Nevertheless, according to McCauley (2015), and consistent with the evolution of life on Earth, some species will benefit from current or future ocean changes. For that reason, many scientists claim that jellyfish will play a key role in the future oceans (Richardson et al., 2009; Boero, 2013). Jellyfish are an ecologically diverse group of animals with circumglobal distribution (Lucas et al., 2014). Occurring in blooms, aside from their negative impact on socio-economic activities (Matsumura et al., 2005; Mariottini and Pane, 2010; Baxter et al., 2011), jellyfish play an important role in marine food webs (Pauly et al., 2009; Riascos et al., 2012; Takao et al., 2014; Ates, 2017; Choy et al., 2017; Dölger et al., 2019; Chi et al., 2021). Jellyfish blooms are often related to degraded ocean environments (Duarte et al., 2013), and anthropogenic stressors such as climate change, eutrophication, and overfishing (Purcell, 2007, 2012; Richardson et al., 2009; Slater et al., 2020). In addition, during the last two decades, the occurrence of jellyfish blooms has been linked to temperature increases (Holst, 2012; Heim-Ballew and Olsen, 2018; Ziegler and Gibbons, 2018). Besides, under an MHW scenario, individual jellyfish need to adjust physiologically due to the thermal stress (Leung et al., 2019). Accordingly, changes in growth, reproduction (Shanks et al., 2020; Gall et al., 2021), feeding behavior (Mitterwallner et al., 2021), and survival in jellyfish can be expected (Pérez et al., 2000). However, abiotic tolerance limits and ranges, that trigger distribution shifts are unknown for most jellyfish (Frolova and Miglietta, 2020).

Currently, a large fraction of jellyfish research is focused on the bloom-forming moon-jellyfish, *Aurelia aurita* (Linnaeus, 1759). This scyphozoan is a cosmopolitan species with a triphasic life cycle (Fuchs et al., 2014). Its metagenic life cycle includes sexual reproductive medusae and planula-type larvae, that settle on the seafloor, and sessile polyps (also called scyphistoma). The polyps reproduce asexually, mainly by different budding modes that evolve into new polyps (Hubot et al., 2017). The understanding of the sessile polyp physiology is poorly developed (Höhn et al., 2017). However, it is largely known that jellyfish bloom outbreaks are linked to benthic polyp dynamics (Boero et al., 2008; Lucas et al., 2012; Gambill and Peck, 2014).

The objective of this research is to study *A. aurita's* polyp metabolism under a simulated severe (III) and extreme (IV) MHW. To accomplish this, we measured the physiological respiration rates and enzymatic respiratory electron transport system activity. Both measurements were carried out kinetically from time courses of O_2 (respiration), and from time courses of tetrazolium reduction, according to Bondyale et al. (2017) and Purcell et al. (2019). Interest in these types of measurements is increasing in the jellyfish research community (Iguchi et al., 2017; Purcell et al., 2019; Aljbour et al., 2019). In addition to these measurements in *A. aurita* polyps, growth, and endpoints of reproduction and survival were measured.

Studying the physiological mechanisms of jellyfish and other marine organisms can elucidate ecosystem changes and future ecological trends due to climate change (Pörtner and Farrel, 2008). Insights on how species and key biological processes respond to temperature changes are needed to understand how food webs and carbon cycling will function in the future oceans (Alcaraz et al., 2013; Boscolo-Galazzo et al., 2021).

This is the first time-course study to follow *A. aurita* polyp metabolism during a simulated MHW and represents an initial step in elucidating how MHWs affect the benthic life stage of the bloom-forming *A. aurita* medusa.

3.3. Material and methods

3.3.1. Polyp culture

The *A. aurita* medusae were collected in February 2019 in Horsea Lake (50˚ 83' 68.26" N / 1˚ 10' 19.11" W), at the inner edge of Portsmouth Harbour, Hampshire, UK. A culture of polyps, from a collected reproductively ripe female *A. aurita* (Linnaeus, 1975), was then maintained at the National Oceanography Center (NOC-Southampton, UK) research aquarium. This stock culture was maintained at 15-16˚C, a salinity of 35 ‰, and fed daily. Around five hundred polyps from the stock culture were transferred to 12-well polycarbonate plates (one polyp per well). Then, the polyps were maintained for 1 month, at 16˚C in a controlled temperature room. This was the same temperature at which the stock culture was maintained. Polyps were kept in darkness, first to let them attach to the new surface, and secondly, to avoid algal growth. Feeding started once polyps were attached (after about 1 week). They were then fed every second day with 24-hour-*Artemia* nauplii (*ad libitum*) for 1 hour (h). The water was replaced afterwards.

3.3.2. Marine heatwave experiment

A Marine heatwave (MHW) is based on the definition by Hobday et al. (2016). MHWs last for at least 5 days, over which the temperature is greater than a seasonally varying threshold value. The experiment started in May 2019, the temperature conditions were chosen according to the summer conditions from the previous year (2018) in South England (UK). Summer 2018 was among the warmest, driest and sunniest summers in the UK in over 100 years (Kendon et al., 2019). Temperatures from an online available MHW tracker (www.marineheatwaves.org) were used to establish the experimental conditions (as in Minuti et al., 2021). This tracker utilised the daily optimally interpolated sea-surface temperature (SST) calculated with the heatwave R package (Schegel et al., 2018) from NOAA OI SST data (Reynolds et al., 2007). For the control polyp culture, the mean summer SST of 16˚C (from year 1982 to 2018) was used (Figure 1).

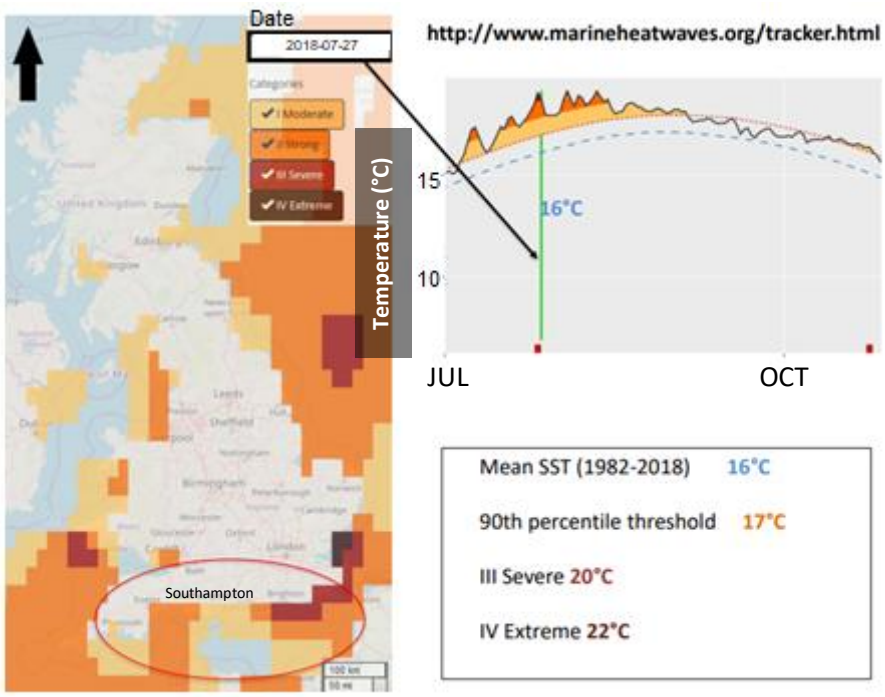


Figure 1. SST temperatures in the southern United Kingdom (Southampton area) on the 27 of July 2018 (Schegel, 2020) and experimental temperatures according to a severe and extreme MHW. (Figure modified from www.marineheatwaves.org/tracker)

The experimental design is illustrated in Figure 2. Two MHW temperatures were chosen, 20˚C and 22˚C, corresponding to a severe (III) and extreme (IV) MHW (S-MHW and E-MHW), respectively. Before exposing the polyps to 20˚C and 22˚ C, they went through a pre-acclimatisation period of 6 days, at 18˚C, while the control group was kept at 16˚C. After this pre-acclimatisation period, the polyps were divided and placed in two incubators, one at 20˚C and one at 22˚C. In addition, three MHW durations were established, 6, 16, and 20 days. According to Oliver et al. (2018), 20 days of MHW corresponds to the mean global MHW duration. After each MHW duration (6, 16, 20 days) the polyps went through a post-acclimatisation period, again at 18˚C for 6 days. Finally, after this period, they continued through a recovery period for 6 days at 16˚C, simulating their initial condition.

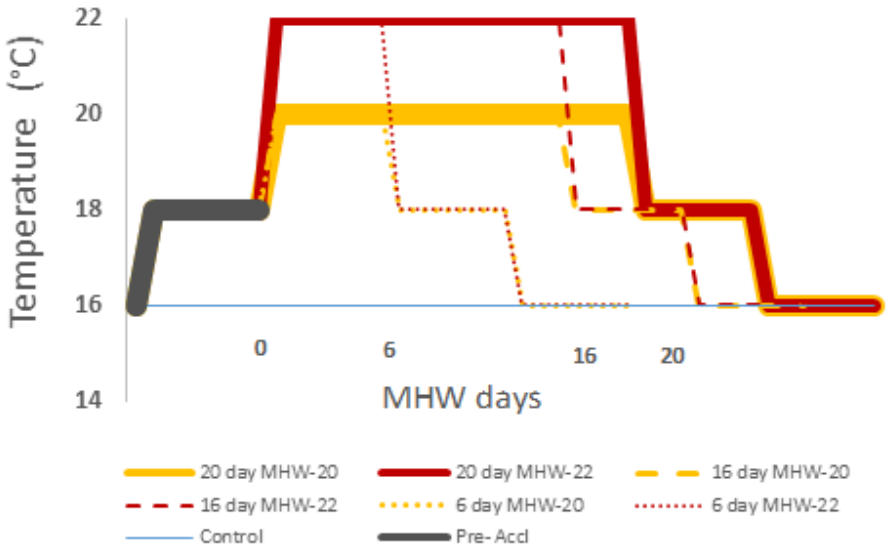


Figure 2. Experimental design of two simulated Marine Heatwaves (MHW) severe (20˚C) and extreme (22˚C). Each MHW counts with 3 durations, 6, 16, and 20 days, respectively, plus a post-acclimatisation period at 18˚C and a recovery period at 16˚C.

3.3.3. Physiological respiration measurements

Respiration (R), based on the fluorescent detection of the molecular oxygen concentration (O_2) over time, was measured with an Oxydish® (OD24, Presens, Germany). For this purpose, 24-well plates with 4 ml glass SensorVials® were used. Each vial contained a sensor spot on the bottom that consisted of an O_2-sensitive fluorescent dye embedded in a tissue-compatible polymer (an optode). The fluorescence emission of the dye depends on the amount of O_2 in the solution and is read non-invasively. Emission values were converted automatically to O_2 concentration (mg/L) using calibration parameters provided with the corresponding software. Respiration measurements were started after the pre-acclimatisation period. After the post-acclimatisation period one respiration measurement was taken and another, after the recovery period.

The respiration measurements were taken inside the different incubators and in the controlled temperature room (for the control group and the recovery period measurements), to maintain a constant experimental temperature. Six polyps were sufficient for each vial to measure respiration, and triplicates were taken. Polyps were transferred carefully from the plates to the vials with a Pasteur pipette. The vials were previously washed and then rinsed with filtered seawater. Blanks only contained filtered seawater. As the sensor spot was installed at the bottom of the vial, to prevent an O_2 gradient and interference with the polyps, the Oxydish was placed vertically in a box and shaken on an orbital shaker. Incubations lasted around 40-70 min and O_2 concentrations were measured every 4 minutes.

3.3.4. Sample storage, sample preparation, and enzymatic analyses

After the respiration measurements, samples were weighed to determine their wet mass (WM), and stored in vials at -80˚C, for further enzymatic analysis. The enzymatic analysis carried out in this study was based on the respiratory electron transport system (ETS) enzymes, principally, NADH dehydrogenase (Complex I, EC 7.1.1.2 (Savenkoff et al., 1995)). The ETS enzymes are responsible for cellular respiration. ETS activity was measured according to Purcell et al. (2019) to determine the potential respiratory activity (Φ) in the polyps. The samples, stored at -80˚C, were homogenized in 0.1M phosphate buffer solution (Packard and Christensen, 2004) using an ultrasonic probe (Cole Parmer) with a Vibracell VCX 130 ultrasonic processor (Sonics) to liberate the enzymes. Then the homogenate was centrifuged at 4000 rpm (1500 g) for 10 min at 0-4˚C. After the centrifugation, 100 µL of the supernatant was mixed in a cuvette with 300 µL of substrate solution containing NADH and NADPH (1.7mM in the same buffer solution) and 100 µL of the indicator INT solution (2 mg INT ml^{-1} in double-distilled H_2O). During the redox reaction, the INT acts as an artificial electron acceptor and is reduced to formazan. The production of formazan is monitored spectrophotometrically (Cary100 UV-Vis Spectrophotometer, Agilent Technologies) at 490 nm for 8 min. The rate of formazan production is stoichiometrically related to the ETS electron flux and potential respiration (Φ). Temperatures were 16, 18, 20, or 22˚C, coinciding with the experimental temperatures.

3.3.5. Growth, survival and reproduction

Growth, survival, and reproduction were followed for the 20-day-MHW experiment. A stereo microscope with an integrated, graduated eyepiece was used for these measurements. To assess somatic growth, the calyx diameter was measured. If the calyx was not round, the average of the maximum and the minimum dimensions were used. One polyp was placed in each well of a 12-well polycarbonate plate and was subjected to the same temperature adaption conditions as the polyps, which were used for the physiological and enzymatic measurements. In total, growth was followed in three plates, one for each temperature (16, 20, and 22˚C). The new budded polyps were removed from the parent polyp once they were solitary polyps, this procedure was performed with tweezers and a Pasteur pipette. Survival was quantified on the same plates that were used to determine growth. The following nomenclature was used: FA for fully active tentacles; RT, for partially retracted tentacles; TRT, for totally retracted tentacles; and C, for cysts. Considering that, healthy polyps flourish fully active tentacles and then evolve into unhealthy polyps. Unhealthy polyps can display retracted, shorter and thicker tentacles, totally retracted tentacles, or even cysts.

To quantify asexual reproduction, again, a three 12-well plate with only one initial polyp per well for each temperature (16, 20, and 22˚C) was used. For asexual reproduction, the following nomenclature was applied: S refers to solitary polyps; SB, to stolonic budding; and DB, to direct budding. For example, if a father polyp displayed two direct buds, it was counted as one solitary polyp (S) and two direct buds (DB).

3.3.6. Statistics

Significant difference between respiration rates, R/Φ ratio, and growth during the simulated MHW, versus control conditions were analysed. Normality was confirmed by the Shapiro-Wilk test and variance homogeneity was confirmed by the Fligner-Killeen or Bartlett tests.

For Fligner Kileen test, the significant differences were confirmed with the Kruskal-Wallis test and with a pairwise comparison using the Tukey and Kramer (Nemenyi) test. Using ANOVA analysis and the Tukey-HSD post-hoc test, the significant differences between treatments were verified, for the Bartlett test. All data were analysed with the R program (R Core Team, 2020).

3.4. Results

The calyx diameter of polyps before starting the simulated heatwave was 1.1 ± 0.1 mm (Figure 3). Control polyps kept at 16˚C grew up to 2.2 ± 0.1 mm, while polyps in S-MHW increased in size until day 6 (1.5 ± 0.1 mm) and then started to shrink until day 20 (1.2 ± 0.1 mm). A similar pattern was observed in the E-MHW, but polyps were already smaller on day 6 of E-MHW. Significant differences in growth between MHW (S and E) and control polyps were found ($p<0.05$).

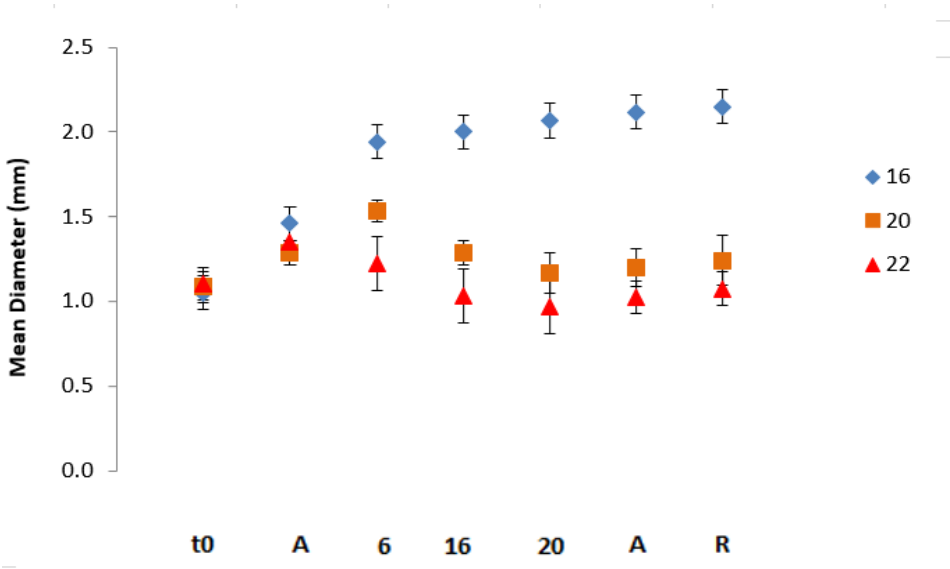


Figure 3. Growth in the control group (blue), 20-day severe (orange), and extreme (red) MHW. At t_0, similar-sized polyps were chosen for this experiment. A indicates the pre- and post-acclimatisation period, and R indicates the recovery period. Numbers, 6, 16, and 20, correspond to MHW durations in days.

Survival was quantified in the same plates as used for growth. FA indicates fully active tentacles, RT partially retracted tentacles, and TRT totally retracted tentacles. All polyps were fully active in the control group, but the number of fully active polyps decreased with increasing temperature (Figure 4). Polyps with RT or TRT were more frequent in E-MHW than in S-MHW. Note that after the recovery period 100% of S- and E- MWH were fully active (not shown).

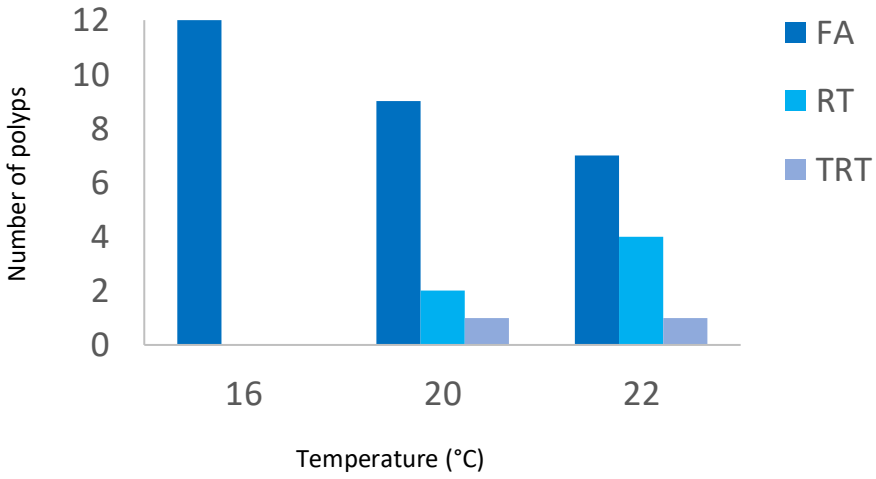


Figure 4. Survival for 20 MHW duration: Control (16˚C), S-MHV (20˚C) and E-MHW (22 ˚C). FA= fully active tentacles. RT= partially retracted tentacles. TRT= totally retracted tentacles. X axis: Control and MHWs. After the recovery period all the polyps from the MHW treatment (severe and extreme, indistinctly) were presented fully active tentacles, as in the control group (not shown).

Reproduction of polyps during the MHW is shown in Figure 5. S indicates solitary polyp, SB, stolonic budding, and DB, direct budding. The number of polyps per plate was higher under MHW conditions (> 20 polyps) than in the control group (16 polyps). In addition, in both MHW's, severe and extreme, reproduction strategies (DB, SB) were more abundant than in the control group. Stolonic budding was only observed under MHW conditions.

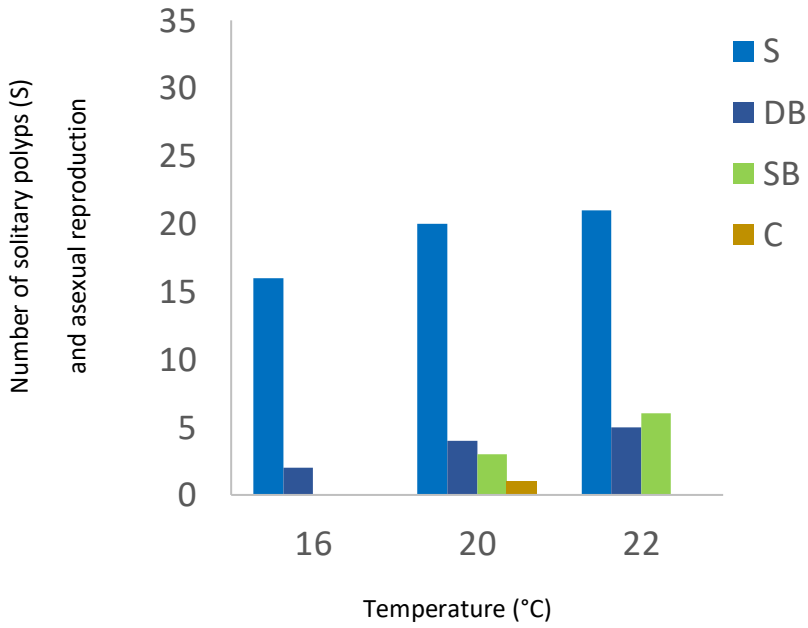
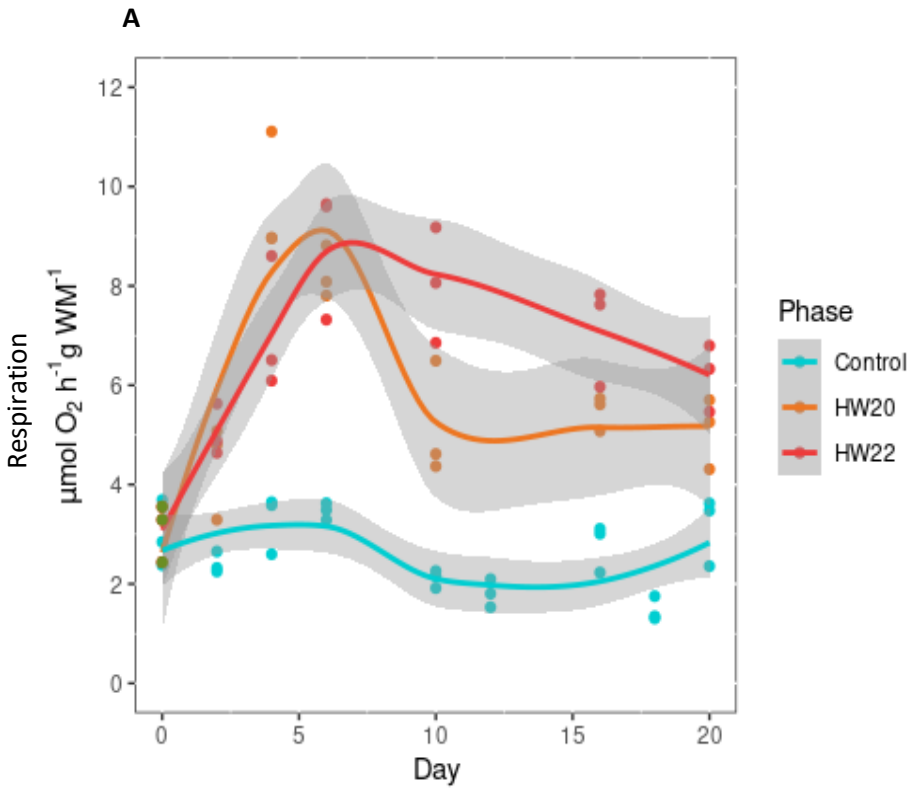


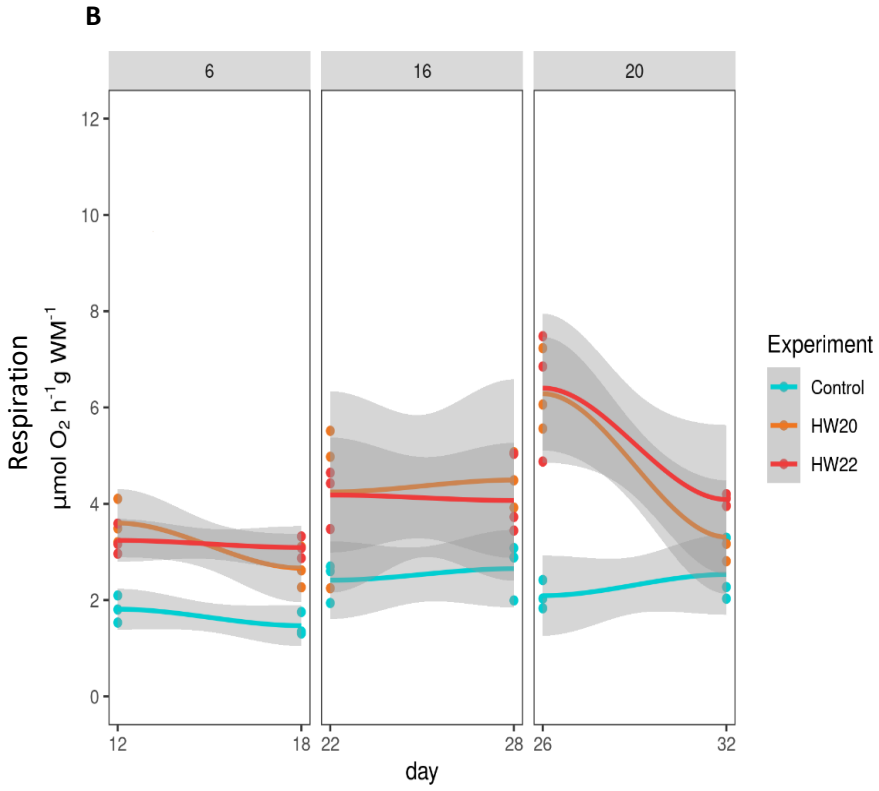
Figure 5. Reproduction for control (16˚C), S-MHV (20˚C), and E-MHW (22˚C) after day 20 of MHW. S= solitary polyps. DB=direct budding. SB= stolonic budding. C=cyst.

Respiratory rates and ratio will be given as mean-values. Wet-mass specific R was 2.55 ± 0.68 µmol O_2 h^{-1} g WM^{-1} for the control group kept at 16˚C throughout the experiment (Figure 6). Before exposing polyps to the severe (S-MHW, 20˚C) and extreme (E-MHW, 22˚C) marine heatwave, a pre-acclimatisation period of 6 days (18˚C) was established (Figure 2) and R increased slightly (3.09 ± 0.48 µmol O_2 h^{-1} g WM^{-1}) with respect to the control polyps (Figure 6 A). During the MHW, R peaked at day 4 (S-MHW) (9.68 ± 1.01 µmol O_2 h^{-1} g WM^{-1}) and day 6 (E-MHW) (8.86 ± 1.31 µmol O_2 h^{-1} g WM^{-1}) (Figure 6 A). In the case of the S-MHW, R decreases rapidly after day 6 and was then stable from day 10 until day 20 (5.24 ± 0.58 µmol O_2 h^{-1} g WM^{-1}). R in the E-MHW decreased slower 0.18 µmol O_2 h^{-1} g WM^{-1} d^{-1} from day 4 to day 20 and did not reach a stable state. After the 6 and 16 day (S-MHW and E-MHW), R decreased in the post-acclimation period (18˚C), however, no decrease was observed during this same period after the 20-day MHW. After the recovery period to the initial temperature at 16˚C, R in S-MHW was between 2.66 – 4.49 µmol O_2 h^{-1} g WM^{-1}, and between 3.1 – 4.1 µmol O_2 h^{-1} g WM^{-1} in the E-MHW. Throughout the MHW duration (S and E), R rates were significantly different from the control conditions ($p<0.05$). After the recovery period, a significant difference was found for the 16-day S-MHW, and the 20-day E-MHW, with respect to the control ($p<0.05$).

Figure 6. Marine Heatwave Experiment (MHW). Time-course of *A. aurita* polyp respiration rates (R) (µmol O_2 g WM^{-1}).



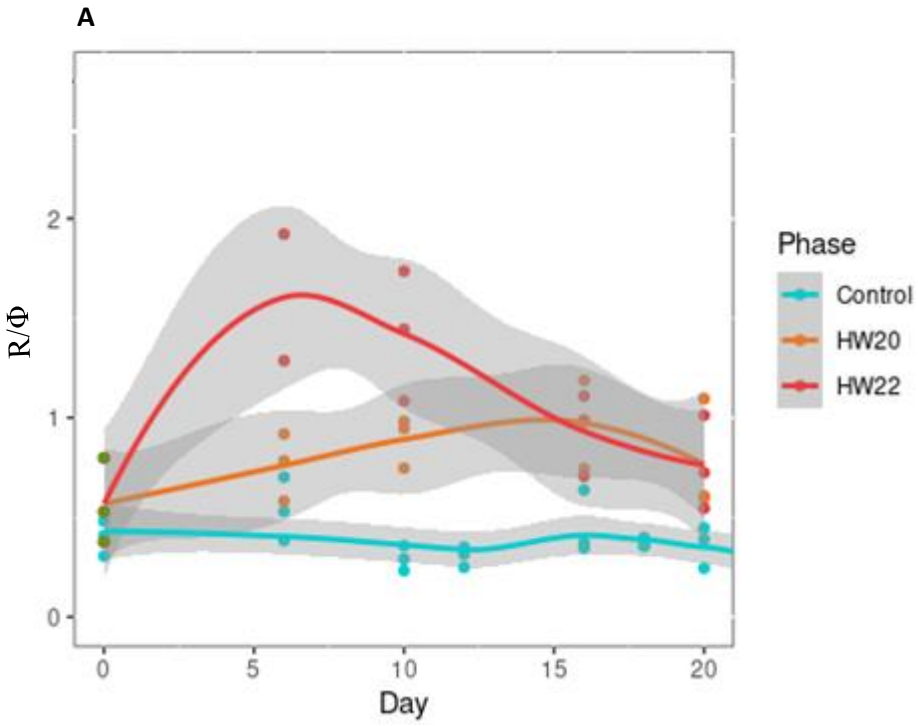
A. R during the MHW (S-MHW-20˚C, E-MHW-22˚C, and control). Green dots ● on day 0 represents the pre-acclimatisation period at 18˚C.



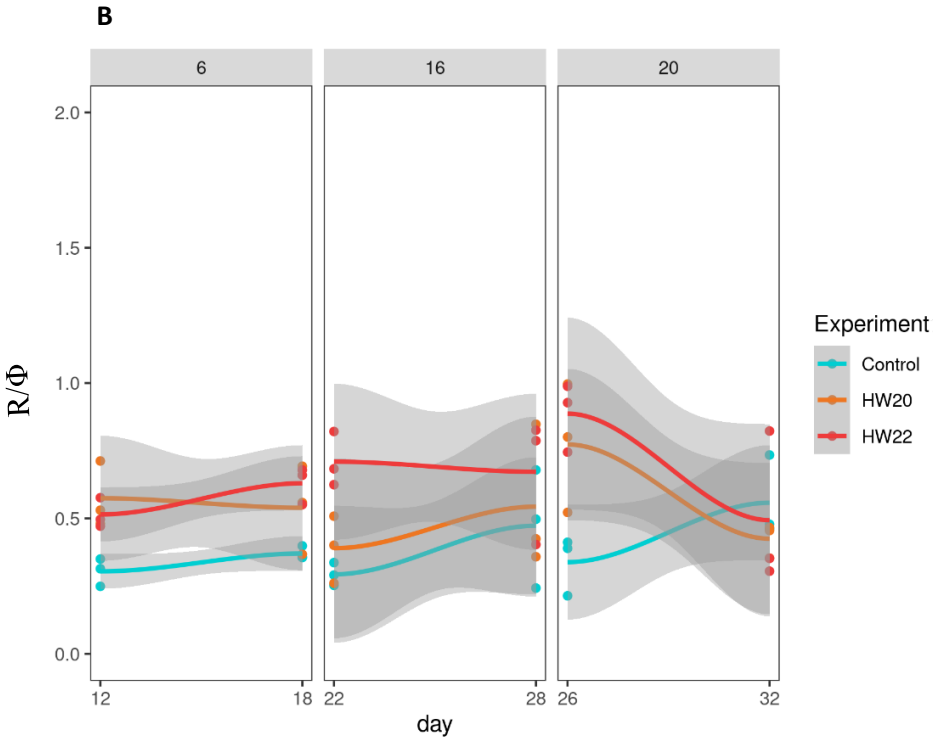
B. R in the recovery period, including post-acclimatisation period (at 18˚C), and recovery (at 16˚C), after 6, 16, and 20 days of MHW duration, respectively.

R/Φ was 0.35 ± 0.08 for the control group kept at 16˚C throughout the experiment (Figure 7). During the pre-acclimatisation period (18˚C), R/Φ rose to 0.57 ± 0.17. In the S-MHW, R/Φ increased steadily until reaching 0.97 ± 0.18 on day 16 and subsequently decreased towards day 20 (0.77 ± 0.23). At 22˚C (E-MHW), R/Φ was above 1 until day 16 (Figure 7 A). R/Φ values > 1 cannot be explained by the theory of the ETS assay. The high ratio indicates that other, unknown, O_2-consuming enzyme reactions occur. R/Φ ratio fell below 1 in the E-MHW after day 16, as it did in the S-MHW. Both R/Φ are significantly different from the control ($p<0.05$). After the recovery period (days 18, 28, and 32) R/Φ was 0.5 ± 0.05 and 0.6 ± 0.08, in S-MHW and E-MHW, respectively. Such ratios allow the jellyfish to regulate their respiration rate efficiently. After the recovery period, only the 6-day E-MHW R/Φ ratio was significantly different from the control ($p<0.05$).

Figure 7. Marine Heatwave Experiment (MHW). Time-course of *A. aurita* polyp R/Φ (unitless)



A. *A. aurita* R/Φ during the MHW (S-MHW-20˚C, E-MHW-22˚C, and control). Green dots ● on day 0 represents the pre- acclimatisation period at 18˚C.



B. R/Φ in the recovery period, including post-acclimatisation period (at 18˚C), and recovery (at 16˚C), after 6, 16, and 20 days of MHW duration, respectively.

3.5. Discussion

In our study polyps kept at 16˚C were larger compared to MHW polyps (Figure 3). Polyps growing larger at lower temperatures has previously been reported (Han and Uye, 2010; Chi et al., 2019) and higher temperatures lead to greater polyp production (Willcox et al., 2007; Hočvar et al., 2018; Treible et al., 2018; Ziegler and Gibbons, 2018). These results in the literature tie in with our results. Asexual reproduction was also greater during the MHW compared to polyps kept at 16˚C (Figure 5). In the case of Chi et al. (2019), polyps, growing at a common summer temperature of 20˚C, were exposed to a simulated summer heatwave (27˚C) for 30 days and the temperature was raised at a rate of 1˚C d^{-1}. In their study, the survival of polyps was affected, the population decreased to 50-75% on day-18 of the MHW, and was even lower after day-30 (0-25%). Here, all polyps survived during the MHW. Although retracted tentacles were observed during the MHWs (Figure 4), polyps did not lose their tentacles, nor did they transform into a non-feeding stage, as observed in Hubot et al. (2017). However, we do not ignore the possibility that strobilation and ephyra-release could be affected after an MHW. Enrique-Navarro et al. (2021), observed that *Cotylorhiza tuberculata* polyps were not greatly affected by future temperature rise scenarios predicted for the Mediterranean Sea, but strobilation and ephyrae were more vulnerable, resulting in a population decrease, malformations, and reduced size, respectively (Enrique-Navarro et al., 2021). Also, Loveridge et al. (2021) found that by simulating a warmer winter, polyps from South England had a limited thermal window during which strobilation occurred successfully. They observed that more ephyra were produced after longer (6 to 8 weeks) and colder winter simulations (4 to 7˚C), and concluded that South England polyps are unlikely to strobilate after experiencing a warmer-than-average winter.

Our study is the first to measure metabolic rates of *A. aurita* polyps during a simulated MHW. Here, as expected, respiration rates increased with temperature in both MHWs (20 and 22˚C). However, the E-MHW stimulated the highest respiration rate over time (Figure 6). The higher respiration during the MHWs coincides with a higher R/Φ ratio, indicating that the respiratory metabolism is working close to its maximum capacity (Figure 7). However, in the recovery period, R/Φ turns back to control conditions. The only available data on R/Φ ratios in *A. aurita* polyps were documented in Purcell et al. (2019). In that study no variability of R/Φ could be observed from well-fed polyps to starved ones, indicating that food availability did not alter this ratio in the short term.

In Minuti et al. (2021) sea urchins (*Heliocidaris erythrogramma*) were exposed to a simulated MHW, followed by a 10-day recovery period. Continued high respiration rates of *H. erythrogramma* in the recovery period suggested an inability to return to metabolic homeostasis and may reflect stress (Minuti et al., 2021). However, in our study, after the recovery period, in both MHWs, respiration rates decreased, reaching initial conditions. Organisms can buffer environmental changes (Pincebourde et al., 2009) because global ocean parameters vary in time and space. This leads to local adaptions and biological parameters, such as ecology, evolution, and plasticity being affected. For example, the same species, under the same scenario, can show species-specific responses to environmental stressors (Vargas et al., 2017). Thermal adaptations of *A. aurita* polyps have been found in Höhn et al. (2017). In their study *A. aurita* polyps, were exposed to 11 temperatures, between 2 and 22˚C, to study physiological tolerance and thermal sensitivity. Respiration curves differed between polyp populations and Höhn et al. (2017) concluded that polyps have different thermal limits due to local adaptions.

Under the growing expectations that jellyfish populations are increasing, future jellyfish research should consider natural variability to define potential stressors. This will allow detection of the limit to plasticity across individuals, populations, and species (Vargas et al., 2017). Climate change is being accelerated due to anthropogenic activity (IPCC, 2014) and climate change models project noticeable increases in MHW events, leading to a quasi-permanent state of MHWs by the end of the century (Plecha et al., 2021). Jellyfish are a diverse group of animals that differ in ecology and physiology, multiple species should be studied for a better understanding of how this part of the zooplankton will respond to increasing MHWs.

3.6. Conclusions

(1) Polyps were able to reproduce more during MHW but were smaller compared to control polyps, and 100% of the polyps were able to survive during the 20-day MHW. Therefore, future increases in MHWs may not impair *A. aurita* polyps.

(2) After experiencing a severe and extreme MHW, in the recovery period, *A. aurita* polyps were able to shift their metabolism to their pre-heatwave respiratory rates.

(3) R/Φ ratio was ≥ 1 during the simulated MHW, but it decreases down to control conditions, suggesting that polyps were able to control their metabolism up to a 20-day MHW.

(4) However, R/Φ > 1 did not follow the constitutive theory of the respiratory ETS methodology. Future research is needed to find potential explanations for how physiological respiration rates can exceed the enzyme activity of the ETS.

3.7. References

Alcaraz, M., Almeda, R., Saiz, E., Calbet, A., Duarte, C. M., Agustí, S., Santiago, R., and Alonso, A., 2013. Effects of temperature on the metabolic stoichiometry of Arctic zooplankton. Biogeosciences, 10(2), 689-697.

Aljbour, S.M., Zimmer, M., Al-Horani, F.A., Kunzmann, A., 2019. Metabolic and oxidative stress responses of the jellyfish *Cassiopea* sp.to changes in seawater temperature. J. Sea Res. 145, 1–7. https://doi.org/10.1016/j.seares.2018.12.002.

Ates, R.M.L., 2017. Benthic scavengers and predators of jellyfish, material for a review. Plankt. Benthos Res. 12, 71–77. https://doi.org/10.3800/PBR.12.71.

Batten, S.D., Raitsos, D.E., Danielson, S., Hopcroft, R., Coyle, K., McQuatters-Gollop, A., 2018. Interannual variability in lower trophic levels on the Alaskan Shelf. Deep Sea Res. Part II Top. Stud. Oceanogr. 147, 58–68. https://doi.org/10.1016/J.DSR2.2017.04.023.

Baxter, E.J., Sturt, M.M., Ruane, N.M., Doyle, T.K., McAllen, R., Harman, L., Rodger, H.D., 2011. Gill Damage to Atlantic Salmon (*Salmo salar*) caused by the Common Jellyfish (*Aurelia aurita*) under Experimental Challenge. PLoS One 6, e18529. https://doi.org/10.1371/JOURNAL.PONE.0018529.

Boero, F., Bouillon, J., Gravili, C., Miglietta, M., Parsons, T., Piraino, S., 2008. Gelatinous plankton: irregularities rule the world (sometimes). Mar. Ecol. Prog. Ser. 356, 299–310. https://doi.org/10.3354/meps07368.

Bondyale-Juez, D. R., Packard, T. T., Viera-Rodríguez, M. A., and Gómez, M., 2017. Respiration: comparison of the Winkler technique, O_2 electrodes, O_2 optodes and the respiratory electron transport system assay. Marine Biology, 164(12), 1-11.

Boscolo-Galazzo, F., Crichton, K. A., Ridgwell, A., Mawbey, E. M., Wade, B. S., and Pearson, P. N., 2021. Temperature controls carbon cycling and biological evolution in the ocean twilight zone. Science, *371*(6534), 1148-1152.

Chi, X., Dierking, J., Hoving, H.-J., Lüskow, F., Denda, A., Christiansen, B., Sommer, U., Hansen, T., Javidpour, J., 2021. Tackling the jelly web: Trophic ecology of gelatinous zooplankton in oceanic food webs of the eastern tropical Atlantic assessed by stable isotope analysis. Limnol. Oceanogr. 66, 289–305. https://doi.org/10.1002/LNO.11605.

Chi, X., Mueller-Navarra, D.C., Hylander, S., Sommer, U., Javidpour, J., 2019. Food quality matters: Interplay among food quality, food quantity and temperature affecting life history traits of *Aurelia aurita* (Cnidaria: Scyphozoa) polyps. Sci. Total Environ. 656, 1280–1288. https://doi.org/10.1016/j.scitotenv.2018.11.469.

Choy, C. A., Haddock, S. H., and Robison, B. H., 2017. Deep pelagic food web structure as revealed by in situ feeding observations. Proceedings of the Royal Society B: Biological Sciences, 284(1868), 20172116.

Dölger, J., Kiørboe, T., Andersen, A., 2019. Dense dwarfs versus gelatinous giants: The trade-offs and physiological limits determining the body plan of planktonic filter feeders. Am. Nat. 194, E30–E40. https://doi.org/10.1086/703656.

Duarte, C. M., Pitt, K. A., Lucas, C. H., Purcell, J. E., Uye, S. I., Robinson, K., Brotz, L., Decker, M. B., Sutherland, K. R., Malej, A., Madin, L., Mianzan, H., Gili, J. M., Fuentes, V., Atienza, D., Pagés, F., Breitburg, D., Malek, J., Graham W. M., and Condon, R. H., 2013. Is global ocean sprawl a cause of jellyfish blooms? Frontiers in Ecology and the Environment, 11(2), 91-97.

Enrique-Navarro, A., Huertas, I. E., León Cobo, M. J., and Prieto, L., 2021. Impact of ocean warming and ocean acidification on asexual reproduction and statolith formation of the symbiotic jellyfish *Cotylorhiza tuberculata*. Plos one, 16(8), e0254983.

Frölicher, T.L., Fischer, E.M., Gruber, N., 2018. Marine heatwaves under global warming. Nature 560, 360–364. https://doi.org/10.1038/s41586-018-0383-9.

Frolova, A., Miglietta, M.P., 2020. Insights on Bloom Forming Jellyfish (Class: Scyphozoa) in the Gulf of Mexico: Environmental Tolerance Ranges and Limits Suggest Differences in Habitat Preference and Resistance to Climate Change Among Congeners. Front. Mar. Sci. 0, 93. https://doi.org/10.3389/FMARS.2020.00093.

Fuchs, B., Wang, W., Graspeuntner, S., Li, Y., Insua, S., Herbst, E.M., Dirksen, P., Böhm, A.M., Hemmrich, G., Sommer, F., Domazet-Lošo, T., Klostermeier, U.C., Anton-Erxleben, F., Rosenstiel, P., Bosch, T.C.G., Khalturin, K., 2014. Regulation of polyp-to-jellyfish transition in *Aurelia aurita*. Curr. Biol. 24, 263–273. https://doi.org/10.1016/j.cub.2013.12.003.

Gall, M.L., Holmes, S.P., Campbell, H., Byrne, M., 2021. Effects of marine heatwave conditions across the metamorphic transition to the juvenile sea urchin (*Heliocidaris erythrogramma*). Mar. Pollut. Bull. 163, 111914. https://doi.org/10.1016/J.MARPOLBUL.2020.111914.

Gambill, M., Peck, M.A., 2014. Respiration rates of the polyps of four jellyfish species: Potential thermal triggers and limits. J. Exp. Mar. Bio. Ecol. 459, 17–22. https://doi.org/10.1016/j.jembe.2014.05.005.

Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., Diaz, D., Harmelin, J. G., Gambi, M. C., Kersting, D. K., Ledoux, J. B., Lejeusne, C., Linares, C, Marschal, C., Pérez, T., Ribes, M., Romano, J. C., Serrano, E., Teixido, N., Torrents, O., Zabala, M., Zuberer, C., and Cerrano, C., 2009. Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Global change biology, 15(5), 1090-1103.

Han, C.-H., Uye, S., 2010. Combined effects of food supply and temperature on asexual reproduction and somatic growth of polyps of the common jellyfish *Aurelia aurita* s.l. Plankt. Benthos Res. 5, 98–105. https://doi.org/10.3800/pbr.5.98.

Hayashida, H., Matear, R.J., Strutton, P.G., 2020. Background nutrient concentration determines phytoplankton bloom response to marine heatwaves. Glob. Chang. Biol. 26, 4800–4811. https://doi.org/10.1111/GCB.15255.

Heim-Ballew, H., Olsen, Z., 2018. Salinity and temperature influence on Scyphozoan jellyfish abundance in the Western Gulf of Mexico. Hydrobiol. 2018 8271 827, 247–262. https://doi.org/10.1007/S10750-018-3771-0.

Hobday, A.J., Alexander, L. V., Perkins, S.E., Smale, D.A., Straub, S.C., Oliver, E.C.J., Benthuysen, J.A., Burrows, M.T., Donat, M.G., Feng, M., Holbrook, N.J., Moore, P.J., Scannell, H.A., Sen Gupta, A., Wernberg, T., 2016. A

hierarchical approach to defining marine heatwaves. Prog. Oceanogr. 141, 227–238. https://doi.org/10.1016/j.pocean.2015.12.014.
Hobday, A. J., Oliver, E. C., Gupta, A. S., Benthuysen, J. A., Burrows, M. T., Donat, M. G., Holbrook, N. J., Moore, P. J., Thomsen, M. S., Wernberg, T., and Smale, D. A., 2018. Categorizing and naming marine heatwaves. Oceanography, 31(2), 162-173.
Hočvar, S., Malej, A., Boldin, B., Purcell, J.E., 2018. Seasonal fluctuations in population dynamics of *Aurelia aurita* polyps in situ with a modelling perspective. Mar. Ecol. Prog. Ser. 591, 155–166. https://doi.org/10.3354/MEPS12387.
Höhn, D.P., Lucas, C.H., Thatje, S., 2017. Respiratory response to temperature of three populations of *Aurelia aurita* polyps in northern Europe. PLoS One 12, 1–13. https://doi.org/10.1371/journal.pone.0177913.
Holst, S., 2012. Effects of climate warming on strobilation and ephyra production of North Sea scyphozoan jellyfish. Jellyfish Bloom. IV 127–140. https://doi.org/10.1007/978-94-007-5316-7_10.
Hubot, N., Lucas, C.H., Piraino, S., 2017. Environmental control of asexual reproduction and somatic growth of *Aurelia* spp. (Cnidaria, Scyphozoa) polyps from the Adriatic Sea. PLoS One 12, 1–16. https://doi.org/10.1371/journal.pone.0178482.
Hughes, T.P., Anderson, K.D., Connolly, S.R., Heron, S.F., Kerry, J.T., Lough, J.M., Baird, A.H., Baum, J.K., Berumen, M.L., Bridge, T.C., Claar, D.C., Eakin, C.M., Gilmour, J.P., Graham, N.A.J., Harrison, H., Hobbs, J.-P.A., Hoey, A.S., Hoogenboom, M., Lowe, R.J., McCulloch, M.T., Pandolfi, J.M., Pratchett, M., Schoepf, V., Torda, G., Wilson, S.K., 2018. Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science (80-.). 359, 80–83. https://doi.org/10.1126/SCIENCE.AAN8048.
Hughes, T.P., Kerry, J.T., Álvarez-Noriega, M., Álvarez-Romero, J.G., Anderson, K.D., Baird, A.H., Babcock, R.C., Beger, M., Bellwood, D.R., Berkelmans, R., Bridge, T.C., Butler, I.R., Byrne, M., Cantin, N.E., Comeau, S., Connolly, S.R., Cumming, G.S., Dalton, S.J., Diaz-Pulido, G., Eakin, C.M., Figueira, W.F., Gilmour, J.P., Harrison, H.B., Heron, S.F., Hoey, A.S., Hobbs, J.-P.A., Hoogenboom, M.O., Kennedy, E. V., Kuo, C., Lough, J.M., Lowe, R.J., Liu, G., McCulloch, M.T., Malcolm, H.A., McWilliam, M.J., Pandolfi, J.M., Pears, R.J., Pratchett, M.S., Schoepf, V., Simpson, T., Skirving, W.J., Sommer, B., Torda, G., Wachenfeld, D.R., Willis, B.L., Wilson, S.K., 2017. Global warming and recurrent mass bleaching of corals. Nat. 2017 5437645 543, 373–377. https://doi.org/10.1038/nature21707.
Loveridge, A., Lucas, C. H., and Pitt, K. A., 2021. Shorter, warmer winters may inhibit production of ephyrae in a population of the moon jellyfish *Aurelia aurita*. Hydrobiologia, 848(3), 739-749.
Kendon, M., McCarthy, M., Jevrejeva, S., Matthews, A., and Legg, T., 2019. State of the UK climate 2018. International Journal of Climatology, 39, 1-55.
Iguchi, N., Iwatani, H., Sugimoto, K., Kitajima, S., Honda, N., Katoh, O., 2017. Biomass, body elemental composition, and carbon requirement of Nemopilema nomurai (Scyphozoa: Rhizostomeae) in the southwestern Japan Sea. Plankt. Benthos Res. 12, 104–114. https://doi.org/10.3800/pbr.12.104.
Leung, J.Y.S., Russell, B.D., Connell, S.D., 2019. Adaptive Responses of Marine Gastropods to Heatwaves. One Earth 1, 374–381. https://doi.org/10.1016/J.ONEEAR.2019.10.025.
Lucas, C.H., Graham, W.M., Widmer, C., 2012. Jellyfish Life Histories: Role of Polyps in Forming and Maintaining Scyphomedusa Populations, in: Advances in Marine Biology. Academic Press, pp. 133–196. https://doi.org/10.1016/B978-0-12-394282-1.00003-X.
Lucas, C.H., Jones, D.O.B., Hollyhead, C.J., Condon, R.H., Duarte, C.M., Graham, W.M., Robinson, K.L., Pitt, K.A., Schildhauer, M., Regetz, J., 2014. Gelatinous zooplankton biomass in the global oceans: Geographic variation and environmental drivers. Glob. Ecol. Biogeogr. 23, 701–714. https://doi.org/10.1111/geb.12169.
Mariottini, G.L., Pane, L., 2010. Mediterranean jellyfish venoms: A review on scyphomedusae. Mar. Drugs 8, 1122–1152. https://doi.org/10.3390/md8041122.
Matsumura, K., Kamiya, K., Yamashita, K., Hayashi, F., Watanabe, I., Murao, Y., Miyasaka, H., Kamimura, N., Nogami, M., 2005. Genetic polymorphism of the adult medusae invading an electric power station and wild polyps of *Aurelia aurita* in Wakasa Bay, Japan. J. Mar. Biol. Assoc. United Kingdom 85, 563–568. https://doi.org/10.1017/S0025315405011483.
McCauley, D.J., Pinsky, M.L., Palumbi, S.R., Estes, J.A., Joyce, F.H., Warner, R.R., 2015. Marine defaunation: Animal loss in the global ocean. Science (80-.). https://doi.org/10.1126/science.1255641.
McKinstry, C.A.E., Campbell, R.W., 2018. Seasonal variation of zooplankton abundance and community structure in Prince William Sound, Alaska, 2009–2016. Deep Sea Res. Part II Top. Stud. Oceanogr. 147, 69–78. https://doi.org/10.1016/J.DSR2.2017.08.016.
Minuti, J.J., Byrne, M., Hemraj, D.A., Russell, B.D., 2021. Capacity of an ecologically key urchin to recover from extreme events: Physiological impacts of heatwaves and the road to recovery. Sci. Total Environ. 785, 147281. https://doi.org/10.1016/J.SCITOTENV.2021.147281.
Mitterwallner, V., Suci, A.N.N., Zamani, N., Lenz, M., 2021. Exposure to moderately elevated temperatures changes food preferences in the tropical marine herbivore Haliotis squamata. Mar. Biol. 2021 1687 168, 1–12. https://doi.org/10.1007/S00227-021-03922-Y.
Oliver, E.C.J., 2019. Mean warming not variability drives marine heatwave trends. Clim. Dyn. 2019 533 53, 1653–1659. https://doi.org/10.1007/S00382-019-04707-2.
Oliver, E.C.J., Benthuysen, J.A., Darmaraki, S., Donat, M.G., Hobday, A.J., Holbrook, N.J., Schlegel, R.W., Gupta, A. Sen, 2021. Marine Heatwaves. https://doi.org/10.1146/annurev-marine-032720-095144 13, 313–342. https://doi.org/10.1146/ANNUREV-MARINE-032720-095144.
Oliver, E.C.J., Donat, M.G., Burrows, M.T., Moore, P.J., Smale, D.A., Alexander, L. V., Benthuysen, J.A., Feng, M., Sen Gupta, A., Hobday, A.J., Holbrook, N.J., Perkins-Kirkpatrick, S.E., Scannell, H.A., Straub, S.C., Wernberg, T., 2018. Longer and more frequent marine heatwaves over the past century. Nat. Commun. 9, 1–12. https://doi.org/10.1038/s41467-018-03732-9.

Pauly, D., Graham, W., Libralato, S., Morissette, L., Deng Palomares, M.L., 2009. Jellyfish in ecosystems, online databases, and ecosystem models. Hydrobiologia 616, 67–85. https://doi.org/10.1007/s10750-008-9583-x.

Pearce AF, Lenanton R, Jackson G, Moore J, Feng M, Gaughan D., 2011. The "marine heat wave" off Western Australia during the summer of 2010/11. Tech. Rep. 222, West. Aust. Fish. Mar. Res. Lab., North Beach, Aust.

Perez, T., Garrabou, J., Sartoretto, S., Harmelin, J. G., Francour, P., and Vacelet, J., 2000. Massive mortality of marine invertebrates: an unprecedented event in northwestern Mediterranean. Comptes rendus de l'Academie des sciences. Serie III, Sciences de la vie, 323(10), 853-865.

Pörtner, H. O., and Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. science, 315(5808), 95-97.

Pörtner, H. O., and Farrell, A. P., 2008. Physiology and climate change. Science, 690-692.

Piatt, J.F., Parrish, J.K., Renner, H.M., Schoen, S.K., Jones, T.T., Arimitsu, M.L., Kuletz, K.J., Bodenstein, B., García-Reyes, M., Duerr, R.S., Corcoran, R.M., Kaler, R.S.A., McChesney, G.J., Golightly, R.T., Coletti, H.A., Suryan, R.M., Burgess, H.K., Lindsey, J., Lindquist, K., Warzybok, P.M., Jahncke, J., Roletto, J., Sydeman, W.J., 2020. Extreme mortality and reproductive failure of common murres resulting from the northeast Pacific marine heatwave of 2014-2016. PLoS One 15, e0226087. https://doi.org/10.1371/JOURNAL.PONE.0226087.

Pincebourde, S., Sanford, E., Helmuth, B., 2009. An intertidal sea star adjusts thermal inertia to avoid extreme body temperatures. Am. Nat. 174, 890–897. https://doi.org/10.1086/648065.

Plecha, S.M., Soares, P.M.M., Silva-Fernandes, S.M., Cabos, W., 2021. On the uncertainty of future projections of Marine Heatwave events in the North Atlantic Ocean. Clim. Dyn. 2021 567 56, 2027–2056. https://doi.org/10.1007/S00382-020-05529-3.

Purcell, J.E., 2012. Jellyfish and ctenophore blooms coincide with human proliferations and environmental perturbations. Ann. Rev. Mar. Sci. https://doi.org/10.1146/annurev-marine-120709-142751.

Purcell, J.E., 2007. Environmental effects on asexual reproduction rates of the scyphozoan *Aurelia labiata*. Mar. Ecol. Prog. Ser. 348, 183–196. https://doi.org/10.3354/meps07056.

Purcell, J.E., Bondyale-Juez, D.R., Romero-Kutzner, V., Martínez, I., Caprioli, R., Tames-Espinosa, M., Almunia, J., Alonso, E., Packard, T.T., Gómez, M., 2019. Food supply effects on the asexual reproduction and respiratory metabolism of Aurelia aurita polyps. Hydrobiologia 846, 135–146. https://doi.org/10.1007/s10750-019-04057-4.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rendina, F., Bouchet, P.J., Appolloni, L., Russo, G.F., Sandulli, R., Kolzenburg, R., Putra, A., Ragazzola, F., 2019. Physiological response of the coralline alga Corallina officinalis L. to both predicted long-term increases in temperature and short-term heatwave events. Mar. Environ. Res. 150, 104764. https://doi.org/10.1016/J.MARENVRES.2019.104764.

Reynolds, R. W., Smith, T. M., Liu, C., Chelton, D. B., Casey, K. S., and Schlax, M. G., 2007. Daily high-resolution-blended analyses for sea surface temperature. Journal of climate, 20(22), 5473-5496

Riascos, J. M., Vergara, M., Fajardo, J., Villegas, V., and Pacheco, A. S., 2012. The role of hyperiid parasites as a trophic link between jellyfish and fishes. Journal of Fish Biology, 81(5), 1686-1695.

Richardson, A.J., Bakun, A., Hays, G.C., Gibbons, M.J., 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends Ecol. Evol. 24, 312–322. https://doi.org/10.1016/j.tree.2009.01.010.

Savenkoff, C., Packard, T.T., Rodier, M., Gérinno, M., Lefèvre, D. and Denis, M., 1995. Relative confribution of dehydrogenases to overall respiratory ETS activity in some marine organisms. Journal of plankton research,17(8), pp.1593-1604.

Scanes, E., Parker, L. M., O'Connor, W. A., Dove, M. C., and Ross, P. M., 2020. Heatwaves alter survival of the Sydney rock oyster, Saccostrea glomerata. Marine pollution bulletin, 158, 111389

Scanes, E., Scanes, P. R., and Ross, P. M., 2020. Climate change rapidly warms and acidifies Australian estuaries. Nature communications, 11(1), 1-11.

Schlegel, R. W., and Smit, A. J., 2018. heatwaveR: A central algorithm for the detection of heatwaves and cold-spells. Journal of Open Source Software, 3(27), 821.

Schlegel, R. W., 2020. Marine Heatwave Tracker. http://www.marineheatwaves.org/tracker. doi: 10.5281/zenodo.3787872 Shanks, A.L., Rasmuson, L.K., Valley, J.R., Jarvis, M.A., Salant, C., Sutherland, D.A., Lamont, E.I., Hainey, M.A.H., Emlet, R.B., 2020. Marine heat waves, climate change, and failed spawning by coastal invertebrates. Limnol. Oceanogr. 65, 627–636. https://doi.org/10.1002/LNO.11331.

Slater, W.L., Pierson, J.J., Decker, M.B., Houde, E.D., Lozano, C., Seuberling, J., 2020. Fewer Copepods, Fewer Anchovies, and More Jellyfish: How Does Hypoxia Impact the Chesapeake Bay Zooplankton Community? Divers. 2020, Vol. 12, Page 35 12, 35. https://doi.org/10.3390/D12010035.

Smale, D.A., Wernberg, T., Oliver, E.C.J., Thomsen, M., Harvey, B.P., Straub, S.C., Burrows, M.T., Alexander, L. V., Benthuysen, J.A., Donat, M.G., Feng, M., Hobday, A.J., Holbrook, N.J., Perkins-Kirkpatrick, S.E., Scannell, H.A., Sen Gupta, A., Payne, B.L., Moore, P.J., 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat. Clim. Chang. 1. https://doi.org/10.1038/s41558-019-0412-1.

Suryan, R.M., Arimitsu, M.L., Coletti, H.A., Hopcroft, R.R., Lindeberg, M.R., Barbeaux, S.J., Batten, S.D., Burt, W.J., Bishop, M.A., Bodkin, J.L., Brenner, R., Campbell, R.W., Cushing, D.A., Danielson, S.L., Dorn, M.W., Drummond, B., Esler, D., Gelatt, T., Hanselman, D.H., Hatch, S.A., Haught, S., Holderied, K., Iken, K., Irons, D.B., Kettle, A.B., Kimmel, D.G., Konar, B., Kuletz, K.J., Laurel, B.J., Maniscalco, J.M., Matkin, C., McKinstry, C.A.E., Monson, D.H., Moran, J.R., Olsen, D., Palsson, W.A., Pegau, W.S., Piatt, J.F., Rogers, L.A., Rojek, N.A., Schaefer, A., Spies, I.B., Straley, J.M., Strom, S.L., Sweeney, K.L., Szymkowiak, M., Weitzman, B.P., Yasumiishi, E.M., Zador, S.G., 2021. Ecosystem response persists after a prolonged marine heatwave. Sci. Reports 2021 111 11, 1–17. https://doi.org/10.1038/s41598-021-83818-5.

Takao, M., Okawachi, H., Uye, S.I., Okawachi, H., 2014. Natural predators of polyps of Aurelia aurita s.l. (Cnidaria: Scyphozoa: Semaeostomeae) and their predation rates. Plankt. Benthos Res. 9, 105–113. https://doi.org/10.3800/pbr.9.105.

Treible, L., Pitt, K., Klein, S., Progress, R.C.-M.E., 2018. Exposure to elevated pCO_2 does not exacerbate reproductive suppression of *Aurelia aurita* jellyfish polyps in low oxygen environments. int-res.com. https://doi.org/10.3354/meps12298.

Uye, S. I., Fujii, N., and Takeoka, H., 2003. Unusual aggregations of the scyphomedusa *Aurelia aurita* in coastal waters along western Shikoku, Japan. Plankton Biology and Ecology, 50(1), 17-21.

Vargas, C.A., Lagos, N.A., Lardies, M.A., Duarte, C., Manríquez, P.H., Aguilera, V.M., Broitman, B., Widdicombe, S., Dupont, S., 2017. Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. Nat. Ecol. Evol. 2017 14 1, 1–7. https://doi.org/10.1038/s41559-017-0084.

Waal, D.B. Van de, Litchman, E., 2020. Multiple global change stressor effects on phytoplankton nutrient acquisition in a future ocean. Philos. Trans. R. Soc. B 375. https://doi.org/10.1098/RSTB.2019.0706.

Weitzman, B., Konar, B., Iken, K., Coletti, H., Monson, D., Suryan, R., Dean, T., Hondolero, D., Lindeberg, M., 2021. Changes in Rocky Intertidal Community Structure During a Marine Heatwave in the Northern Gulf of Alaska. Front. Mar. Sci. 8, 115. https://doi.org/10.3389/FMARS.2021.556820/BIBTEX.

Willcox, S., Moltschaniwskyj, N.A., Crawford, C., 2007. Asexual reproduction in scyphistomae of *Aurelia* sp.: Effects of temperature and salinity in an experimental study. J. Exp. Mar. Bio. Ecol. 353, 107–114. https://doi.org/10.1016/j.jembe.2007.09.006.

Ziegler, L., Gibbons, M., 2018. Environmental responses of jellyfish polyps as drivers of medusa populations off the coast of Namibia. https://doi.org/10.2989/1814232X.2018.1500397 40, 323–329. https://doi.org/10.2989/1814232X.2018.1500397.

CHAPTER

4

Metabolism of *A. aurita*: From polyp to medusa life stage

Inde ferunt, totidem qui vivere debeat annos, corpore de patrio parvum phoenica renasci

They say, the new phoenix, destined to live the same number of years as its parent, will be reborn from the ashes of the old phoenix.

In Metamorphoseon Libri by Publius Ovidius Naso (Ovid), 8 AD

4.1 Abstract

Many scientists claim that jellyfish will play a key role in future oceans. Here, we study the physiology of metamorphosing *A. aurita*. This medusa is a ubiquitous, cosmopolitan scyphozoan jellyfish that transforms its body through a triphasic life cycle. To assess the different features of its metabolism, respiratory O_2 consumption, NH_4^+ excretion, the enzymatic activity of the respiratory electron transport system (i.e., potential respiration, Φ), isocitrate dehydrogenase (IDH), and glutamate dehydrogenase (GDH), were measured, as *A. aurita* morphed from benthic polyp to juvenile medusa life stage. In addition, biochemical components (protein, lipid, and carbohydrate) and growth (ephyra to medusa) were determined. The findings showed that all wet mass-specific respiratory vary through the different life stages ($p<0.05$). Benthic polyps and strobila presented the highest metabolic rates. In strobila, the energy reserves initially accumulated in the first stages and decreased towards strobilation. The low R/Φ ratio (0.23 ± 0.09) did not differ among life stages ($p>0.05$), indicating that *A. aurita's* respiration was stable during metamorphosis and suggests flexibility in shifting its metabolism in response to environmental variability.

4.2 Introduction

Jellyfish are ancient cnidarians that date back to the Ediacaran period, 94 million years before the Cambrian, a time when “the metazoans inherited the world” (Cloud and Glaessner, 1982). At this time, metagenesis began and metamorphosis appears to have evolved up to eight distinct times in metazoa (Hadfield, 2000). Cnidarian metagenesis is one of the most ancient complex life cycles in metazoans (Marques and Collins, 2004; Kraus et al., 2015) and may have contributed to Cnidarian success (Pitt et al., 2013). It consists of an alternation between the sessile polyp form, attached to a hard substratum, and the motile, pulsating, medusoid form, that swims through the ocean’s water column. The polyp form has been considered to be the ancestral cnidarian adult body plan, and the medusa form, a later secondary derivative (Haeckel, 1879; Brooks, 1886; Korschelt and Heider, 1890; Hadži, 1953; Remane, 1954; Salvini-Plawen, 1987; Collins, 2002).

Scyphozoans, a class of cnidarians often called, “real jellyfish”, have an even more complicated life cycle, a triphasic one, with motile planula larvae. These bilaterally symmetrical larvae are produced by sexually mature medusa and become a sessile benthic polyp, once it attaches to a substrate. Polyps have different ways of asexual reproduction and can metamorphose to pelagic medusae through a process called strobilation. Strobilation starts by segmentation of the polyp's body by apical transversal constrictions. It culminates in the apical release of planktonic, star-shaped, ephyrae that grow to medusae (Lucas et al., 2012).

Currently, many scientists claim that jellyfish will play a key role in the future oceans (Richardson et al., 2009; Boero et al., 2016). The scenario of our future oceans is affected more and more by global warming and anthropogenic activity (Jackson et al., 2001; Purcell et al., 2007; McCauley et al., 2015) with a direct influence on marine ecosystems. It has been shown that marine

invertebrates can benefit from current or future ocean changes (McCauley et al., 2015). During metamorphosis, however, jellyfish may be particularly vulnerable to environmental stressors (Lowe et al., 2021). Because of this, global climate change may affect jellyfish survival and performance through physiological, ecological, and behavioral mechanisms (Lowe et al., 2021).

Furthermore, there is new evidence that transgenerational changes can affect jellyfish populations differently (Lu et al., 2020). In this way, the biology of jellyfish populations may be shifted unpredictably by climate change. However, jellyfish ecophysiology research mostly focuses on a single life stage (Klein et al., 2014; Algueró-Muñiz et al., 2016; Dong and Sun, 2018; Chuard et al., 2019). In contrast, marine climate-change research is starting to avoid single life-stage studies, to focus on the adaptive capacity of populations in future ocean scenarios (Pineda et al., 2012; Foo and Byrne, 2016; Pandori and Sorte, 2019; Aalto et al., 2020; van der Lee et al., 2020; Olguín-Jacobson et al., 2021).

In that sense, forecasting how jellyfish populations may respond to environmental changes requires considering their life cycles (Dong and Sun, 2018; Goldstein and Steiner, 2020). In addition, little is known about the persistence and potential of the responses that promote adaptation to global change scenarios across multiple generations (Gibbin et al., 2017). Therefore, jellyfish research that integrates all life stages is needed, to predict population-size trends in the context of future climate change (Dong and Sun, 2018).

The scyphozoan, *Aurelia aurita*, is a well-studied widespread, almost cosmopolitan species with a triphasic life cycle (Fuchs et al., 2014). The objective of this research is to study *A. aurita's* life cycle metabolism, under controlled laboratory conditions to characterise, biochemically and physiologically, the polyp-to-juvenile medusa transitions, and identify high energy demanding stages. To accomplish this, we have measured metabolic

rates (respiration (R) and ammonia excretion), and an enzyme-based index corresponding to the major energy metabolism pathways (Roznere et al., 2021). Metabolic rates and metabolic enzyme activities are the most commonly measured physiological responses in the literature (Seebacher et al., 2015). These activities theoretically reflect the maximum capacity to produce or consume a specific metabolite.

Here, based on in-vitro enzyme assays, they were measured kinetically (Fernández-Urruzola et al., 2011; Purcell et al., 2019). Specifically, potential oxygen consumption (Φ), based on the respiratory electron transport system (ETS); potential CO_2 production (IDH), based on the Krebs cycle enzyme isocitrate dehydrogenase activity and; an index of NH_4^+-excretion, based on glutamate dehydrogenase (GDH) were measured. Interest in these types of measurements is increasing in the jellyfish research community (Iguchi et al., 2017; Aljbour et al., 2019; Purcell et al., 2019). In addition to these measurements, biochemical composition (carbohydrates, lipids, and protein), and growth (from ephyrae to medusae) were measured. All measurements were made during a more than 3-month time course, that spanned the polyp-to-young medusa transition.

Although little is known about the physiological and biochemical changes during the metamorphosis of Scyphozoa, it appears reasonable to think that the development of swimming ability would be accompanied by metabolic adaptations. Therefore, we expected changes in metabolic and enzyme activities during the different life stage transitions. We hypothesised, first, that physiological rates would increase, and second, that the energy content of the three major biochemical constituents, as well as their storage, would shift, as the *A. aurita* morphed through its life-stage transitions.

This is the first long-term study to follow *A. aurita* metabolism, from polyp to medusa, under controlled laboratory conditions. It represents an initial step in

elucidating how environmental factors could affect life-stages and their transitions.

4.3 Material and Methods

4.3.1 Culture and experimental design

Polyps, sexually produced from *A. aurita* planulae, were obtained from the Poema del Mar Aquarium (Gran Canaria, Spain). The stock culture was maintained in an 8-L tank at the University of Las Palmas de Gran Canaria at 23˚C, 33‰ salinity, and pH 8.2. It was fed *Artemia* sp. nauplii *ad libitum* once daily. To start the experiment, around 2000 polyps were placed in three 4.3 L aquaria, and were acclimatized for 3 weeks to a temperature of 20˚C. During this time, they were daily fed 48h-*Artemia* sp. nauplii (Hassan and Rahman, 2016), enriched with Selco (Easy DHA Selco, INVE Aquaculture). The aquaria were checked for physical and chemical changes periodically and on every experimental day. Specifically, temperature, O_2, pH, nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and salinity (S‰) were monitored. Seawater was refreshed daily (around 10% of the seawater was replaced) and completely renewed (100%) on experimental days.

The polyps and strobilae were maintained in the aquaria. Ephyrae, released from the strobilae, were transferred from the aquaria to 1.7 L Kreisel-tanks. During the acclimatisation period (stock culture) and throughout the entire experiment, the organisms were kept in an incubator (NUVE ES120) at 20˚C.

In this study, we define five different life stages: polyp (P), strobila (S), ephyra (E), metaephyra (ME), and medusa (M). All measurements were performed on these life stages. In addition, growth from ephyrae to medusae was followed by measuring the mean bell-diameter (D) photographically. Biochemical

composition (protein, lipid, and carbohydrates) was also measured from polyp to medusa life stage. Results of growth and biochemical composition will be presented and discussed in part I of this chapter.

The experiment lasted 108 days. Benthic life stage (polyp) measurements started at day – 52 (Table 1), and strobilae were measured in four different stages (Figure 1) (day – 21 to -3). Scyphozoan planktonic life forms, ephyra to medusa transition, were followed from ephyrae release (day 0) until day 56. After the measurements in the polyp life stage, strobilation was induced by lowering the temperature in the incubator from 20˚C to 10˚C, 1˚C per day.

The strobilation induction period lasted around 7 weeks. Strobilae stages (Figure 1, modified from Kuniyoshi et al., 2012) were measured on day -21 (stage I), day -11 (stage II), day -6 (stage III), and day -3 (stage IV). Subsequently, the first ephyrae released from strobilae were measured on day 0. The sample ages, during the ephyrae-to-medusae transition and when measurements were made, are shown in Table 1.

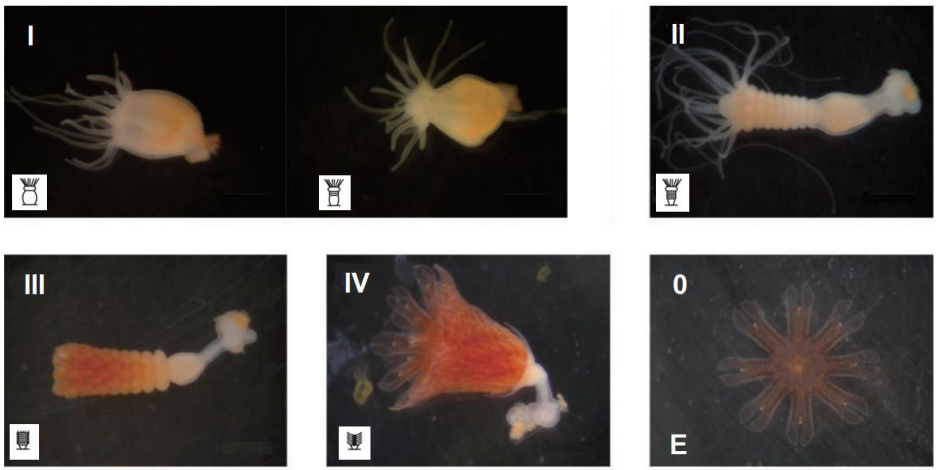


Figure 1. Strobilae stages measured in this study, and release of ephyrae (day 0, stage E=ephyrae). (Figure modified from Kuniyoshi et al. 2012)

Table 1. Timeline and related life stages, number of organisms used in incubations, and bottle volume. P= Polyp, S=Strobila, E= Ephyra, ME= Metaephyra, M= Medusa.

T(Day)	**Life stage**		**Number of organisms**	**Incubation volume (mL)**
- 52	**Polyp** (P)		10	5
-21	**Strobila (S)** See Figure 1	I	10	5
-11		II	10	5
-6		III	10	5
-3		IV	10	5
0 to 4	**Ephyra** (E)		15	5
7 to 26	**Metaephyra** (ME)		10	5
30 to 56	**Medusa** (M)		3-5	56

4.3.2 Experimental routine

The experiment started in October 2020. This routine was always followed: first, the physical and chemical conditions of the water in the aquaria or Kreisels were checked. Then the culture was fed for 1 h (Purcell and Kremer, 1983; Fernández-Urruzola et al., 2011), with a known concentration of enriched 48-h-old *Artemia* sp. nauplii. Avoiding the egestion of food during the experimental performance was crucial because it could lead to erroneous metabolic calculations. Food ingestion depended on the life stage (Figure 2). Food ingestion was almost absent during the final stages of strobilae (Figure 2). In this way, feeding ceased before strobilation and until day 4 of ephyrae release.

After 1 h, the approximate food consumed was determined (Figure 2). Ingestion rates, per h, were calculated by subtracting final, from initial, *Artemia* sp. nauplii concentrations. For this, three 5-mL aliquots were taken, and the *Artemia* sp. were counted. Simultaneously, the experimental organisms (polyps/strobilae/ephyrae or medusae) were separated from the culture tank and acclimated for half an hour in filtered seawater, as in Fernández-Urruzola et al. (2011). Once the organisms were acclimated, they were placed carefully in the respiration incubation flasks to determine oxygen (O_2) consumption and NH_4^+ excretion.

In the case of the stages from ephyrae to medusae, the mean diameter (D) was determined photographically with a stereoscopic microscope before incubating the samples. The number of the organisms and the volume of the incubation flasks depended on the organism biomass (Table 1). The different sized incubation flasks ensured an adequate organism-biomass/seawater-volume ratio (Purcell et al., 2010). The incubation time was fixed at 1h. A short incubation time minimizes enzymatic induction and repression, as well as starvation effects (Férnandez-Urruzola et al., 2011). Furthermore, it should

be within the time *A. aurita* takes to digest its prey (1-2.3 h, Uye and Shimauchi, 2005). Also, NH_4^+ excretion rates are constant during a 2 h period (Purcell and Kremer, 1983; Nemazie et al.,1993). Once the incubations were concluded, wet mass (WM) was measured on an analytical balance and then the samples were stored for enzyme and biochemical analysis at -80˚C. This procedure was repeated, in triplicate, for each of the 3 aquaria/Kreisels. That meant 9 samples, in total, and a blank for each incubation. Methodology and results of *A. aurita*'s metabolism will be presented and discussed in part II of this chapter.

4.3.3 Statistics

Significant difference between, biochemical composition, respiration rates, excretion rates, and ratios, through the different life stages, were analysed. Normality was confirmed by the Shapiro-Wilk test and variance homogeneity was confirmed by the Fligner-Killeen or Bartlett tests. For Fligner Kileen test, the significant differences were confirmed with the Kruskal-Wallis test and with a pairwise comparison using the Tukey and Kramer (Nemenyi) test. Using ANOVA analysis and the Tukey-HSD post-hoc test, the significant differences between treatments were verified, for the Bartlett test. All data were analysed with the R program (R Core Team, 2020).

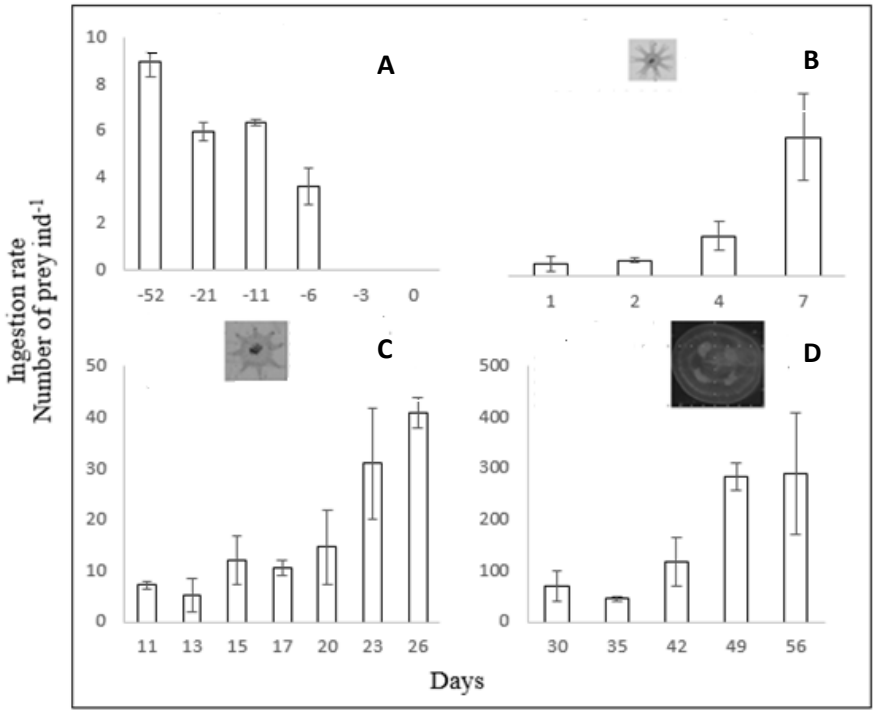


Figure 2. Ingestion rate (number of prey ingested per individual, per h). A. Polyps and strobilae B. Ephyrae C. Metaephyrae D. Juvenile medusae.

PART I

I.1. Material and methods: Biochemical components

The determination of protein, lipid, and carbohydrates was based on the spectrophotometric methods proposed by Lowry et al. (1951), Knight et al. (1972), and Dubois et al. (1956), respectively. Our modifications are given below.

Protein

Protein content was determined according to Martínez et al. (2020). 100µL of homogenate was mixed with 500 µL of the Rutter solution (Rutter, 1967) with sodium dodecyl-sulphate sodium salt (SDS) (Markwell et al., 1978). After 10 min, 50 µL of Folin solution was added, then the solution was well mixed and left in darkness for 40 min. The absorbance was measured at 750 nm. Bovine serum albumin was used as the standard (0-500µg/mL).

Lipid

Lipid content, as an index of total lipids, was measured based on Knight et al. (1972), Barnes and Blackstock (1973), De Coen and Janssen (1997), and Marsh and Weinstein (1966). First, lipids were extracted following the Bligh and Dyer (1959) methodology, using a chloroform: methanol: water ratio of 1:1:0.9 (this ratio takes into account the water present in the sample). The mixture was centrifuged for 10 min at 2600 g, and 4˚C (Gerber et al., 2018). Then, 100 µL of the bottom phase were then pipetted out and added to 500 µL of H_2SO_4 (95%). This solution was kept at 200˚C for 15 min, without mixing. After this time, the solution was allowed to cool down for 5 min and then mixed well. After that, 40 µL of the solution was mixed with 1 mL of phospho-vanillin reagent and incubated for 15 min at 37˚C. The solution was then

cooled for 5 min and its absorbance was measured at 525 nm. Commercial olive oil (0 - 4.8 mg mL^{-1}) dissolved in chloroform was used as standard.

Carbohydrates

Carbohydrates were determined following Dubois et al. (1956). 150 µL of a 5% phenol solution and 750 µL of a 95% concentrated sulphuric acid were added to 150 µL of homogenate. After 10 min, the solution was well mixed and incubated at 30˚C for another 10 min. Then, the sample was cooled to room temperature for 5 min and absorbance was read spectrophotometrically at 485 nm. Glucose (0-1.5 mg mL^{-1}), dissolved in homogenate buffer solution served as the standard.

I.2. Results

I.2.1 Growth

After the liberation of the ephyrae, we followed the evolution of growth (Figure 3), based on size and WM, as it transitions to juvenile medusa during 56 days. Growth was separated into three groups: ephyra, metaephyra, and medusa. Linear equations for growth rate were the following: ephyrae (growth = 0.2114 mm day^{-1} + 1.08, R^2=0.95), metaephyrae (growth= 0.0532 mm day^{-1} + 3.747, R^2=0.93), and medusae (growth= 0.5263 mm day^{-1} – 9.58, R^2=0.98).

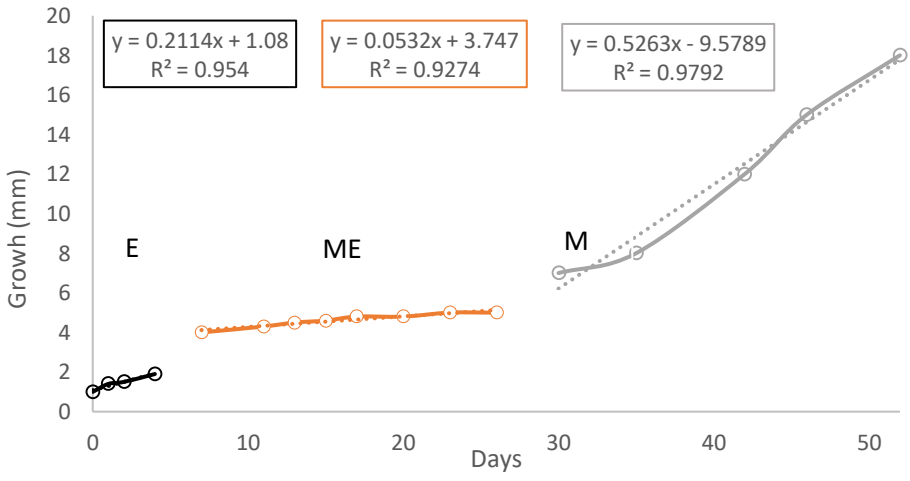


Figure 3. Growth, mean diameter (mm) from the ephyrae (E) through metaephyrae (ME) and the medusae (M) life stage.

I.2.2 Biochemical composition

In Figure 4, WM-specific biochemical composition data (protein, lipids, and carbohydrates) are shown. A gradual increase of organic resources was observed during the strobila life stage. Lipid content begins to decrease after day -11 (Figure 4, strobila II stage). In contrast, protein and carbohydrates decrease after day -6 (Figure 4, strobila III stage). Carbohydrate concentrations are low throughout the subsequent life stages around 1.2 µg mg WM^{-1}, after day 4 (ephyrae and medusae). Protein increases slightly (day 0 to 2) after its first peak (day -11 to -6), and then decreases by 8 µg mg WM^{-1} to day 7. In contrast, lipids remain low after day 2 (3.2 µg mg WM^{-1}).

Biochemical composition per individual is shown in Figures 5 A, B, and C. Protein, lipids, and carbohydrates are higher in strobilae than in polyps, low in ephyrae and metaephyrae, and gradually increase again with size in the medusae life stage. In contrast to protein (Figure 5 A) and carbohydrates (Figure 5 C), lipids (Figure 5 B) are consumed during strobila development. However, the concentrations of all three compounds are significantly different between polyps and ephyra ($p<0.0.5$). Significant difference could also be found between medusa, ephyra, and metaephyra ($p<0.05$).

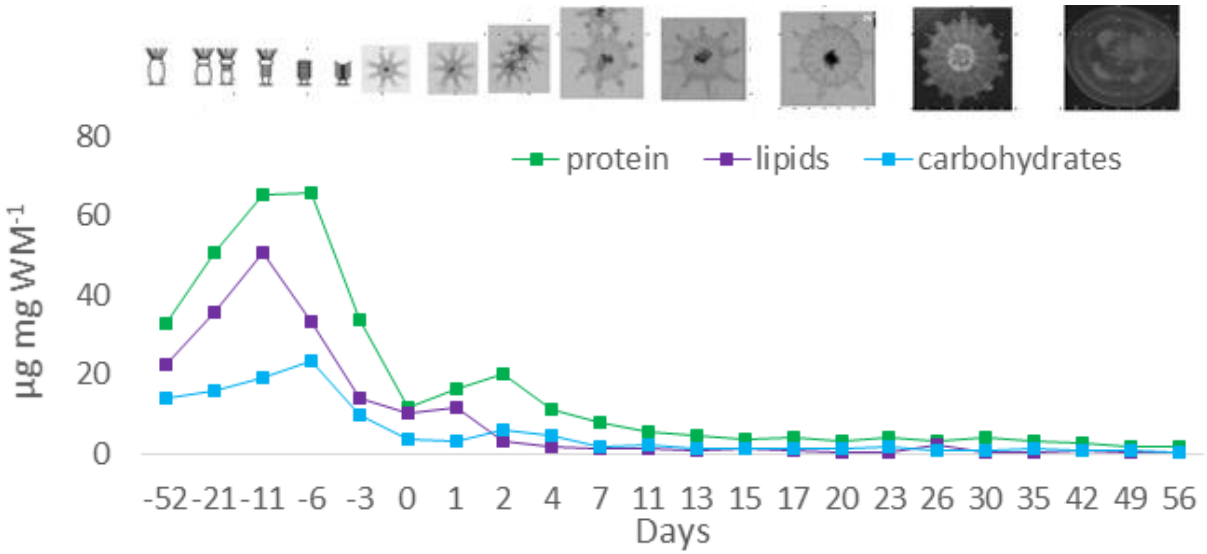


Figure 4. Weight-specific protein, lipids, and carbohydrate composition of *A. aurita* life stages (from polyp to medusa).

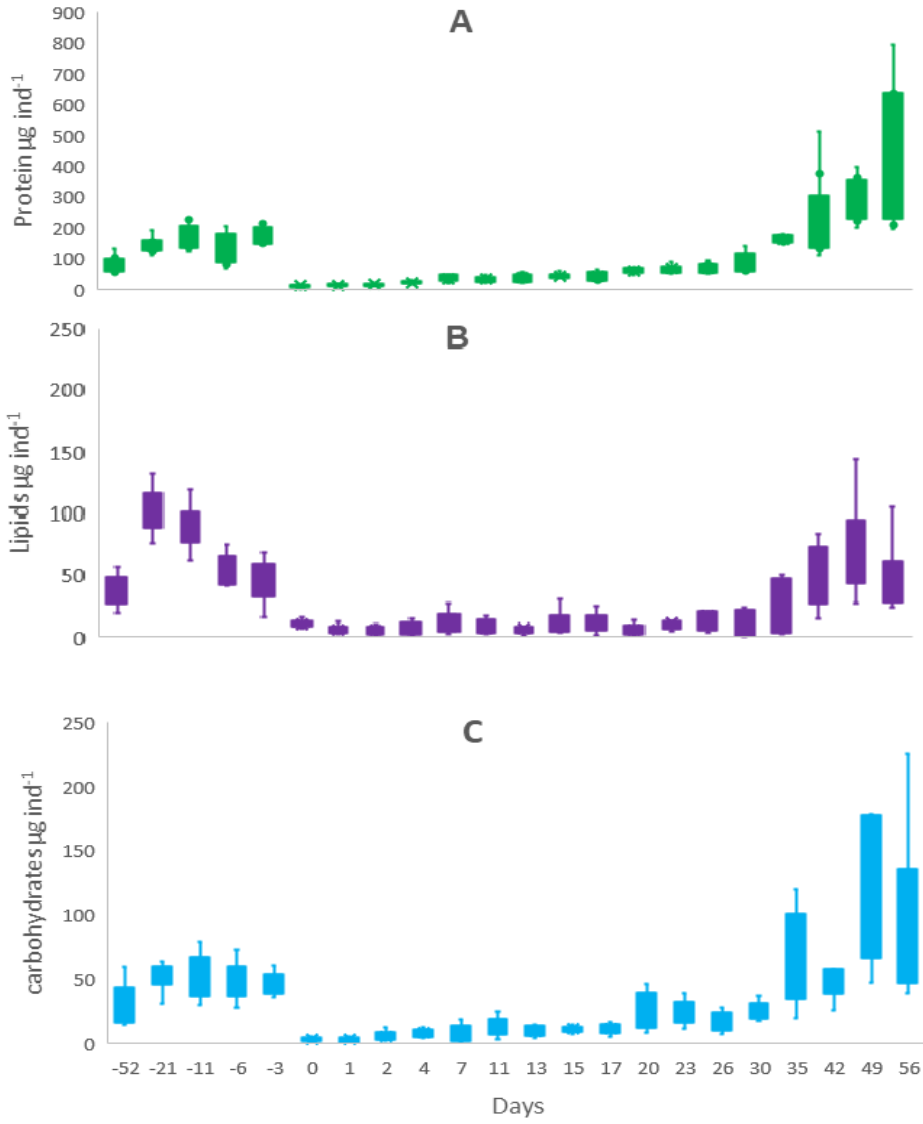


Figure 5. Protein, lipids, and carbohydrate composition of *A. aurita* life stages (from polyp to medusa). **A.** Protein content per individual. **B.** Lipid content per individual and **C.** Carbohydrate composition per individual. Note the different scales on the y-axis.

I.3. Discussion

In this study, the life stage transition from polyp to ephyra, induced by a shift in temperature from 20˚C to 10˚C, took 52 days (day -52 to 0). Kuniyoshi et al. (2012) induced strobilation by shifting the temperature down from 25˚C to 10˚C (for 59 days). The daily growth rate from ephyra through metaephyrae to medusa (Figure 3, from day 0 to day 56), more than doubled, from 0.2210 mm day^{-1} to 0.5263 mm day^{-1}, but was almost non-existent during the metaephyral transition 0.0532 mm day^{-1}.

The growth rate of *A. aurita* can differ in the natural environment. For example, *A. aurita* from open waters are reported to be larger than those from closed or semi-closed environments. Ishii and Båmstedt (1998) collected wild *A. aurita* (4 cm bell diameter) from a semi-enclosed bay, south of Bergen (Norway). They studied *A. aurita* growth rates in the wild for 56 days, and also in cultured *A. aurita*, under saturated and starved food conditions. The wild *A. aurita* growth rates were 0.05 cm day^{-1} during these 56 days, while the cultured well-fed ones grew at rates between 0.16 cm day^{-1}, and the starved ones shrunk 0.02 cm day^{-1}. These investigators showed that, in *A. aurita*, food availability controls growth, as well as additionally maturity. Interestingly, wild *A. aurita* were smaller and yet, showed sexual maturity, whereas cultured medusae were larger, but showed no evidence of sexual maturity. Ishii and Båmstedt (1998) suggested that food scarcity changed the energy allocation towards reproduction. However, medusa in this experiment were smaller (2 cm) and did not reach a mature state.

Several articles have studied the relative composition of the main proximate components in *A. aurita* (Larson, 1986*;* Schneider, 1989; Matsakis and Conover, 1991*;* Lucas, 1994; Båmstedt et al., 1994). Jellyfish are high in water, rich in protein, and low in carbohydrates and lipids (Arai, 1997; Abdullah et al., 2015; Khong et al., 2016). Lucas (1994) very extensively

analyses the biochemical components in *A. aurita* from ephyrae to medusae. Lucas (1994) characterised their medusa by high protein, intermediate lipids, and low carbohydrate content.

However, during metamorphosis, proteins, lipids, and carbohydrates are mobilised as energy sources (Figure 3). Normally, lipids and carbohydrates are considered the main sources of biological energy during metamorphosis (Agrell and Lundquist, 1973), but here, we found that proteins contributed the largest quantity of free-energy equivalents to the metamorphosing *A. aurita*. This was followed by lipids, and then carbohydrates (Figure 4). These energy reserves (WM-specific) accumulated during the polyp and the first strobilae stages, and were mostly consumed in the final strobilae stages, and as ephyrae grew. The cold temperatures required for strobilation may provide the conditions needed to reduce metabolic energy consumption, and convert the food consumed to lipid storage for the resulting ephyrae. At the last stage of strobilae (stage IV, day -3) individuals have lost their tentacles and do not ingest food. Feeding ceases before strobilation (Russell, 1970), and strobilae use stored food at this stage. For comparison, in the non-feeding stage of insect metamorphosis, lipids and glycogen constitute the predominant source of energy (Agrell and Lundquist, 1973; Beenakkers et al., 1981).

According to our results, WM-specific energy reserves were exhausted at the end of the ephyra life stage (day 7). Ephyrae do have high energy requirements (Lucas et al., 2012). However, in the experiments by Fu et al. (2014), starved ephyrae survived for 44-92 days. Once ephyrae evolve to metaephyrae, the water content starts to increase (Pitt et al., 2013). Kotova et al. (2015), pointed out that the main difference between polyps, strobilae, ephyrae, and medusa, is the mesoglea (Kotova et al., 2015). The mesoglea is mostly an acellular tissue (Kotova et al., 2015). One of its principal components is the protein,

mesoglein. Transcripts of mesoglein appear at the polyp stage, rises in ephyrae, and reach their maximum in adult medusae (Kotova et al., 2015).

In addition to providing energy for metamorphosis, these biochemicals provided reserves for maintenance after metamorphosis (Nestel et al., 2003). For example, in the sea lamprey (*Petromyzon marinus)*, WM-specific lipid reserves were low in non-metamorphosing and high in pre-metamorphosing, as well as in medium metamorphosing lampreys, respectively (Boyle and Beamish, 1977). This is similar, in the case of *A. aurita*, if we consider that the medusa is a non-metamorphosing stage, polyps and strobila are pre-metamorphosing stages, and ephyra is a metamorphosing stage. However, this scyphozoan's metagenic life cycle is unique. It has a sessile benthic life stage where one polyp can metamorphose into several planktonic free-swimming ephyrae by transverse segmentation of the polyp body. From strobilae- to ephyrae-metamorphosis, new structures are formed that are absent in the parent polyp. The most important new structures that enable ephyrae to swim are the peripheral rhopalia with statoliths (gravity receptors), striated muscles, a new nerve net, and arms with lappets (Thompson, 1993).

Shifts from benthic to planktonic life were also observed in González-Valdovinos et al. (2019), by studying the digestive capacity of the cannonball jellyfish (*Stomolophus meleagris*). Their results suggest that polyps hydrolyse carbohydrates, best; ephyrae, proteins; and medusae, lipids. They explain that the high digestive activity of amylase and aminopeptidase in polyps is an advantage because they are sessile and have lower chances to encounter food. Our study was only conducted until juvenile medusae, however, the lipid content should be higher in mature medusa because of the development of gonads. In addition, nematocysts, which are generally found in the tentacles surrounding the mouth of medusae, contain lipoprotein toxins (Lane, 1960), phospholipases, and phosphodiesterases (Stillway and Lane, 1971). This

would also lead to an increase in lipids as medusae grow. Here, metaephyra and medusa had the lowest WM- specific energy content throughout the different life stages.

Part II

II. 1. Material and methods: Metabolism

This study was designed to investigate *A. aurita* metabolism throughout the scyphozoan's life cycle. To accomplish this, the following measurements were made: respiration (Lilley and Lombard, 2015); NH_4^+ excretion (Fernández-Urruzola et al., 2011); respiratory ETS activity (Φ (Purcell et al., 2019)); isocitrate dehydrogenase activity (IDH (Berdalet et al., 1995; Tames-Espinosa et al., 2018)); and glutamate dehydrogenase (GDH) activity (Fernández-Urruzola et al., 2011).

II.1.1 *Physiological measurements*

II. 1.1.1 Respiration (R)

R was determined using O_2-sensitive optodes according to Lilley and Lombard (2015) and Bondyale et al. (2017). These optodes are based on an O_2-sensitive fluorescent dye, embedded in a tissue-compatible polymer installed inside the incubation vessels. We used the Fibox-4 system by Presens. The fluorescence emission of the optode is inversely proportional to the amount of O_2 in the solution and is read out non-invasively by a fiber-optic probe outside the incubation chamber. The incubation flasks were carefully inverted (three times) before each measurement, to avoid O_2 concentration gradients. Respiratory O_2 consumption rate (RO_2) as ($\Delta[RO_2]/\Delta t$) was determined as the change in O_2 concentration ($\Delta[O_2]$) during incubation time (Δt). One flask, filled with filtered seawater without organisms, served as a control (Osma et al., 2016). Incubations were performed in darkness at 20˚C±0.2˚C for polyps, ephyrae, and medusae. Strobilae were incubated at 10˚C±0.2˚C, following the strobilation induction period.

II. 1.1.2 Ammonium excretion

Ammonium excretion (Solorzano, 1969; Fernández-Urruzola et al., 2011) was determined after the R incubations. An aliquot with 1 mL of seawater from the R incubation flasks was taken before starting the R experiment and 1 mL when the R incubation was concluded. The water sample was then mixed with 40 µL of 95% phenol solution, 40 µL of 0.5% sodium nitroprusside, and 100 µL oxidizing solution (a solution of sodium hypochlorite and sodium citrate). These reagents were added separately and mixed thoroughly after each addition. A standard curve (0-30 µM) was prepared beforehand with NH_4Cl (Sigma, A4514). The reaction time for the standard curve and sample solution after the addition of the reagents was 1.5 h. After this time, the standard curve and the samples were analysed spectrophotometrically (Cary100 UV-Vis Spectrophotometer, Agilent Technologies) at 640 nm.

This NH_4^+ excretion method is extremely sensitive, e.g. sweat, perfume, and tobacco residues can contaminate the sample. Accordingly, the day before the experiment, all the glassware was cleaned with diluted hydrochloric acid and then placed in a dishwasher with distilled water at 65˚C. The glassware was then kept in plastic bags and dried in a drying oven, to avoid contamination until the analysis.

II.1.2 *Enzymatic measurements*

The samples, after being stored at -80˚C, were homogenized in 0.1M phosphate buffer solution, pH 8.2, (Packard 1971, Packard and Christensen, 2004) using an ultrasonic probe (Cole Parmer) with a Vibracell VCX 130 ultrasonic processor (Sonics) to liberate the enzymes. Then the homogenate was centrifuged at 4000 rpm (1500g) for 10 min at 0-4˚C (Gómez et al., 1996). The enzymatic analyses (ETS, IDH, and GDH) need to be carried out within 1 h of homogenization, to avoid denaturation of the enzymes, and loss of

enzyme activity (Packard et al., 1974; Thuesen and Childress, 1994). After these measurements, the remaining homogenate was stored at -20˚C to further determine the biochemical composition.

II.1.2.1 ETS Activity

The respiratory ETS activity was measured according to Purcell et al. (2019) to determine the potential respiratory activity. ETS enzymes are responsible for cellular respiration and they reflect the potential respiration (Φ), the maximum capacity of R (Packard, 1985a, b). After centrifugation, 100 µL of the supernatant fluid was mixed in a cuvette with 300 µL of substrate solution containing NADH and NADPH (1.7mM) in substrate buffer solution (but at pH 8.6) and 100 µL of the indicator INT solution (2 mg INT ml^{-1} in double-distilled H_2O). INT accepts electrons from the ETS before cytochrome oxidase and produces intense red formazan dye during the reaction. The formazan production is followed at 490 nm for 8 min; the slope of the time-course reflects the ETS activity (Figure 1 in Packard and Christensen, 2004). The rate of formazan production (slope) is stoichiometrically related to the ETS activity via the molar attenuation coefficient (12.8 mM^{-1} cm^{-1}) of the formazan. The formazan production rate (µmol) is related by a factor of 2 to the ETS activity (µmol e^-) and 0.5 to Φ (Packard 1985).

II.1.2.2 IDH Activity

The IDH activity was determined according to Tames-Espinosa et al. (2018). After centrifugation, 100 µL of the sample-homogenate supernatant-fluid was pipetted into a spectrophotometer cuvette. Then, 100 µL of 0.5 mM $NADP^+$ (Sigma N0505) were added to a 300 µL mixture containing 3 mM DL-trisodium-isocitrate (Sigma l1252) and 6 mM $MgCl_2$ (Panreac 131396). These reagents were prepared in the same phosphate buffer solution (pH 8.2), described above. IDH activity is the rate at which $NADP^+$-dependent

isoenzymes of IDH oxidisreude isocitrate and reduce $NADP^+$ while producing CO_2. NADPH and CO_2 are related stoichiometrically, mole for mole, in the reaction (Eq.1)

$$\text{Isocitrate} + NADP^+ \leftrightarrow \text{alpha-ketoglutarate} + NADPH + CO_2 \qquad (1)$$

The IDH activity was followed by the time-dependent NADPH production at 340 nm for 10 min. Respiratory potential CO_2 production (IDH) was calculated by multiplying IDH activity by 3 (Roy and Packard, 2001). This operation takes into account two other CO_2-producing enzymes in the Krebs Cycle, pyruvate dehydrogenase, and alpha-ketoglutarate dehydrogenase.

II.1.2.3 GDH Activity

Glutamate dehydrogenase (GDH) activity, was measured according to Bidigare and King (1981) and Fernández-Urruzola et al. (2011). After centrifugation, 40 µL of the supernatant fluid of the sample-homogenate was pipetted into a 96-well microplate. Then 60 µL of a 5 mM NAD^+ 50 µL 10 mM ADP and 100 µL 125 mM sodium glutamate were added. All substrates and activators were prepared in the substrate buffer solution (pH 8.6). The fluorescence was converted to activity (µmol NH_4^+ per time) from a standard curve, which was prepared from pure GDH (1.4.1.3) extracted from bovine liver (Sigma G2626). The curve ranged from 0.039-0 international units (U) of GDH activity ml^{-1}, where one U equals the amount of enzyme that converts 1 µmol NAD^+ to NADH, and 1 µmol NADH converts to 1 µmol NH_4^+. Throughout this chapter, we will report our GDH activity as µmol NH_4^+ h^{-1} g WM^{-1}. The fluorescence increase, resulting from NADH produced by the reduction of NAD^+, was followed fluorometrically at 360 nm excitation and

460 nm emission wavelengths for 10 min (Microplate reader, FLUO Star Omega, BMG Labtech) at 20˚C (Fernández-Urrozola et al., 2011) (as before, strobilae were analysed at 10˚C).

II.2 Results

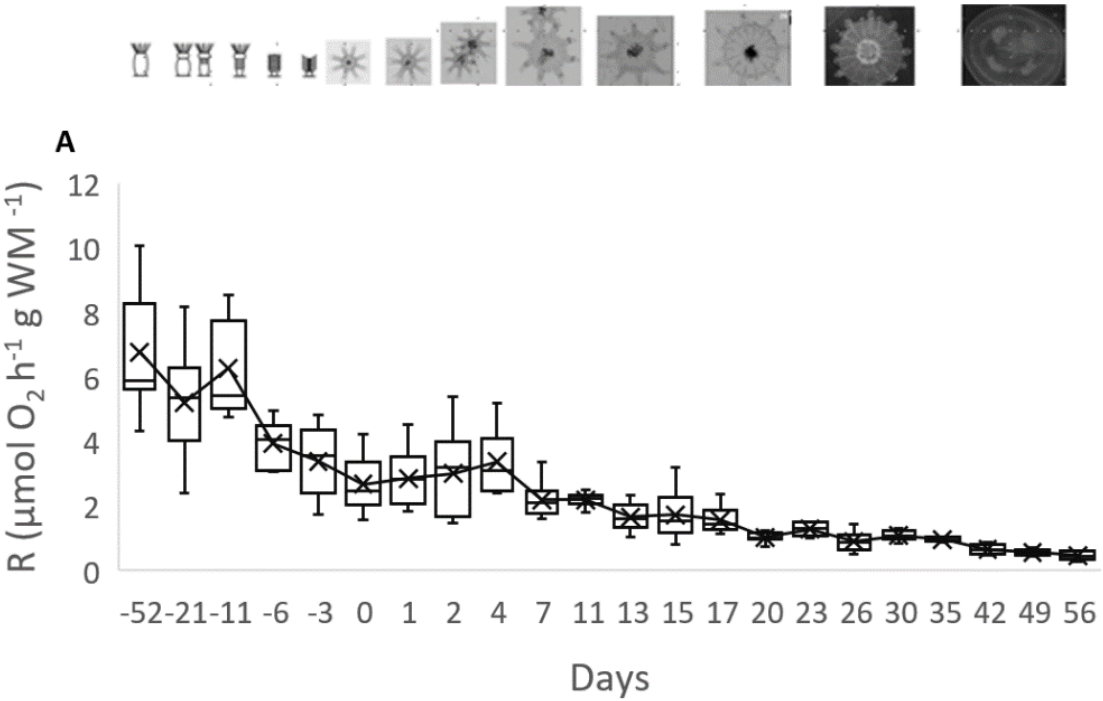
II.2.1 O_2 consumption rates

R and Φ rates differ throughout *A. aurita's* life stage transitions (Figure 6 A and B, Table 2). WM-specific R decreased steadily from polyp to medusa life stage (Figure 6 A). On one hand, polyps and strobilae have the highest R, 6.78 and 4.78 µmol O_2 h^{-1} g WM^{-1}, respectively. A subtle increase in respiration rates can be observed at the first stages of strobilae, both in R and Φ. However, R of the first two stages of strobilae (stage I, and II) is very similar to those of polyps (note that these stages keep their tentacles and are therefore still able to eat). This increase in oxygen consumption in strobilae corresponds to days -21 and -11 for R, and day - 6, for Φ.

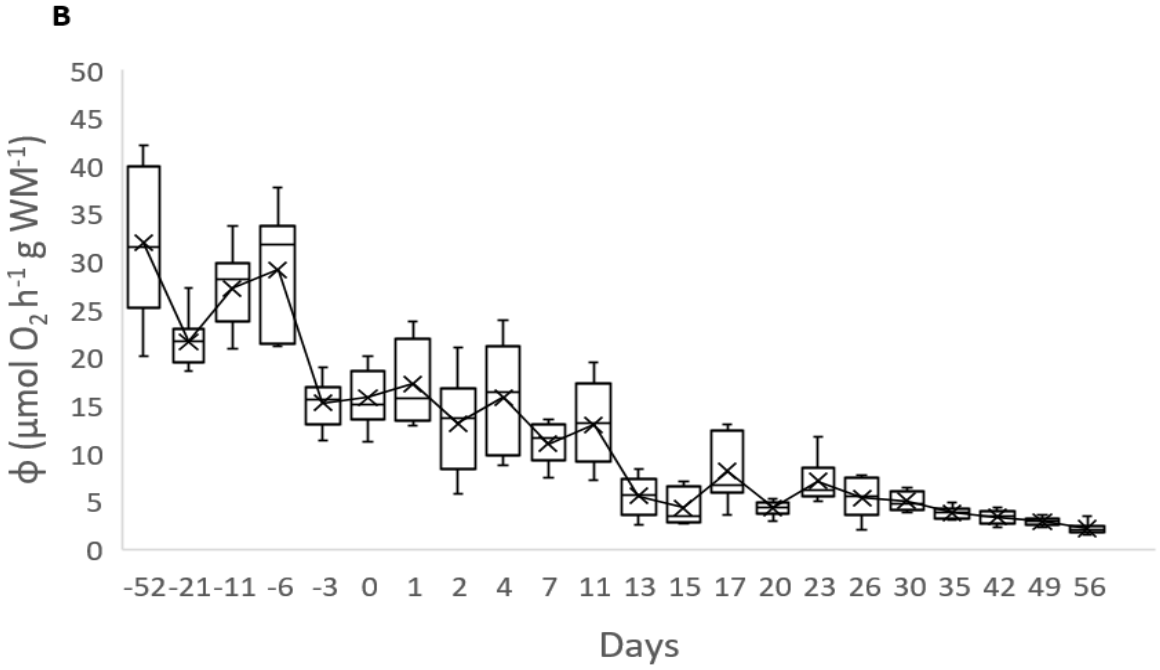
Due to the strobilation induction protocol, all strobilae were kept at 10˚C (day -21 to -3), while the rest of the experiment, with *A. aurita's* different life-forms, was performed at 20˚C. When converting strobilae respiration rates from 10˚C to 20˚C, applying the Arrhenius equation, these Rs are almost 3-fold higher than the respiration rates measured at 10˚C (not shown). This 3-fold increase in R can be applied to all physiological and enzymatic measurements when the Arrhenius equation is applied. However, we decided not to correct metabolic rates (physiological and enzymatic), as strobilation under lower temperatures is a natural condition. On the other hand, R decreases after day 4 (ephyra stage), while Φ waits until day 11 before decreasing (Figure 6 A and B). Medusae had the lowest R (0.75 µmol O_2 h^{-1} g WM^{-1}, Table 2) of all the different life stages. Protein-specific respiration rates follow a different

trend (Table 2). The highest protein-specific R corresponds to metaephyrae and medusae (0.3 µmol O_2 h^{-1} mg $protein^{-1}$). Polyps and ephyrae had a respiration rate of 0.2 µmol O_2 h^{-1} mg $protein^{-1}$ and strobilae 0.1 µmol O_2 h^{-1} mg $protein^{-1}$. WM-R and WM- Φ showed significant difference throughout the life stages ($p<0.05$) (with exception of polyps and strobila, where no significant difference could be found). Respiration rates normalised by the number of individuals are also shown in Table 2. No significant difference could be found in the R/Φ ratio (0.23 ± 0.09) during the complete experiment (including all life stages that have been studied, Figure 6 C).

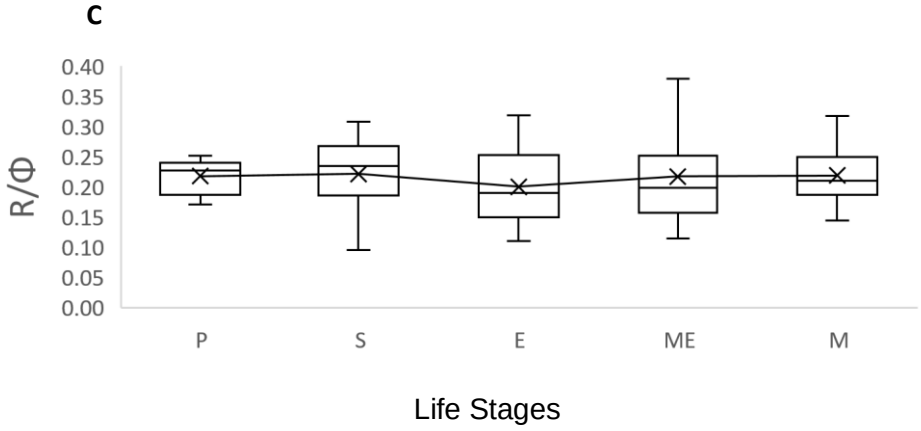
Figure 6. Respiratory metabolism rates and R/Φ ratio



A. Physiological O_2 consumption rate (R) (µmol O_2 h^{-1} g WM^{-1}).



B. Potential respiration, enzymatic O_2 consumption rates (Φ) (µmol O_2 h^{-1} g WM^{-1}).



C. R/Φ ratio (dimensionless). P=polyps; S=strobilae; E=ephyrae; ME=metaephyrae; M=medusae. Note the different scales on the y-axis. Note also, that for the strobilae, R and Φ were measured at 10˚C while R and Φ, for all the others, were measured at 20˚C.

II.2.2 Potential CO_2 production rates

Potential CO_2 production rates (IDH) are shown in Figure 7. As Φ (Figure 6 B), IDH drops from polyp to strobila life stage. Remember that strobilae were kept at 10˚C and the rest of the life stages at 20˚C. However, no IDH increase was observed for the strobilae. This is in contradiction to the shifts in Φ as the life forms changed (Figure 6 B). The WM specific-IDH decreased as ephyrae shifted to the medusae life stage (Figure 7, Table 2). It decreased from 34.75 ± 7.27 µmol CO_2 h^{-1} g WM^{-1} in the polyps to 3.95 ± 2.03 CO_2 h^{-1} g WM^{-1} in the medusae. WM-IDH showed significant difference throughout the life stages ($p<0.05$) (with exception of polyps and strobila, where no significant difference could be found). Protein-specific IDH rates followed a different trend (Table 2). They were high during metaephyra and medusa life stages (1.5 µmol CO_2 h^{-1} mg $protein^{-1}$), medium in polyps, and ephyrae (1.1 and 0.7 µmol CO_2 h^{-1} mg $protein^{-1}$, respectively), and low in the strobila life stage (0.4 µmol CO_2 h^{-1} mg $protein^{-1}$). IDH rates normalised by the number of individuals are also shown in Table 2.

Table 2. *A. aurita* physiological rates and enzymatic activities during life stage transitions. R= O_2 consumption (µmol O_2), A= NH_4^+ excretion (µmol NH_4^+), Φ= potential O_2 consumption (µmol O_2), IDH= potential CO_2 production (µmol CO_2), GDH: potential NH_4^+ excretion (µmol NH_4^+)

		Polyps		Strobilae		Ephyrae		Metaephyrae		Medusae	
	n=	9		33		45		65		39	
		mean	± *sd*	mean	± *sd*	mean	± *sd*	mean	± *sd*	mean	± *sd*
R	g WM^{-1}	6.78	*1.75*	4.78	*1.75*	2.83	*0.98*	1.49	*0.56*	0.75	*0.26*
	mg $protein^{-1}$	0.21	*0.04*	0.1	*0.03*	0.23	*0.07*	0.31	*0.07*	0.3	*0.06*
	$individual^{-1}$	0.016	*0.05*	0.016	*0.05*	0.004	*0.03*	0.015	*0.05*	0.064	*0.042*
A	g WM^{-1}	0.51	*0.27*	0.53	0.43	0.28	0.17	0.3	0.16	0.17	0.07
	mg $protein^{-1}$	0.02	0.01	0.01	0.01	0.02	0.01	0.06	0.03	0.06	0.02
	individual-1	1.2E-03	7.2E-04	1.3E-03	8E-04	3.1E-03	2.6E-04	2.9E-03	1.9E-03	8.8E-03	4.8E-03
Φ	g WM^{-1}	31.97	7.48	23.3	6.67	15.49	4.55	7.33	3.88	3.41	1.19
	mg $protein^{-1}$	0.97	0.17	0.46	0.08	1.1	0.3	1.39	0.49	1.38	0.27
	$individual^{-1}$	0.07	0.02	0.07	0.01	0.02	0.001	0.065	0.03	0.34	0.24
IDH	g WM^{-1}	34.75	7.27	18.98	6.72	10.18	3.32	7.06	3.18	3.95	2.03
	mg $protein^{-1}$	1.06	0.201	0.38	0.137	0.72	0.185	1.44	0.42	1.53	0.529
	$individual^{-1}$	0.08	0.021	0.058	0.022	0.01	0.004	0.067	0.034	0.37	0.21
GDH	g WM^{-1}	37.6	11.8	49.34	31.53	12.56	5.2	4.7	2.27	2.91	1.81
	mg $protein^{-1}$	1.14	0.32	0.98	0.72	0.96	0.33	0.96	0.44	1.06	0.57
	$individual^{-1}$	0.09	0.03	0.15	0.11	0.02	0.01	0.05	0.03	0.21	0.13

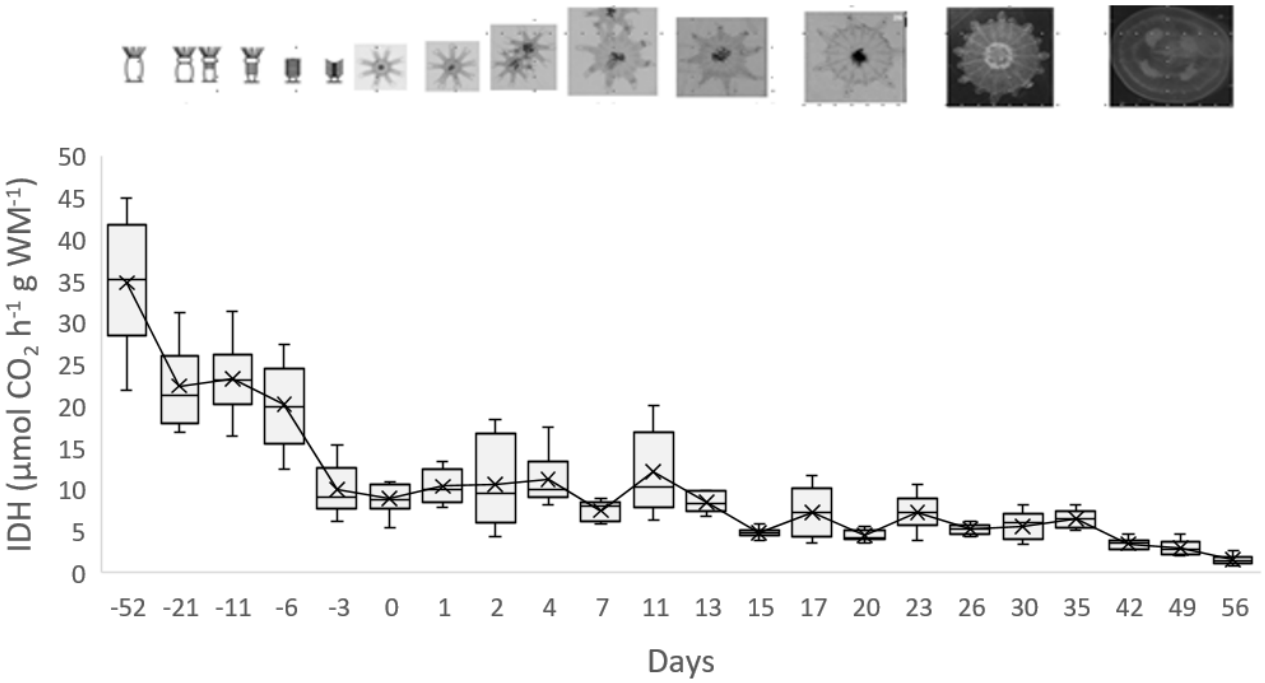
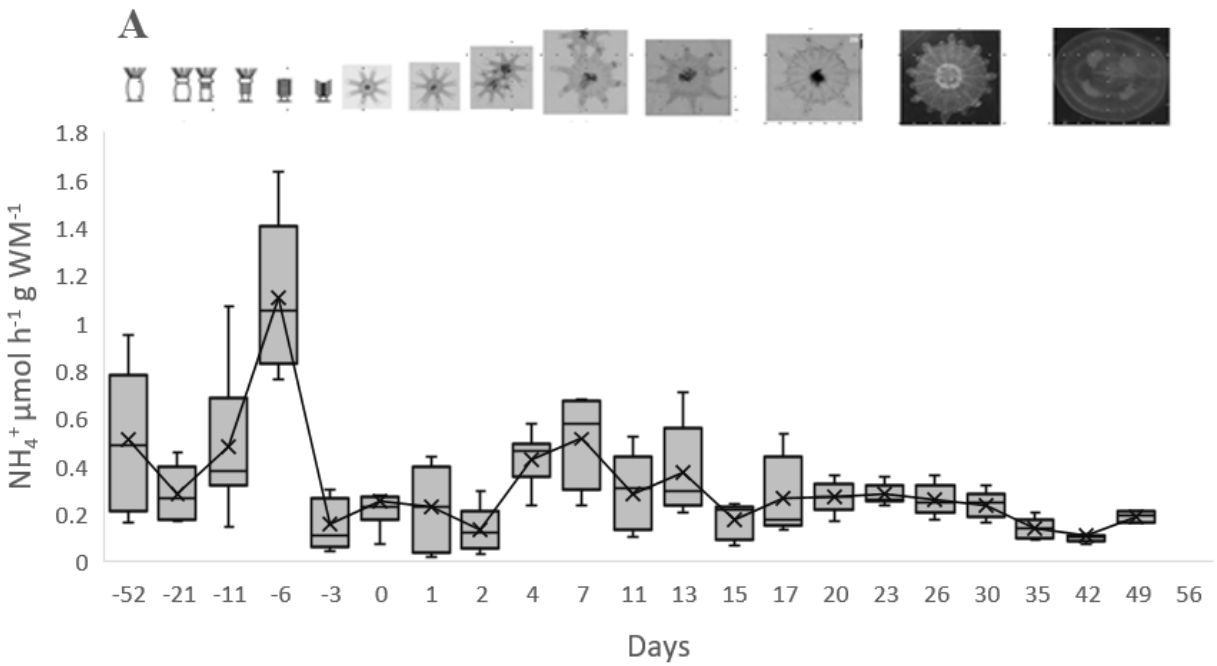


Figure 7. Potential CO_2 production rates (IDH) (µmol CO_2 h^{-1} g WM^{-1}).

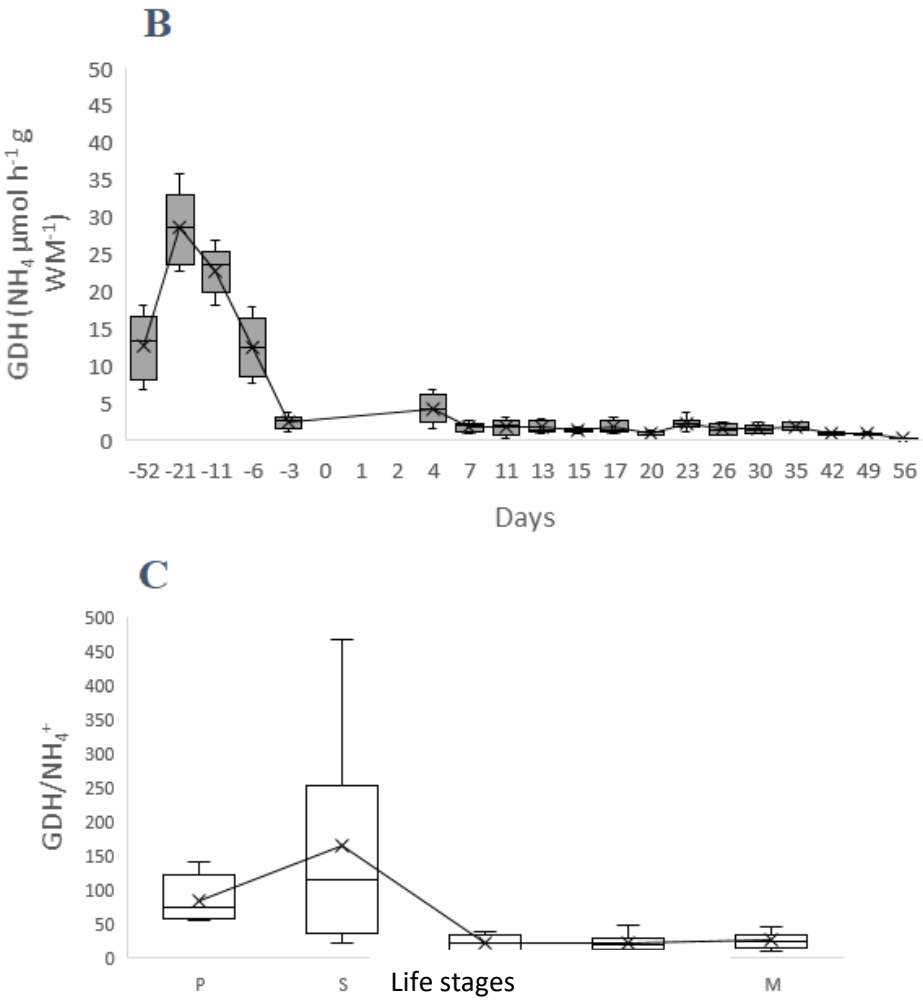
II.2.3 NH_4^+ excretion

Weight-specific NH_4^+ excretion rates, from polyp to ephyra stages, varied from 0.5 to 1 µmol NH_4^+ h^{-1} g WM^{-1} (Figure 8 A). Polyp excretion rate was 0.5 µmol NH_4^+ h^{-1} g WM^{-1}. Excretion rates then decreased towards the first stage of strobila (0.25 µmol NH_4^+ h^{-1} g WM^{-1}, at day -21). They peaked on day - 6 at about 1 µmol NH_4^+ h^{-1} g WM^{-1} and then decreased again on day -3 to about 0.2 µmol NH_4^+ h^{-1} g WM^{-1}. For their first two days, ephyrae had similar rates to day -3 (strobilae, stage IV). We noted that ephyrae did not ingest food after their release and for their next 24 h of free-swimming life. An increase in excretion rates could be observed from day 4 to day 13 (0.4 µmol NH_4^+ h^{-1} g WM^{-1}). Then, from day 15 onwards, excretion rates decreased (0.2 µmol NH_4^+ h^{-1} g WM^{-1}). Only the medusa life stage showed a significant difference in WM-NH_4^+ excretion rates ($p<0.05$). GDH activity (Figure 8 B) trended similarly with the physiological NH_4^+ excretion rates (Figure 8 A). The highest variability in GDH could be observed, as in NH_4^+ excretion rates, from the polyp to the ephyra stage (Figure 8 B). However, GDH activity increased to a maximum (30 µmol NH_4^+ h^{-1} g WM^{-1}) in the first stage of strobilae (day -21), then, from day -21 to day -3 (strobilae life stage) it gradually decreased. On day -3 (strobilae stage IV) GDH activity was 2.5 µmol NH_4^+ h^{-1} g WM^{-1}. No GDH activity could be measured at the release of ephyrae on day 0 until day 2 because it was under the limit of detection. From day 4 onwards GDH activity did not show any variability, while steadily maintaining its activity at about 2.5-1 µmol NH_4^+ h^{-1} g WM^{-1}. WM-GDH and GDH/NH_4^+, in the benthic life stages (polyps and strobila were significantly different from the planktonic life stages (ephyra to medusa) ($p<0.05$).

Figure 8. NH_4^+ excretion rates and GDH activity



A. NH_4^+ excretion rates (µmol NH_4^+ h^{-1} g WM^{-1})



B. GDH activity (µmol NH_4^+ h^{-1} g WM^{-1}) and **C.** GDH/NH_4^+ ratio (dimensionless). Note the different scales on the y-axis.

II.3 Discussion

In many marine invertebrates and amphibians, metamorphosis is an ancestral condition, contrary to insects, which evolved from direct development (Truman and Riddiford, 1999). Among marine invertebrates, complex life cycles are characteristic of diverse phyla such as Porifera, Platyhelminthes, Annelida, Mollusca, Arthropoda, Bryozoa, Echinodermata, Chordata (fish, tunicates, salps, doliolids), and Cnidaria (Thorson, 1950; Helm, 2018). However, metabolism studies of metamorphosis in cnidarian and particularly in scyphozoan are scarce. Gambill and Peck (2014) concluded in their study that mass-specific respiration rates of *A. aurita* polyps, ephyrae, and medusae are similar. However, their results are mainly based on previously published data from other studies. Here, we have measured *A. aurita*'s respiratory rates during life stage transitions from polyp to medusa. In our study, weight-specific respiration rates differ among life stages (Figure 6 A, Table 2) ($p<0.05$).

We argue that the transition from polyps to strobilae and then to ephyrae is energetically demanding, as shown by the large energy consumption (Figure 4), especially lipid consumption (Figure 5 B), and also from an increase in respiration rates (Figure 6 A) (also observed in Nestel et al., 2003). Respiration rates decreased in strobilae (Figure 6 A) once ingestion ceased (Figure 2). We deduced that this behaviour is caused by strobilae losing the tentacles from their previous polyp phase and, thus, were not able to eat anymore. Russel (1970) supported this deduction. At the last stage of strobila (day - 3) about 10 well-formed, but still attached, ephyrae were visible per strobila. Strobilae (kept at 10˚C) and ephyrae, during their first 4 days at 20˚C had similar weight-specific respiration rates (4 µmol O_2 h^{-1} g WM^{-1}).

Respiration rates measured in metamorphosing holometabolous insects (egg, larva, pupa, and adult life stage) usually follow a U-shaped curve (Agrell and

Lundquist, 1973). Energy consumption is high during the first stages of metamorphosis, declines toward the mid-pupal stage, and increases again towards the last phases of the larval-adult transformation. This U-shaped R curve can be observed during the polyp-to-ephyra transition, however, it does not apply to the metaephyrae and medusae life stage. We think that it is due to the increasing water content when the mesoglea is developed.

Cnidarians, like most aquatic animals, excrete primarily NH_4^+ (Hubot et al., 2021; Wright, 1995). NH_4^+ is largely formed and excreted as a by-product of amino-acid catabolism (Weihrauch and Allen, 2018). Lower NH_4^+ excretion rates imply lower rates of amino acid catabolism. Amino acids cannot be stored in animal tissue, so their excess, those that cannot be used in protein production, are catabolised to NH_4^+ and then excreted. Only a few NH_4^+ excretion experiments have been carried out in *A. aurita* (Schneider, 1989; Nemazie et al., 1993; Shimauchi and Uye, 2007; Hubot et al., 2021). Shimauchi and Uye (2007) concluded in their work, that weight-specific metabolic rates of gelatinous zooplankton tend to be biomass independent. Ammonium excretion from wild *A. aurita* (Schneider, 1989) (Kiel Bight, Baltic Sea) were lower than in our study (0.52-1.70 µmol NH_4^+ g WM^{-1} d^{-1}), as they are normalised per day. However, we have observed that excretion rates cease with time after ingestion in *A. aurita*, and extrapolation from hour to day is not direct. This is the first study that measures NH_4^+ excretion in *A. aurita* from polyp to medusae life stage.

Enzymes, as indices of rate processes in biological oceanography, are becoming a useful tool in metamorphosis studies (García-Esquivel et al., 2001). Theoretically, ETS, IDH, and GDH activities could provide quantitative information on the maximum rates attainable in the respiratory electron transport system, and the TCA Cycle. Furthermore, they should provide a basis for comparison with the metabolic rates that were concurrently

measured in this study (RO_2 and NH_4^+ excretion). The maximal activity of the respiratory enzymes, ETS and IDH, which also reflect their concentration, shifts during metamorphosis (Figures 6 B and 7 A). These enzymes are located in the mitochondria, and one potential explanation is that the number of mitochondria varies during metamorphosis. Mitochondria need to be enumerated to test this idea. An alternative explanation, for mitochondria loss, could be apoptosis, programmed cell death.

Metamorphosis of the cnidarian *Hydractinia* (Seipp et al., 2001; Wittig et al., 2011) involves extensive apoptosis, suggesting that programmed cell death plays a significant role during coral metamorphosis. Apoptosis was observed in metamorphosing *A. aurita* planula larvae (Yuan et al., 2008). The metamorphosis from planula to polyp includes cell differentiation, migration, proliferation, and nervous system reorganization (Yuan et al., 2008). In addition, during strobilation, extensive cell divisions occur, according to histological studies (Chuin, 1930; Suzuki, 2020). Therefore, it could be expected that some cell structures are lost and others are newly created during *A. aurita's* life stage transition. Different enzyme concentrations through metamorphosis have also been observed in weight-specific ETS of Pacific oyster (*Crassostrea gigas*) (García-Esquivel et al., 2001). They showed that ETS concentrations differ within 24 days of post-settlement and displayed annual variability of R/Φ (0.45-0.80).

In addition, the R/Φ ratio can provide insight into the stress condition of an organism (St- Amand et al., 1999). It shows how much of an organism's energy-transforming potential is being utilized. In our study, the R/Φ ratio is low throughout life stages transitions. In other words, all life stages have a low-basal metabolism compared to their potential (Figure 6 C). Purcell et al. (2019) have already indicated that *A. aurita* polyps have a low R/Φ ratio independently of food supply and starvation. These results from Purcell et al.

(2019) were unexpected due the evidence in the literature that diet affects the metabolism in marine organisms (Hernández-León and Gómez, 1996; Romero-Kutzner et al., 2015; Martínez et al., 2010). Thus, the R/Φ ratio throughout *A. aurita's* life cycle (polyp to medusae) could indicate consolidated transitions because, despite the energetically costly metamorphosis, *A. aurita* does not shift far away from basal metabolism. However, this study has been conducted under controlled laboratory conditions.

The GDH/NH_4^+ excretion ratio in ephyrae to medusae was 24.4±14.8 (n=107). This ratio agrees well with the observations by other investigators (Bidigare and King 1981; Bidigare et al., 1982; Hernández-León and Torres, 1997; Fernández-Urruzola et al., 2011). It shows a decreasing trend from polyps and strobilae towards ephyrae and medusae life stages (Figure 8 C). This fact suggests that life stages or age, act unevenly on the physiological rate and enzymatic activity (Fernández-Urruzola et al., 2011). GDH is an important enzymatic link between amino acids and the tricarboxylic acid cycle (Nestel et al., 2003). Most amino acids are first transaminated to form ionic glutamate. Glutamate is then deaminated, enzymatically, by GDH to form NH_4^+ and alpha-ketoglutarate. Alex (1971) showed that GDH increases its affinity (K_M: Michaelis-Menten constant) for coenzymes during tadpole to frog metamorphosis. This affinity favors the production of NH_4^+ and alpha-ketoglutarate. A change in affinity for coenzymes could be an additional explanation of why different enzyme concentrations are present in *A. aurita's* life stage transitions. Besides, GDH activity has been studied in the metamorphosing fruit fly *Ceratitis capitata* (Nestel et al., 2003) and the silkworm *Bombyx mori* (Sivaprasad and Bhuvaneswari, 2018). In those studies, GDH activity was high during the early pupa stages and decreased towards the adult life stage, similar to what we have observed in this study, where GDH activity is high during the metamorphosing stages and low in the

non-metamorphosing medusae stage. However, GDH activity could not be detected between day 0, when ephyrae were released, and day 4. In contrast, IDH (Figure 7) decreased, while GDH was not detectable, but NH_4^+ excretion was measurable (Figure 8 B). On one hand, according to Reitzer (2003), GDH is only active at high intracellular NH_4^+ concentrations due to the low affinity of the enzyme for NH_4^+. On the other hand, IDH (Figure 7), is involved in the synthesis of lipid energy storage (Koh et al., 2004) and during the ephyra life stage these lipids reserves are being exhausted (Figure 4). Overall, our results show shifts in metabolic rates (physiological and enzymatic) and biochemical composition (Part I), throughout life-stage transition.

In the ocean where variability is more likely, and even more under the current climate change scenario, the physiological response of *A. aurita* may change. For example, it has been shown that temperature is a principal environmental controller of the scyphozoan life-cycle (Willcox et al., 2007; Purcell et al., 2012; Schiariti et al., 2014; Pascual et al., 2015) and its implication on metamorphosing *A. aurita* should be taken into account in future research.

4.4. Conclusions (Part I and II)

(1) Here we have identified metabolic and biochemical changes during the transition from sessile to planktonic lifestyles in *A. aurita*. WM-specific respiration, excretion, and enzymatic rates vary throughout the different life stages ($p<0.05$). The highest rates have been observed in the benthic life stages. In addition, during the strobila stages, energy reserves were accumulated and decreased towards strobilation.

(2) As hypothesised, shifts in metabolic rates could be observed, high respiratory and excretory metabolic activity was shown in polyps during metamorphosis.

(3) Rapid drops in respiratory activity (R, Φ, IDH) and excretory activity (NH_4^+ production rates and GDH activity) were observed during the final day of the strobila metamorphosis. These changes in activity matched with changes in the ingestion (almost absent from day -6 to day 2) and coincided with changes in the biochemical components, where protein, lipids, and carbohydrate content nearly doubled during the initial phase of the strobila metamorphosis and then dropped during the phases closer to the liberation of the ephyrae. The last stages of strobila and young ephyrae could, therefore, be more vulnerable to environmental variability. The second metamorphosis, from ephyra to medusa, experienced less intense changes than the ones described above, showing how severe, at a physiological and anatomical level, the transition from polyp to ephyra is.

(4) We observed stability in the R/Φ ratio (0.2) from polyp to medusae life stage. Metamorphosis in *A. aurita*, despite being energetically costly, might therefore be a metabolic consolidated process if environmental conditions are stable. This stability has previously been reported in *A. aurita* polyps under different food regimes and starvation (Purcell et al., 2019). Based on the stability of the R/Φ ratio, specific ETS activity seems to be a reliable respiration indicator for jellyfish, such as *A. aurita*.

(5) This study represents an initial step in elucidating how metabolism and energy reserves shift during life stage transitions. Hence, future ecophysiological research, designed to elucidate future jellyfish population dynamics should not be focused on single life-stage studies. In future research, environmental stressors and transgenerational effects on the metabolism should also be taken into account.

4.5. References

Aalto, E.A., Barry, J.P., Boch, C.A., Litvin, S.Y., Micheli, F., Woodson, C.B., De Leo, G.A.D., 2020. Abalone populations are most sensitive to environmental stress effects on adult individuals. Mar. Ecol. Prog. Ser. 643, 75–85. https://doi.org/10.3354/meps13320.

Abdullah, A., Nurjanah, N., Hidayat, T. and Aji, D.U., 2015. Fatty acid profile of jellyfish (*Aurelia aurita*) as a source raw material of aquatic result rich beneft. International Journal of Chemical and Biomolecular Science,1(1), pp.12-16.

Agrell, I. P., and Lundquist, A. M., 1973. Physiological and biochemical changes during insect development. In The physiology of insecta (pp. 159-247). Academic Press.

Alex, D., 1971. Isoenzymes in Amphibian metamorphosis (Master's thesis, Calif. State College, Hayward.).

Algueró-Muñiz, M., Meunier, C.L., Holst, S., Alvarez-Fernandez, S., Boersma, M., 2016. Withstanding multiple stressors: ephyrae of the moon jellyfish (*Aurelia aurita*, Scyphozoa) in a high-temperature, high-CO_2 and low-oxygen environment. Mar. Biol. 163, 1–12. https://doi.org/10.1007/s00227-016-2958-z.

Aljbour, S.M., Zimmer, M., Al-Horani, F.A., Kunzmann, A., 2019. Metabolic and oxidative stress responses of the jellyfish *Cassiopea* sp.to changes in seawater temperature. J. Sea Res. 145, 1–7. https://doi.org/10.1016/j.seares.2018.12.002

Arai, M., 1997. A functional biology of Scyphozoa.

Båmstedt, U., Martinussen, M. B., and Matsakis, S., 1994. Trophodynamics of the two scyphozoan jellyfishes, *Aurelia aurita* and *Cyanea capillata*, in western Norway. ICES Journal of Marine Science, 51(4), 369-382.

Barnes, H., and J. Blackstock. 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilun method for 'total'lipids. J. Exp. Mar. Bio. Ecol. 12: 103–118.

Beenakkers, A.M.T., Van der Horst, D.J., Van Marrewijk, W.J.A., 1981. Role of Lipids in Energy Metabolism, in: Energy Metabolism in Insects. Springer US, pp. 53–100. https://doi.org/10.1007/978-1-4615-9221-1_3.

Berdalet, E., Packard, T., Lagace, B., Roy, S., St-Amand, L. and Gagne, J.P., 1995. CO_2 production, O_2 consumption and isocitrate dehydrogenase in the marine bacterium *Vibrio natriegens*. Aquatic Microbial Ecology, 9(3), pp.211-217.

Bidigare, R. R., and King, F. D., 1981. The measurement of glutamate dehydrogenase activity in *Praunus flexuosus* and its role in the regulation of ammonium excretion. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 70(3), 409-413.

Bidigare, R.R., King, F.D., Biggs, D.C., 1982. Glutamate dehydrogenase (GDH) and respiratory electron-transport-system (ETS) activities in Gulf of Mexico zooplankton. J. Plankton Res. 4, 895–911. https://doi.org/10.1093/plankt/4.4.895.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Bondyale-Juez, D. R., Packard, T. T., Viera-Rodríguez, M. A., and Gómez, M., 2017. Respiration: comparison of the Winkler technique, O_2 electrodes, O_2 optodes and the respiratory electron transport system assay. Marine Biology, 164(12), 1-11.

Boero, F., Brotz, L., Gibbons, M. J., Piraino, S., and Zampardi, S., 2016. 3.10 Impacts and effects of ocean warming on jellyfish. Explaining Ocean Warming: Causes, Scale, Effects and Consequences. Gland: IUCN, 213-237.

Brooks, W., 1886. The life history of the hydromedusae: a discussion of the medusae and of the significance of metagenesis. Mem Bost. Soc Nat Hist 3, 359–430.

Chuard, P.J.C., Johnson, M.D., Guichard, F., 2019. Ocean acidification causes mortality in the medusa stage of the cubozoan Carybdea xaymacana. Sci. Rep. 9, 1–6. https://doi.org/10.1038/s41598-019-42121-0.

Chuin, T. T., 1930. Le cycle évolutif du scyphistome de *Chrysaora*. Trav. Sta. Biol. Roscoff, 8: 1-179.

Cloud, P., Glaessner, M.F., 1982. The Ediacarian Period and System: Metazoa Inherit the Earth, science.sciencemag.org.

Collins, A.G., 2002. Phylogeny of medusozoa and the evolution of cnidarian life cycles. J. Evol. Biol. 15, 418–432. https://doi.org/10.1046/j.1420-9101.2002.00403.x.

De Coen, W.M., Janssen, C.R., 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. J. Aquat. Ecosyst. Stress Recover. 6, 43–55.

Dong, Z., Sun, T., 2018. Combined effects of ocean acidification and temperature on planula larvae of the moon jellyfish *Aurelia coerulea*. Mar. Environ. Res. 139, 144–150. https://doi.org/10.1016/j.marenvres.2018.05.015.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., and Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical chemistry, 28(3), 350-356.

Fernández-Urruzola, I., Packard, T.T., Gómez, M., 2011. GDH activity and ammonium excretion in the marine mysid, *Leptomysis lingvura*: Effects of age and starvation. J. Exp. Mar. Bio. Ecol. 409, 21–29. https://doi.org/10.1016/j.jembe.2011.07.035.

Foo, S. A., and Byrne, M., 2016. Acclimatization and adaptive capacity of marine species in a changing ocean. Advances in marine biology, 74, 69-116.

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

https://doi.org/10.1016/j.cub.2013.12.003.
Gambill, M., Peck, M.A., 2014. Respiration rates of the polyps of four jellyfish species: Potential thermal triggers and limits. J. Exp. Mar. Bio. Ecol. 459, 17–22. https://doi.org/10.1016/j.jembe.2014.05.005.
Gibbin, E.M., Massamba N'Siala, G., Chakravarti, L.J., Jarrold, M.D., Calosi, P., 2017. The evolution of phenotypic plasticity under global change. Sci. Rep. 7, 1–8. https://doi.org/10.1038/s41598-017-17554-0.
Goldstein, J., Steiner, U.K., 2020. Ecological drivers of jellyfish blooms – The complex life history of a 'well-known' medusa (*Aurelia aurita*). J. Anim. Ecol. 89, 910–920. https://doi.org/10.1111/1365-2656.13147.
González-Valdovinos, M., Ocampo, L., Tovar-Ramírez, D., 2019. Evaluation of digestive capacity in the polyp, ephyrae, and medusae stages of the cannonball jellyfish *Stomolophus meleagris*. Hydrobiologia 828, 259–269. https://doi.org/10.1007/s10750-018-3817-3.
Hadfield, M.G., 2000. Why and how marine-invertebrate larvae metamorphose so fast. Semin. Cell Dev. Biol. 11, 437–443. https://doi.org/10.1006/scdb.2000.0197.
Hadži, J., 1953. An attempt to reconstruct the system of animal classification. Syst. Zool. https://doi.org/10.2307/sysbio/2.4.145
Haeckel, E., 1879. Das system der Medusen: Erster Teil einer Monographie der Medusen.
Helm, R.R., 2018. Evolution and developmen5t of scyphozoan jellyfish. Biol. Rev. 93, 1228–1250. https://doi.org/10.1111/brv.12393.
Hernández-León, S., Gómez, M., 1996. Factors affecting the respiration/ETS ratio in marine zooplankton. J. Plankton Res. 18, 239–255. https://doi.org/10.1093/plankt/18.2.239.
Hernández-León, S., and Torres, S., 1997. The relationship between ammonia excretion and GDH activity in marine zooplankton. Journal of plankton research, 19(5), 587-601.
Höhn, D.P., Lucas, C.H., Thatje, S., 2017. Respiratory response to temperature of three populations of *Aurelia aurita* polyps in northern Europe. PLoS One 12, 1–13. https://doi.org/10.1371/journal.pone.0177913.
Hubot, N.D., Giering, S.L.C., Füssel, J., Robidart, J., Birchill, A., Stinchcombe, M., Dumousseaud, C., Lucas, C.H., 2021. Evidence of nitrification associated with globally distributed pelagic jellyfish. Limnol. Oceanogr. 9999, lno.11736. https://doi.org/10.1002/lno.11736.
Iguchi, N., Iwatani, H., Sugimoto, K., Kitajima, S., Honda, N., Katoh, O., 2017. Biomass, body elemental composition, and carbon requirement of *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) in the southwestern Japan Sea. Plankt. Benthos Res. 12, 104–114. https://doi.org/10.3800/pbr.12.104.
Ishii, H., and Båmstedt, U., 1998. Food regulation of growth and maturation in a natural population of *Aurelia aurita* (L.). Journal of Plankton Research, 20(5), 805-816.
Jackson, J.B.C., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J., Warner, R.R., 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science (80-.). https://doi.org/10.1126/science.1059199.
Khong, N.M.H., Yusoff, F.M., Jamilah, B., Basri, M., Maznah, I., Chan, K.W., Nishikawa, J., 2016. Nutritional composition and total collagen content of three commercially important edible jellyfish. Food Chem. 196, 953–960. https://doi.org/10.1016/J.FOODCHEM.2015.09.094.
Klein, S.G., Pitt, K.A., Rathjen, K.A., Seymour, J.E., 2014. Irukandji jellyfish polyps exhibit tolerance to interacting climate change stressors. Glob. Chang. Biol. 20, 28–37. https://doi.org/10.1111/gcb.12408.
Knight, J. A., S. Anderson, and J. M. Rawle. 1972. Chemical Basisof the Sulfo-phospho-vanillin Reactionfor EstimatingTotal Serum Lipids. Clin. Chem. 18: 199–202.
Koh, H.J., Lee, S.M., Son, B.G., Lee, S.H., Ryoo, Z.Y., Chang, K.T., Park, J.W., Park, D.C., Song, B.J., Veech, R.L., et al., 2004. Cytosolic $NADP^+$-dependent Isocitrate Dehydrogenase Plays a Key Role in Lipid Metabolism. Journal of Biological Chemistry 279, 39968– 39974.
Korschelt, E., Heider, K., 1890. Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Tiere.
Kotova, A. V., Kovtun, O.A., Podgornaya, O.I., Adonin, L.S., 2015. Mesoglein differences between two jellyfish populations of the genus Aurelia. Int. Aquat. Res. 7, 123–131. https://doi.org/10.1007/s40071-015-0096-8.
Kraus, J.E., Fredman, D., Wang, W., Khalturin, K., Technau, U., 2015. Adoption of conserved developmental genes in development and origin of the medusa body plan. Evodevo 6, 23. https://doi.org/10.1186/s13227-015-0017-3.
Kuniyoshi, H., Okumura, I., Kuroda, R., Tsujita, N., Arakawa, K., Shoji, J., Saito, T., and Osada, H., 2012. Indomethacin induction of metamorphosis from the asexual stage to sexual stage in the moon jellyfish, *Aurelia aurita*. Bioscience, biotechnology, and biochemistry, 76(7), 1397-1400.
Lane, C. E., 1960. The toxin of Physalia nematocysts. Annals of the New York Academy of Sciences, 90(3), 742-750.
Larson, R. J.,1986. Water content, organic content, and carbon and nitrogen composition of medusae from the northeast Pacific. Journal of experimental marine biology and ecology, 99(2), 107-120.
Lilley, M. K. S., and Lombard, F., 2015. Respiration of fragile planktonic zooplankton: extending the possibilities with a single method. Journal of Experimental Marine Biology and Ecology, 471, 226-231.
Lowe, W.H., Martin, T.E., Skelly, D.K., Woods, H.A., 2021. Metamorphosis in an Era of Increasing Climate Variability. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2020.11.012.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Lu, Y., Lucas, C., Loveridge, A., 2020. Transgenerational acclimation influences asexual reproduction in *Aurelia aurita* jellyfish polyps in response to temperature. Mar. Ecol. Prog. Ser. 656, 35–50. https://doi.org/10.3354/meps13517.

Lucas, C.H., 1994. Biochemical composition of *Aurelia aurita* in relation to age and sexual maturity. J. Exp. Mar. Bio. Ecol. 183, 179–192. https://doi.org/10.1016/0022-0981(94)90086-8.

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

Markwell, M.A.K., Haas, S.M., Bieber, L.L., Tolbert, N.E., 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. 87, 206–210. https://doi.org/10.1016/0003-2697(78)90586-9.

Marques, A.C., Collins, A.G., 2004. Cladistic analysis of Medusozoa and cnidarian evolution. Invertebr. Biol. 123, 23–42. https://doi.org/10.1111/j.1744-7410.2004.tb00139.x.

Marsh, J. B., and D. B. Weinstein., 1966. Simple charring method for determination of lipids. J. Lipid Res. **7**: 574–576.

Martínez, I., Gómez, M., Packard, T.T., 2010. Potential respiration is a better respiratory predictor than biomass in young *Artemia salina*. J. Exp. Mar. Bio. Ecol. 390, 78–83. https://doi.org/10.1016/j.jembe.2010.05.011.

Martínez, I., Herrera, A., Tames-espinosa, M., Bondyale-juez, D.R., Romero-Kutzner, V., Packard, T.T., Gómez, M., 2020. Protein in marine plankton: A comparison of spectrophotometric methods. J. Exp. Mar. Bio. Ecol. 526, 151357. https://doi.org/10.1016/j.jembe.2020.151357.

Matsakis, S., and Conover, R. J., 1991. Abundance and feeding of medusae and their potential impact as predators on other zooplankton in Bedford Basin (Nova Scotia, Canada) during spring. Canadian Journal of Fisheries and Aquatic Sciences, 48(8), 1419-1430.

McCauley, D.J., Pinsky, M.L., Palumbi, S.R., Estes, J.A., Joyce, F.H., Warner, R.R., 2015. Marine defaunation: Animal loss in the global ocean. Science (80-.). https://doi.org/10.1126/science.1255641.

Nemazie, D. A., Purcell, J. E., and Glibert, P. M., 1993. Ammonium excretion by gelationous zooplankton and their contribution to the ammonium requirements of microplankton in Chesapeake Bay. Marine Biology, 116(3), 451-458.

Nestel, D., Tolmasky, D., Rabossi, A., Quesada-allué, L.A., 2003. Lipid, Carbohydrates and Protein Patterns During Metamorphosis of the Mediterranean Fruit Fly, Ceratitis capitata (Diptera: Tephritidae), academic.oup.com.

Olguín-Jacobson, C., Pitt, K.A., Carroll, A.R., Melvin, S.D., 2021. Chronic pesticide exposure elicits a subtle carry-over effect on the metabolome of *Aurelia coerulea* ephyrae. Environ. Pollut. 275, 116641. https://doi.org/10.1016/j.envpol.2021.116641.

Osma, N., Fernández-Urruzola, I., Gómez, M., Montesdeoca-Esponda, S., and Packard, T. T., 2016. Predicting in vivo oxygen consumption rate from ETS activity and bisubstrate enzyme kinetics in cultured marine zooplankton. Marine Biology, 163(7), 1-14.

Packard, T. T., 1971. The Measurement of Respiratory Electron-transport Activity in Marine Phytoplankton. . Mar. Res. 29 (3), 235–244

Packard, T. T., 1985. Measurement of electron transport activity of microplankton. Williams, P.J.LeB., Jannasch, H.W. (Eds.), Advances in Aquatic Microbiology. Academic Press, New York, pp. 207–261

Packard, T. T., 1985. Oxygen consumption in the ocean: measuring and mapping with enzyme analysis. In: Zirino, A. (Ed.), Mapping Strategies in Chemical Oceanogra phy. American Chemical Society, Washington, D.C.

Packard, T., Harmon, D., and Boucher, J., 1974. Respiratory electron transport activity in plankton from upwelled waters. Tethys, 6(1-2), 213-222.

Packard, T. T., and Christensen, J. P., 2004. Respiration and vertical carbon flux in the Gulf of Maine water column. Journal of Marine Research, 62(1), 93-115.

Pandori, L.L.M., Sorte, C.J.B., 2019. The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. Oikos 128, 621–629. https://doi.org/10.1111/oik.05886.

Pascual, M., Fuentes, V., Canepa, A., Atienza, D., Gili, J.M., Purcell, J.E., 2015. Temperature effects on asexual reproduction of the scyphozoan *Aurelia aurita* s.l.: Differences between exotic (Baltic and Red seas) and native (Mediterranean Sea) populations. Mar. Ecol. 36, 994–1002. https://doi.org/10.1111/maec.12196.

Pineda, M.C., McQuaid, C.D., Turon, X., López-Legentil, S., Ordóñez, V., Rius, M., 2012. Tough Adults, Frail Babies: An Analysis of Stress Sensitivity across Early Life-History Stages of Widely Introduced Marine Invertebrates. PLoS One 7, e46672. https://doi.org/10.1371/journal.pone.0046672.

Pitt, K.A., Duarte, C.M., Lucas, C.H., Sutherland, K.R., Condon, R.H., Mianzan, H., Purcell, J.E., Robinson, K.L., Uye, S.I., 2013. Jellyfish Body Plans Provide Allometric Advantages beyond Low Carbon Content. PLoS One 8, 1–10. https://doi.org/10.1371/journal.pone.0072683.

Purcell, J. E., and Kremer, P., 1983. Feeding and metabolism of the siphonophore *Sphaeronectes gracilis*. Journal of Plankton Research, 5(1), 95-106.

Purcell, J. E., Fuentes, V., Atienza, D., Tilves, U., Astorga, D., Kawahara, M., and Hays, G. C., 2010. Use of respiration rates of scyphozoan jellyfish to estimate their effects on the food web. Jellyfish Blooms: New Problems and Solutions, 135-152.

Purcell, J.E., Atienza, D., Fuentes, V., Olariaga, A., Tilves, U., Colahan, C., Gili, J.M., 2012. Temperature effects on asexual reproduction rates of scyphozoan species from the northwest Mediterranean Sea. Hydrobiologia 690, 169–180. https://doi.org/10.1007/s10750-012-1047-7.

Purcell, J.E., Bondyale-Juez, D.R., Romero-Kutzner, V., Martínez, I., Caprioli, R., Tames-Espinosa, M., Almunia, J., Alonso, E., Packard, T.T., Gómez, M., 2019. Food supply effects on the asexual reproduction and respiratory metabolism of Aurelia aurita polyps. Hydrobiologia 846, 135–146. https://doi.org/10.1007/s10750-019-04057-4.

Purcell, J.E., Uye, S.I., Lo, W.T., 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: A review. Mar. Ecol. Prog. Ser. 350, 153–174. https://doi.org/10.3354/meps07093.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reitzer, L., 2003. Nitrogen assimilation and global regulation in Escherichia coli. Annual Reviews in Microbiology, 57(1), 155-176.

Remane, A., 1954. Die Geschichte der Tiere.

Richardson, A.J., Bakun, A., Hays, G.C., Gibbons, M.J., 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends Ecol. Evol. 24, 312–322. https://doi.org/10.1016/j.tree.2009.01.010

Romero-Kutzner, V., Packard, T.T., Berdalet, E., Roy, S.O., Gagné, J.P., Gómez, M., 2015. Respiration quotient variability: Bacterial evidence. Mar. Ecol. Prog. Ser. https://doi.org/10.3354/meps11062.

Roy, S., and Packard, T., 2001. CO_2 production rate predicted from isocitrate dehydrogenase activity, intracellular substrate concentrations and kinetic constants in the marine bacterium *Pseudomonas nautica*. Marine Biology, 138(6), 1251-1258.

Roznere, I., Sinn, B.T., Daly, M., Watters, G.T., 2021. Freshwater mussels (Unionidae) brought into captivity exhibit up-regulation of genes involved in stress and energy metabolism. Sci. Rep. 11, 2241. https://doi.org/10.1038/s41598-021-81856-7.

Russel, F.S., 1970. The medusae of the British Isles II. Pelagic Scyphozoa with a supplement to the first volume on hydromedusae. Cambridge University Press, Cambridge, 284 pp.

Rutter, W.J., 1967. Protein determinations in embryos, in: Wilt, F.H., Wessels, N.K. (Eds.), Methods in Developmental Biology. New York, pp. 671–683.

Salvini-Plawen, L. V., 1987. Mesopsammic cnidaria from Plymouth (with systematic notes). J. Mar. Biol. Assoc. United Kingdom 67, 623–637. https://doi.org/10.1017/S0025315400027338.

Seipp, S., Schmich, J., and Leitz, T., 2001. Apoptosis–a death-inducing mechanism tightly linked with morphogenesis in *Hydractina echinata* (Cnidaria, Hydrozoa).

Schiariti, A., Morandini, A.C., Jarms, G., Von Glehn Paes, R., Franke, S., Mianzan, H., 2014. Asexual reproduction strategies and blooming potential in Scyphozoa. Mar. Ecol. Prog. Ser. 510, 241–253. https://doi.org/10.3354/meps10798.

Schneider, G., 1989. The common jellyfish *Aurelia aurita*: standing stock, excretion and nutrient regeneration in the Kiel Bight, Western Baltic. Mar. Biol. 100, 507–514. https://doi.org/10.1007/BF00394827.

Seebacher, F., White, C.R., Franklin, C.E., 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. Nat. Clim. Chang. 5, 61–66. https://doi.org/10.1038/nclimate2457.

Shimauchi, H., Uye, S.I., 2007. Excretion and respiration rates of the scyphomedusa *Aurelia aurita* from the Inland Sea of Japan. J. Oceanogr. 63, 27–34. https://doi.org/10.1007/s10872-007-0003-z.

Sivaprasad, S., and Bhuvaneswari, E., 2018. Energetics of pupal-adult metamorphosis in the silkworm, *Bombyx mori*: An analysis of transdeamination parameters in the fat body and haemolymph. Journal of Applied and Natural Science, 10(2), 746-752.

Solorzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. ATS (11-1) GEN 10, PA 20. Limnology and oceanography, 14(5), 799-801.

Stillway, L. W., and Lane, C. E., 1971. Phospholipase in the nematocyst toxin of *Physalia physalis*. Toxicon, 9(3), 193-195.

Suzuki, K., 2020. Ecological Studies of the Scyphozoan Jellyfish Aurelia coerulea: Implications for the Mechanisms of Their Outbreaks.

Tames-Espinosa, M., Martínez, I., Romero-Kutzner, V., Bondyale-Juez, D. R., Packard, T. T., and Gomez, M., 2018. $NADP^+$-dependent isocitrate dehydrogenase activity in marine plankton. Marine Chemistry, 204, 86-94.

Thorson, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. Biological reviews, 25(1), 1-45.

Thuesen, E. V., and Childress, J. J., 1994. Oxygen consumption rates and metabolic enzyme activities of oceanic California medusae in relation to body size and habitat depth. The biological bulletin, 187(1), 84-98.

Thompson, W. E., 1993. Protein expression during Aurelia development (Doctoral dissertation, Rutgers The State University of New Jersey-New Brunswick).

Truman, J. W., and Riddiford, L. M., 1999. The origins of insect metamorphosis. Nature, 401(6752), 447-452.

Uye, S. I., and Shimauchi, H., 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(3), 237-248.

van der Lee, G.H., Kraak, M.H.S., Verdonschot, R.C.M., Verdonschot, P.F.M., 2020. Persist or perish: critical life stages determine the sensitivity of invertebrates to disturbances. Aquat. Sci. 82, 24. https://doi.org/10.1007/s00027-020-0698-0.

Weihrauch, D., and Allen, G. J., 2018. Ammonia excretion in aquatic invertebrates: new insights and questions. Journal of Experimental Biology, 221(2), jeb169219.

Willcox, S., Moltschaniwskyj, N.A., Crawford, C., 2007. Asexual reproduction in scyphistomae of *Aurelia* sp.: Effects of

temperature and salinity in an experimental study. J. Exp. Mar. Bio. Ecol. 353, 107–114. https://doi.org/10.1016/j.jembe.2007.09.006.
Wittig, K., Kasper, J., Seipp, S., and Leitz, T., 2011. Evidence for an instructive role of apoptosis during the metamorphosis of *Hydractinia echinata* (Hydrozoa). Zoology, 114(1), 11-22.
Wright, P.A., 1995. Nitrogen excretion: three end products, many physiological roles. J. Exp. Biol. 198, 273–281. https://doi.org/10.1242/jeb.198.2.273.

CHAPTER

5

Conclusions

Research is formalised curiosity.

Zora Neale Hurston (1942)

5.1 Chapter 2

(1) Changes in respiration rates (O_2 consumption and CO_2 production) were observed due to the nutritional effect on the physiological state. Bacteria under substrate limitation had higher RQs than when grown under nutritional sufficiency conditions.

(2) Metabolic changes in bacteria, due to nutritional conditions, should be taken into account if an RQ is to be used.

(3) Predictions of the balance between ocean autotrophy and heterotrophy, carbon flux calculations, and ecosystem models, could be affected if RQ variability can be found in trophic levels above prokaryotes.

5.2. Chapter 3

(4) After experiencing a severe and extreme marine heatwave (MHW), in the recovery period *A. aurita* polyps were able to shift their metabolism back to the initial values of their pre-heatwave respiratory rates.

(5) An $R / \Phi \geq 1$ ratio was found during the MHW, but it decreases until reaching control conditions in the recovery period. This suggests that *A. aurita* polyps can control their metabolism in an MHW of 20 days.

(6) However, $R/\Phi > 1$ does not follow the theory that respiratory ETS activity is constitutive, i.e. that is not easily activated or repressed. Future research is needed to find possible explanations for how physiological respiration rates can exceed the enzymatic activity of the respiratory ETS.

5.3. Chapter 4

(7) We have identified metabolic and biochemical changes during life stage transitions of metamorphosing *A. aurita*. The highest WM-specific metabolic rates have been observed in the benthic life stages.

(8) The strobila life stage accumulates energy reserves, which decrease towards strobilation, the release of ephyrae. In addition, respiratory and excretory activity showed rapid falls during the last days of strobila metamorphosis. We hypothesise, that the later stages of the strobila and the first ephyra could be more vulnerable to environmental variability.

(9) The R/Φ (0.2) ratio was stable during life stage transitions, from polyp to ephyra and from ephyra to medusa. This could indicate that the metamorphosis in *A. aurita* is a consolidated metabolic process if the environmental conditions are stable. Stability in the R/Φ ratio has been previously seen in *A. aurita* polyps under different food regimes and starvation (Purcell et al., 2019).

(10) The R/Φ ratio seems to be a good indicator of respiration for *A.aurita* during metamorphosis.

CHAPTER

6

Resumen

El polvo enamorado

Poema de Josefina Pla (1968)

6.1 Introducción

6.1.1. Metabolismo, antes del oxígeno

La historia del planeta Tierra cubre aproximadamente 4.5 billones de años y según evidencias geológicas, la existencia de una hidrosfera se remonta a 4,3 billones de años (Mojzsis et al., 2001). La vida se originó en el agua a partir de moléculas orgánicas prebióticas (Fleury et al., 2017). Estas moléculas fueron producto de la actividad volcánica subacuática y subaérea. Los científicos han buscado análogos actuales que se asemejen al entorno de la Tierra prebiótica. Estos análogos incluyen: El Parque Nacional Yellowstone en Wyoming (EE. UU.), Hell's Gate cerca de Rotorua (Nueva Zelanda), Kamchatka (Russia), Dallol en la depresión de Danakil (Etiopía), El Tatio (Chile) y las fumarolas hidrotermales oceánicas (Deamer, 2021). Los ambientes prebióticos tienen varias características en común: abundancia de compuestos orgánicos, presencia de minerales, gradientes de temperaturas elevadas y, un potencial redox, con ambiente reductor. Aun así, el origen y la evolución de la materia orgánica en la Tierra todavía despierta la curiosidad de los científicos. Al investigar estos análogos modernos, los científicos han aprendido que el oxígeno no era esencial para la vida. Esto se debe a que el oxígeno estaba ausente o en bajas concentraciones en la atmósfera primitiva de la Tierra (Kasting, 1993; Zimorski et al., 2019; Ozaki y Reinhard, 2021).

A diferencia del oxígeno, la energía, sí es fundamental para la vida; permite la existencia, la reproducción y la supervivencia. Todos los organismos, autótrofos y heterótrofos, han desarrollado vías bioquímicas para recolectar, convertir y almacenar energía: Principalmente a través de la síntesis, primero de acetil fosfato (AcP) (Whicher et al., 2018), y luego de adenosín trifosfato (ATP) (Carlson et al., 2007), para mantener las funciones fisiológicas. Todas las células tienen, mayoritariamente, las mismas vías catabólicas para descomponer la materia orgánica en energía celular.

Los procesos catabólicos son ubicuos en todos los organismos (Lane 2006), utilizan y controlan: la glucólisis, el ciclo de los ácidos tricarboxílicos (TCA o ciclo de Krebs) y el sistema respiratorio de transporte de electrones (ETS). Estas vías metabólicas son los pasos principales que componen la respiración celular. En las células, la producción de ATP está controlada e iniciada por gradientes de protones, asociados con donantes de protones y electrones, que son los encargados de transferir las corrientes eléctricas a través del ETS respiratorio (Figura 1).

Fue Ochoa (1942) quien propuso que la respiración está acoplada a la producción de ATP, y luego, Mitchell (1961) añadió que es la acción del ETS respiratorio quién genera el ATP. Esto llevó a Mitchell a ganar el Premio Nobel en 1977, por su explicación quimiosmótica de la producción de ATP. Los donantes de electrones (electropositivos) y los aceptores (electronegativos) varían en todo el entorno abiótico (Falkowski et al., 2008). Por ejemplo, en organismos anaerobios, el H_2 puede actuar como donante de electrones y el CO_2 como aceptor de electrones, y así se alimenta la metanogénesis (Canfield et al., 2006). Mientras que, en la respiración aeróbica, el O_2 actúa como aceptor de electrones. La diferencia más destacada entre el metabolismo anaeróbico y aeróbico, es la velocidad a la que se transforma la energía.

Además, el metabolismo abarca un sistema de enzimas entrelazadas, que catalizan todas las reacciones bioquímicas. Aun así, se desconoce en gran medida cómo surgieron estos sistemas de enzimas (Noda-García et al., 2018; Becerra, 2021). Varias enzimas que transportan electrones al ETS respiratorio, se han podido rastrear hasta el último ancestro común universal (LUCA), e incluyen: NADH deshidrogenasa, succinato deshidrogenasa e hidrogenasa (Castresana et al., 1999). LUCA, un tipo de organismo unicelular, fue el precursor de todos los seres vivos actuales. Se cree que LUCA, a pesar de su

metabolismo anaeróbico, utilizó trazas de oxígeno para respirar (Weiss et al., 2016). El conocimiento del metabolismo de LUCA proporciona evidencia de que los procesos respiratorios, vinculadas al ETS y a la síntesis ATP, evolucionaron hace alrededor de 3,5 billones de años (Castresana et al., 1999; Noda-García et al., 2018).

La ciencia de la bioquímica evolutiva considera que, desde las enzimas individuales hasta las redes metabólicas, se evolucionó a partir de reacciones catalizadas geoquímicamente para cumplir con condiciones fisiológicas y ambientales específicas. En este sentido, el metabolismo no es solo un fenómeno molecular, celular y ecológico, sino que es un fenómeno planetario, que evolucionó a partir de la actividad geoquímica en la Tierra prebiótica (Goldford y Segrè, 2018).

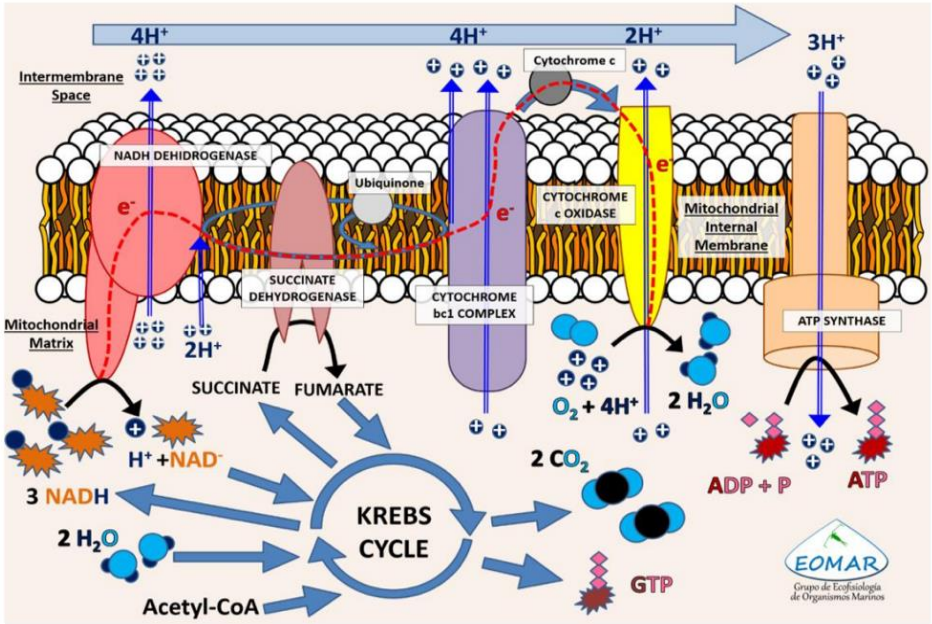


Figura 1. Representación de las principales vías bioquímicas implicadas en la respiración aeróbica localizadas en las mitocondrias: el ciclo de los ácidos tricarboxílicos (TCA o ciclo de Krebs), el sistema de transporte de electrones (ETS) y la síntesis de ATP. El ciclo de Krebs está vinculado a la glucólisis, mediante el producto final de la misma, el ácido pirúvico. Este ácido pirúvico se combina con la coenzima A (CoA) para formar acetil coenzima A (Acetil CoA), y así entrar en el ciclo de Krebs. Por cada acetil-CoA, el ciclo de Krebs libera 3 moléculas de NADH, 1 molécula de $FADH_2$, CO_2 y guanosín trifosfato (GTP). Los complejos enzimáticos del ETS oxidan NADH y $FADH_2$, generando así un flujo de electrones. Este flujo de electrones provoca un bombeo de protones (H^+) en la membrana de la mitocondria. Luego, en el complejo IV (citocromo c oxidasa) los electrones, reducen el O_2 para formar H_2O en presencia de protones (H^+). De esta manera, se genera un gradiente de pH mediante el bombeo de H^+ a través de la membrana, y el consumo de protones para formar agua. Este gradiente de pH a través de la membrana

mitocondrial interna es una reserva de energía libre, e impulsa la síntesis de ATP. El acoplamiento de la transferencia de electrones y el bombeo de H^+, con la síntesis de ATP, fue propuesto por Peter Mitchell (1961) en su hipótesis quimiosmótica. La aparición de las mitocondrias coincide con los primeros protozoos hace 2,5 billones de años y se necesitaron otros 2 billones de años para la evolución de la vida multicelular. Ilustración de la figura de D.R. Bondyale-Juez.

6.1.2. Evolución del metabolismo aeróbico en eucariotas

Inconcebiblemente, las cianobacterias, fotoautótrofas, al producir O_2, modificaron la química de la atmósfera terrestre. Con el tiempo, la atmósfera pasó de ser una atmósfera reductora a una atmósfera oxidante, y las cianobacterias utilizaron agua como agente reductor y liberaron oxígeno molecular. Este cambio conllevó a la mayor revolución en la historia de la vida, la oxigenación del planeta, o también llamado la Gran Oxidación (GOE) (Vernadsky, 1926; Olejarz et al., 2021). Las concentraciones de oxígeno comenzaron a aumentar con la fotosíntesis oxigénica, hace alrededor de 2,5 billones de años (Planavsky et al., 2014; Krause et al., 2018), fomentando así el metabolismo aeróbico (Wignall y Twitchett 1996; Vaquer-Sunyer y Duarte, 2008) (Figura 2).

Este oxígeno era originalmente tóxico para las células primitivas (Margulis y Sagan, 1986). Un estudio reciente de Canfield et al. (2021), sugiere que el oxígeno, aunque tóxico para los procariotas aero-intolerantes, no fue un impedimento para el desarrollo de los primeros ecosistemas eucariotas. Por ejemplo; se cree que la melatonina es un antioxidante antiguo que hizo que el oxígeno fuera metabólicamente tolerable (Manchester et al., 2015).

Una vez que los organismos se han adaptado al oxígeno, en la respiración aeróbica se utiliza el oxígeno como aceptor de electrones. Los organismos a partir de ese momento eran capaces de descomponer la materia orgánica en agua y dióxido de carbono. De esta manera, la respiración aeróbica llega a liberar mucha más energía que la respiración anaeróbica. Más tarde los ecosistemas eucariotas evolucionaron con la disponibilidad de nutrientes (Brocks et al., 2017; Eckford-Soper y Canfield, 2020), desarrollo de la alimentación, depredación, multicelularidad y organización celular (Knoll et al., 2011).

Los primeros animales, según restos fósiles y biomarcadores, consistieron principalmente en esponjas del filo Porifera, y medusas del filo Cnidaria (Cloud y Glaessner, 1982; Narbonne, 2005). En particular, abundaban los cnidarios sésiles, bentónicos y formadores de colonias (Narbonne, 2005). Esto se remonta a hace unos 550 millones de años, en el momento en que "los metazoos heredaron el mundo" (Figura 2).

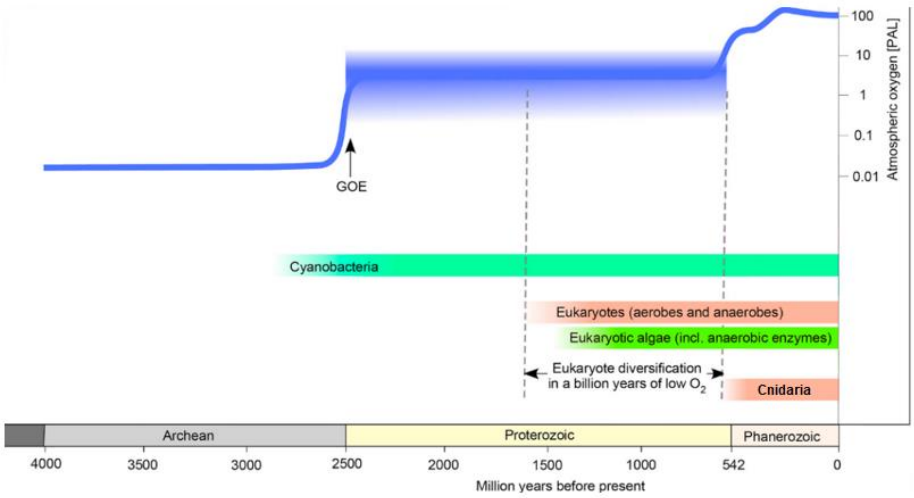


Figura 2. Resumen de la acumulación de oxígeno en la historia de la Tierra y la evolución de la vida. Nomenclatura: Porcentaje del nivel de oxígeno atmosférico (PAL), la Gran Oxidación (GOE). (Modificado de Zimorski et al. 2019)

6.1.3. Medusozoos, primeros metazoos

Desde el LUCA, que presentaba un metabolismo respiratorio anaeróbico, hasta la aparición de los primeros animales que respiran O_2 en la Tierra, pasaron 3,5 billones de años. La diversificación del filo Cnidaria resulta ser uno de los eventos evolutivos más antiguos (Cartwright et al., 2007; Han et al., 2016). Este filo tiene un sistema nervioso y muscular simple, y hoy en día se cree que representa la forma más antigua de las neuronas y los músculos (Steinmetz et al., 2012; Marlow y Arendt 2014; Moroz, 2021).

Los análisis filogenéticos sostienen que los cnidarios son un clado hermano de los bilaterales, que suponen el 99% de los animales existentes (Simion et al., 2017; Whelan et al., 2017). El mismo análisis también reveló que la división en el filo Cnidaria, en Anthozoa y Medusozoa, ocurrió en el Precámbrico, alrededor de 641,8-531,5 millones de años (Dos Reis et al., 2015).

Los Medusozoa, que incluyen Scyphozoa, Cubozoa e Hydrozoa, tienen una estructura del genoma mitocondrial única (Bridge et al., 1992). Los cnidarios no tienen sistema vascular ni órgano excretor. Su único sistema de transporte es pasivo, y depende de la difusión. El O_2 se difunde a través de las capas celulares que están expuestas al medio acuoso, proporcionando de esta manera el O_2 para la respiración (Graham, 1988). La campana de las medusas, tiene dos capas, la ectodermis y la endodermis. Estas dos capas están separadas por una mesoglea acelular (Holstein et al., 2011), capaz de retener altas concentraciones de O_2, generando una reserva que puede servir para condiciones ambientales desfavorables (por ejemplo, la hipoxia) (Thuesen, 2005). Numerosas medusas incorporan un pólipo bentónico, sésil y/o una fase de vida planctónica (Gold et al., 2018) (Figura 3). Los pólipos también pueden incorporar O_2 disuelto del agua por difusión a través de su tejido corporal (Han et al., 2016).

La capacidad de movilidad de las medusas se considera una innovación biológica, que tuvo lugar durante la transición Precámbrico-Cámbrico (hace alrededor de 550 millones de años) (Collins, 2002; Narbonne, 2005; Marques y Collins, 2005; Collins et al., 2006;). Gold et al. (2018) plantean que los Medusozoa han desarrollado su compleja etapa de vida planctónica reorganizando las vías genéticas ya presentes en el último antepasado común de los cnidarios. Por ende, se considera que los cambios en las fases de vida son adaptaciones a nuevos nichos ecológicos (Gold et al., 2018).

La metagénesis de los Medusozoa es uno de los ciclos de vida más complejos y antiguo de los metazoos (Marques y Collins, 2004; Kraus et al., 2015), y puede incluir varias fases de vida (medusa, larva plánula, pólipo, estróbila y éfira) (Figura 3). Se estima que el ciclo de vida de las medusas puede haber contribuido a su éxito durante los últimos 500 millones de años (Pitt et al., 2013). Las distintas etapas de vida tienen la capacidad de proliferar en altas densidades, de forma que la proliferación de poblaciones de pólipos por reproducción asexual (gemación), y la producción de éfiras (estrobilación), pueden determinar la población de medusas planctónicas.

Según los registros fósiles, la morfogénesis de los Medusozoa se ha mantenido estable a lo largo del tiempo, lo que ha dado como resultado una similaridad en las formas de las medusas. Con el cambio climático antropogénico, se espera que los Medusozoa, que han sobrevivido alrededor de 0,5 billones de años, puedan recuperar el papel de depredador pelágico exclusivo (Parson y Lalli, 2002; Richardson et al., 2009).

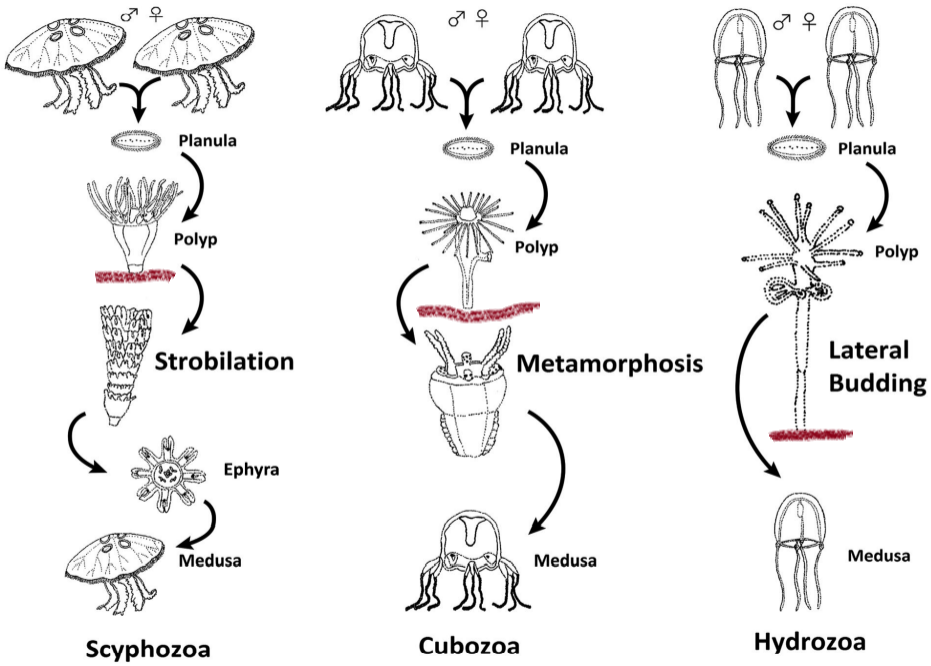


Figura 3. Principales fases de vida en los Medusozoa (Scyphozoa, Cubozoa e Hydrozoa). La etapa de vida pólipo es bentónica. *Budding* es un tipo de reproducción asexual, que forma nuevos pólipos a partir del plano corporal parental. (Modificado de Boero et al. 2016)

6.1.4. El papel del bacterioplancton y los medusozoos en los océanos modernos

Como hemos visto en los anteriores apartados, la presencia de energía, en forma de materia orgánica, y el metabolismo, permitieron la evolución de la vida. El principal aporte de energía en el ecosistema marino proviene del sol, y la biota marina juega un papel clave en la composición del agua de mar (Sverdrup et al., 1942; Redfield et al., 1963). Toda la vida que se conoce, se compone de compuestos orgánicos, que circulan a través de sistemas abióticos y bióticos. En otras palabras, la energía solar es fijada por productores primarios (autótrofos) y luego se transfiere a través de interacciones depredador-presa a niveles tróficos más altos. Ya se ha mencionado anteriormente que, todos los heterótrofos pueden oxidar la materia orgánica ingerida, la pueden transformar en energía celular a través de la respiración, y así regulan su metabolismo para el crecimiento y la reproducción.

Los microorganismos fueron los primeros habitantes del planeta. Los microbios heterótrofos pueden transformar la materia orgánica disuelta (MOD/COD-carbono orgánico disuelto), bien transformando esta energía en carbono orgánico particulado (MOP/COP-carbono orgánico particulado) como productores de biomasa, o a través de la respiración microbiana. A través de la respiración, la MOD/COD se convierte en dióxido de carbono (CID) (Williams, 1981; Azam et al., 1983; Ducklow et al., 1986; Robinson, 2019), de manera que se crean los flujos asociados de materia y energía en la columna de agua (Figura 3). Fue Pomeroy, en 1974, quien propuso por primera vez que el bacterioplancton desempeñaba un papel clave en la red trófica marina, porque consumen la mayor parte de la energía procedente de la producción primaria (Pomeroy 1974). Resumiendo, en los sistemas tróficos oceánicos modernos, las bacterias heterótrofas son responsables de hasta >

59% de la respiración comunitaria (Robinson y Williams, 2005) y son clave en el equilibrio entre el almacenamiento de carbono orgánico y el CO_2.
Ampliando la escala a los metazoos, las medusas fueron los primeros animales en habitar los océanos, y son cada vez más reconocidas como organismos con gran influencia en el medio marino, puesto que sus interacciones tróficas son extensas (Pauly et al., 2009; Riascos et al., 2012; Takao et al., 2014; Ates, 2017; Choy et al., 2017). Las medusas cuando generaran aglomeraciones, se las conoce como *blooms* de medusas. Suponen una fuente de alimento para muchas especies marinas (Lamb et al., 2017), y juegan un papel relevante como depredadores, alimentándose en gran medida de larvas de peces y mesozooplancton (Behrends y Schneider, 1995; Lynam et al., 2005). Además, las medusas también se consideran como un importante agente en el transporte de carbono a través de la columna de agua, ya que periódicamente caen en dicha columna como MOP/COP, un fenómeno conocido como *jelly-falls* (Figura 4) (Billet et al., 2006; Lebrato et al., 2013, 2012, 2019). También pueden liberar MOD/COD en la columna de agua, estando vivas o muertas (Condon et al., 2011).

Esto significa que, si las medusas forman *blooms*, pueden limitar el carbono biodisponible a niveles tróficos más altos y promover el bucle microbiano, generando alternancias en el ecosistema marino (Condon et al., 2011). Sin embargo, se desconoce la materia orgánica producida y excretada por las medusas (Tinta et al., 2021) y depende de la fisiología y las condiciones ambientales de las fases de vida (Pitt el al., 2009).

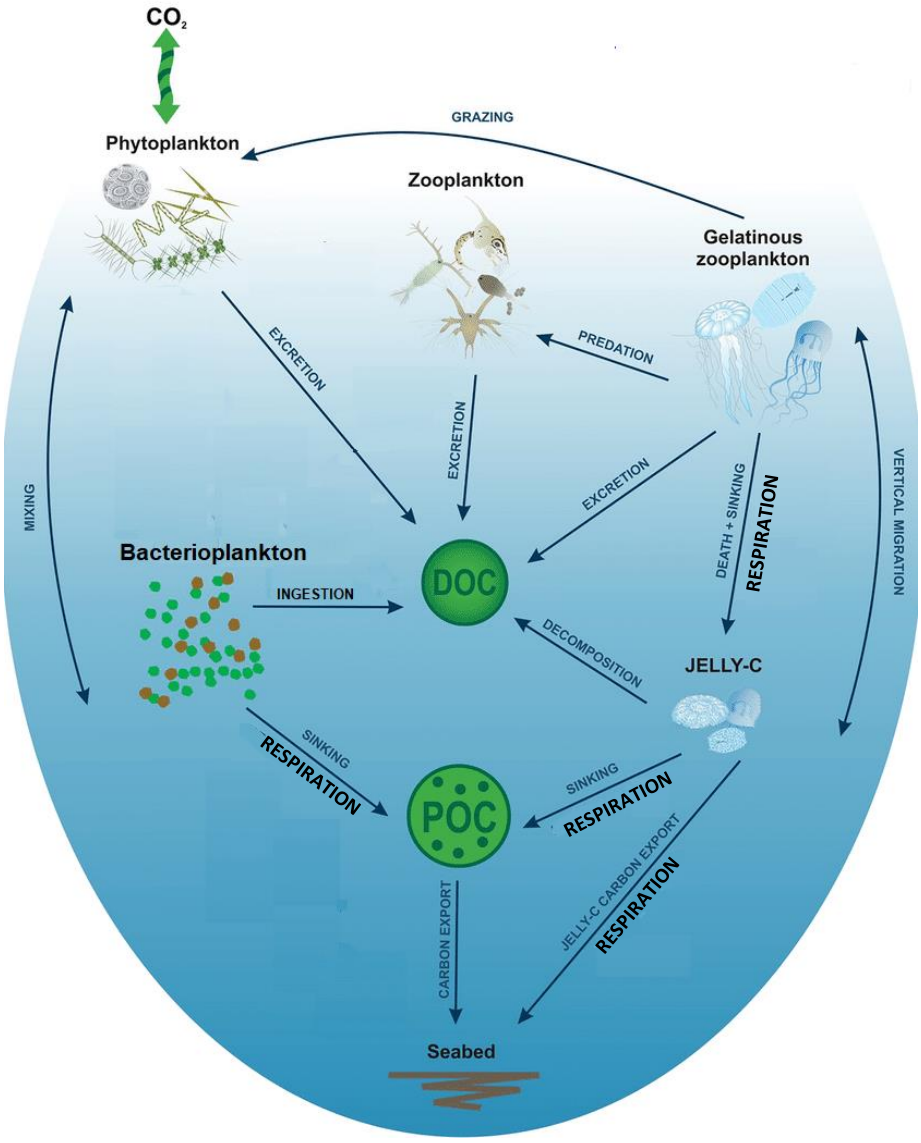


Figura 4. Ciclo de carbono (COD y COP) derivado del bacterioplancton y de las medusas (Jelly-C) Se muestran los procesos físicos (hundimiento, descomposición) o fisiológicos (respiración, excreción) que transportan partículas biogénicas desde la capa superior del océano hasta el ecosistema bentónico. (Modificado de Lebrato et al. 2019).

6.1.5. Metabolismo, océano y cambio climático

Los flujos de materia orgánica (o flujos de energía) en el mar están controlados en parte por la biota marina (Figura 4). La fisiología es la respuesta bioquímica de un organismo hacia su entorno, y puede arrojar luz sobre estos procesos de flujos orgánicos. Hace casi 60 años, Redfield et al. (1963), recalcaron que el estudio del metabolismo de los organismos marinos puede desvelar la descripción químico-física del mar. Hoy en día sabemos que la regulación de O_2 y CO_2 en la atmósfera se ve afectada parcialmente por la mayor reserva oceánica de carbono orgánico, en forma de MOD/COD (Fakhraee et al., 2021).

La estimación de la materia orgánica en el océano se realiza de forma rutinaria utilizando trampas de sedimentos. Se trata de contenedores que recogen las partículas que se hunden y caen hacia el fondo del mar. Sin embargo, todo flujo puede variar en el tiempo y el espacio. Además, ningún ecosistema está cerrado y las masas de agua contienen subregiones geográficas e hidrográficas (Figura 5) (Longhurst, 1998) que pueden afectar a la recolección de partículas.

La medición del metabolismo respiratorio es una alternativa para estimar el flujo de materia orgánica en los océanos. La respiración en el interior del océano se nutre de la transformación de la energía (autótrofa) en las aguas superiores. Este flujo de energía continúa a través de la columna de agua, hasta alcanzar el lecho marino (Sverdrup et al., 1942, Riley 1951, Redfield 1963). Aunque la variabilidad en el metabolismo asociada a diferentes organismos y especies puede reflejar requerimientos de energía alternos, las tasas de transformación de energía en grandes cuerpos de agua presentan cierta regularidad (Redfield, 1963).

Por lo tanto, se considera que la respiración es un buen indicador para estimar el flujo de materia orgánica en el océano, ya que es la respuesta a la materia orgánica rica en energía.

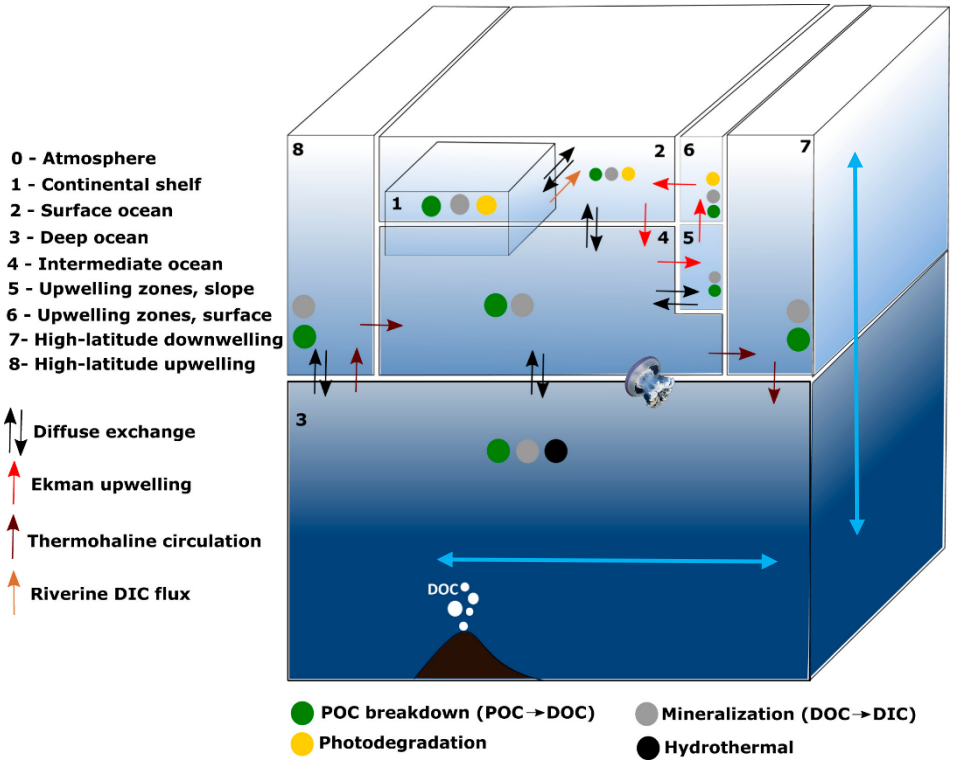


Figura 5. Modelo de caja para el ciclo de carbono a partir de masas de agua diferenciadas según su origen geográfico e hidrográfico. La degradación del COP (punto verde) y la mineralización (punto gris) son procesos controlados por la respiración. La presencia de materia orgánica en cada caja es el resultado de mecanismos de transporte físicos (difusión, afloramiento, circulación termohalina y flujo fluvial). El bacterioplancton y las medusas (plancton gelatinoso) se distribuyen por todas las cajas de masa de agua (flecha azul). (Modificado de Fakhraee et al. 2021)

Desde Harvey (1928, 1957), investigar el metabolismo de los organismos marinos ha sido una de las principales áreas de interés en Oceanografía. Actualmente, el interés de la comunidad científica trata de investigar el papel que juega el océano como sumidero de CO_2 durante el cambio climático antropogénico (Falkowsi y Wilson, 1992; McKinley et al., 2016). Por esta razón, es necesario abordar el estado metabólico del océano (Del Giorgio y Duarte; Del Giorgio y Williams, 2005; Ducklow et al., 2013; Duarte et al., 2013).

El metabolismo de los organismos marinos tiene la capacidad de amortiguar los cambios ambientales (Pincebourde et al., 2009). Sin embargo, se desconoce en gran medida cómo afecta el cambio climático antropogénico a los ecosistemas marinos (Hoegh-Guldberg y Bruno, 2010). Si se desconoce la respuesta metabólica, desde individuos a los ecosistemas, en todas sus posibles facetas, la cuantificación y anticipación de los efectos del cambio climático se convierte en imposible. Por lo tanto, se necesita estudiar el metabolismo, para poder hacer predicciones de cómo el cambio climático afecta a las especies, comunidades, poblaciones y ecosistemas, respectivamente.

Uno de los impactos más importantes del cambio climático para muchas especies es el calentamiento de los océanos (Hughes et al., 2017; Smale et al., 2019; Scanes et al., 2020 a, b). Esto se debe a que todas las tasas metabólicas se ven afectadas por la temperatura. La temperatura aumenta la velocidad de las reacciones metabólicas. Además, el metabolismo cumple una cierta ventana de tolerancia que depende de la especie y su hábitat. De esta manera, las olas de calor marinas (marine heatwave, MHW), eventos prolongados de aguas extremadamente cálidas, un fenómeno creciente en los océanos, está provocando severos efectos en la biota marina (Suryan et al., 2021). En un escenario de MHW, los individuos deben adaptarse fisiológicamente al estrés

térmico (Leung et al., 2019). Como consecuencia, se esperan cambios en el crecimiento, la reproducción (Shanks et al., 2020; Gall et al., 2021), la alimentación (Mitterwallner et al., 2021) y en la supervivencia (Pérez et al., 2000).

Durante las MHW, debido a las columnas de agua altamente estratificadas, hay menos flujo de nutrientes y se espera una producción primaria más débil (Hayashida et al., 2020). Esto puede generar entornos con escasez de energía. Sin embargo, según McCauley (2015), algunas especies podrán beneficiarse de los cambios oceánicos actuales y futuros. El bacterioplancton y las medusas son organismos potencialmente importantes en el futuro océano, como lo han sido durante millones de años. Por ello, estudiar su metabolismo es de gran interés.

6.1.5.1. Desafíos futuros

La Década de los Océanos de las Naciones Unidas (año 2021-2030), tiene como meta investigar para avanzar hacia un océano futuro sostenible y saludable. Baltar et al. (2019), propusieron la combinación de estudios de evolución y de metabolismo, para permitir predicciones futuras sobre los efectos adversos del cambio climático en la biota marina. Esta propuesta está fundamentada en que: los cambios ambientales estimulan la evolución (por ejemplo, el metabolismo aeróbico) y la evolución, a su vez, estimula los cambios en el medio ambiente (por ejemplo, GOE).

Por lo tanto, es fundamental comprender las respuestas metabólicas a los cambios ambientales. Sin embargo, en ecología, el metabolismo respiratorio se aborda comúnmente empleando la Teoría Metabólica de la Ecología (MTE) (Brown et al., 2004). Esta teoría se basa en la ley de Kleiber que relaciona la respiración con la biomasa. En otras palabras, en esta teoría, la biomasa consta como un factor importante que controla la respiración. De hecho, existe

correlación entre la respiración y la biomasa (Kleiber, 1932, 1961). Esto se debe a que la biomasa empaqueta las mitocondrias y las enzimas respiratorias (Packard y Gómez, 2008; Martínez et al, 2010).

Se conoce, desde la década de 1950, que las mitocondrias son las centrales energéticas de la respiración (Siekevitz, 1957). Sin embargo, la relación entre la respiración y las enzimas mitocondriales puede presentar alteraciones, por ejemplo, por la fase de vida, la edad, el sexo, el estado reproductivo, el estado nutricional, etc. (Dods et al., 2008; Martínez et al., 2010). Establecer correlaciones a partir de la biomasa no puede predecir estas fluctuaciones en la respiración. De este modo, se ha detectado una no linealidad entre la biomasa y la respiración en diferentes taxones (Kolokotrones et al., 2010): bacterias (Aguiar-Gonzaléz et al., 2012), plantas (Reich et al., 2006; Mori et al., 2010) y zooplancton marino (Packard y Gómez, 2008; Martínez et al., 2010).

La MTE se ha aplicado anteriormente para calcular la respiración total del plancton en el océano (López-Urrutia et al., 2006) y recientemente los flujos de materia orgánica derivada de las medusas (Luo et al., 2020). Aunque Baltar et al. (2019) aboga por una integración del metabolismo en los estudios de los ecosistemas marinos, proponen la aplicación de MTE para cumplir con este propósito. Mediante la aplicación de la MTE se puede evitar la manipulación de las frágiles medusas para estudios ecofisiológicos, sin embargo, el metabolismo no se llega a medir directamente.

Por el contrario, en la bioquímica moderna se sabe que el ETS respiratorio controla la respiración, desde los primeros trabajos de David Keilin en la década de 1920 (con los citocromos como transportadores de electrones) (Keilin, 1966) y el trabajo cinético sobre la respiración y el transporte de electrones de Chance y Williams (1954; 1955). La respiración refleja la

velocidad a la que la enzima citocromo oxidasa utiliza los electrones, para reducir el oxígeno en agua en el ETS respiratorio.

En definitiva, los procesos y conexiones metabólicas son antiguos. Toda la vida que hoy existe, evolucionó a partir del metabolismo hace billones de años. Por tanto, las reacciones bioquímicas pueden proporcionar una mejor comprensión del metabolismo respiratorio que la biomasa en sí misma (Keilin, 1966; Mahler y Cordes, 1971; Nelson y Cox, 2005). En este trabajo proponemos una alternativa a la MTE, que sí refleja el metabolismo de un organismo, desde un enfoque enzimático.

6.1.7. Ecofisiología de organismos marinos: ensayos enzimáticos y fisiológicos

En el estudio de la ecofisiología, los métodos bioquímicos implican la determinación de la actividad enzimática vinculada a las vías metabólicas en los organismos marinos, que incluyen, principalmente: Sistema de transporte de electrones (ETS) y ciclo de Krebs. Así, los ensayos enzimáticos se han convertido en una herramienta importante para controlar el metabolismo del zooplancton.

Nuestro grupo de investigación (EOMAR) está especializado en el estudio del metabolismo de los organismos marinos, siguiendo un enfoque enzimático y fisiológico. En las últimas décadas se han desarrollado diferentes metodologías *in vitro* que incluyen: el ensayo del sistema de transporte de electrones respiratorio (ETS) (consumo potencial de O_2, Φ) (Packard, 1971; Gómez et al., 1996; Osma et al., 2016; Bondyale et al., 2017), $NADP^+$-isocitrato deshidrogenasa (IDH) (producción potencial de CO_2) (Tames-Espinosa et al., 2018) y glutamato deshidrogenasa (GDH) (Fernández-Urrozola et al., 2011). Estos ensayos enzimáticos requieren la adición de

sustratos (en concentración saturada) según su velocidad máxima (V_{max}), siguiendo el modelo cinético propuesto por Michaelis y Menten (1910). De esta forma se asegura la reproducibilidad de la reacción (Maldonado et al., 2012) porque la oxidación de los sustratos es idéntica en todas las células vivas (Vosjan, 1982).

Packard et al. (1971) propuso un método enzimático basado en el ETS como proxy para medir la respiración. Es una metodología diseñada para todos los organismos vivos y a cualquier profundidad que, además de la aplicación oceanográfica, se ha empleado también para estudiar el metabolismo en cultivos de laboratorio. Los organismos experimentales varían desde bacterias (Christensen et al., 1980; Romero Kutzner et al., 2015), zooplancton (Gómez et al., 1996; Herrera et al., 2011) hasta medusas (Purcell et al., 2019). Estos ensayos de ETS siguen principalmente el procedimiento propuesto en Owens y King (1975). En esta metodología, los electrones transferidos a través del ETS son recogidos por un aceptor de electrones artificial, la sal de tetrazolio (INT) (al reducirse genera formazán que tiñe la solución de rojo). La tasa de producción de INT-formazán revela la capacidad de consumo de oxígeno de un organismo, o la respiración potencial (Φ). Estequiométricamente, la tasa de producción de INT-formazán (µmol) está relacionada por un factor de 2 con la actividad de ETS (µmol e^-) y de 0,5 a Φ (Packard 1985).

Tames-Espinosa et al. (2018) desarrollaron la metodología de la $NADP^+$-isocitrato deshidrogenasa (NADP-IDH) en plancton marino, como proxy para estimar la producción de CO_2 respiratorio. La actividad de NADP-IDH es la velocidad a la que las isoenzimas (dependientes de $NADP^+$) reducen el $NADP^+$, mientras producen CO_2. La producción de CO_2 respiratorio potencial se puede calcular multiplicando la actividad de NADP-IDH por el factor 3 (Roy y Packard, 2001). Esto se debe a que la NADP-IDH es solo una de las tres principales enzimas que producen CO_2 asociadas con el ciclo de Krebs.

Las otras dos enzimas son la piruvato deshidrogenasa y el alfa-cetoglutarato (Walsh y Koshland, 1984; Holms, 1986; Packard et al., 1996).

En la naturaleza, las células pueden presentar una concentración de sustrato limitada; por lo tanto, este tipo de mediciones *in vitro* podrían no corresponderse a las tasas metabólicas *in vivo*. De esta manera, también se miden las tasas fisiológicas, para comprender mejor cómo se relacionan estas tasas *in vitro*/*in vivo*, en diferentes condiciones. Sin embargo, hasta la fecha no se ha desarrollado una metodología asequible y viable para medir la producción de CO_2 respiratorio. Por lo tanto, la producción de CO_2 respiratorio se convierte comúnmente a partir de las tasas de consumo de O_2, asumiendo un cociente respiratorio (RQ) constante de 1 (González et al., 2003; Bühring et al., 2006). El RQ representa la relación entre la producción de CO_2 respiratorio y el consumo de O_2 de un organismo.

La relación entre tasas fisiológicas y enzimáticas puede variar (Båmstedt, 1979; Hernández-León y Gómez, 1966; Herrera et al., 2011), sin embargo, la variabilidad está asociada con tasas fisiológicas que son más sensibles a factores abióticos como por ejemplo, temperatura, pH, salinidad o contaminación (Finlay, 1983) y a factores biológicos como podrían ser la masa corporal, sexo, o la calidad y cantidad de alimentos. Esta variabilidad agrega un valor adicional al enfoque enzimático *in vitro*, ya que establece una línea de base para estudiar el metabolismo.

A gran escala, esta variabilidad en los ratios respiratorios (entre tasas fisiológicas y enzimáticas) es menor (Finlay et al., 1983; Arístegui y Montero 1995). Por ejemplo, en la Figura 6, los datos de las relaciones respiratorias de las medusas, medidos en nuestro laboratorio, mantienen una relación de más de 10 órdenes de magnitud y encajan en la figura de Finlay et al. (1983) donde se representa la relación de la tasa respiratoria y la actividad del ETS para diferentes grupos de organismos.

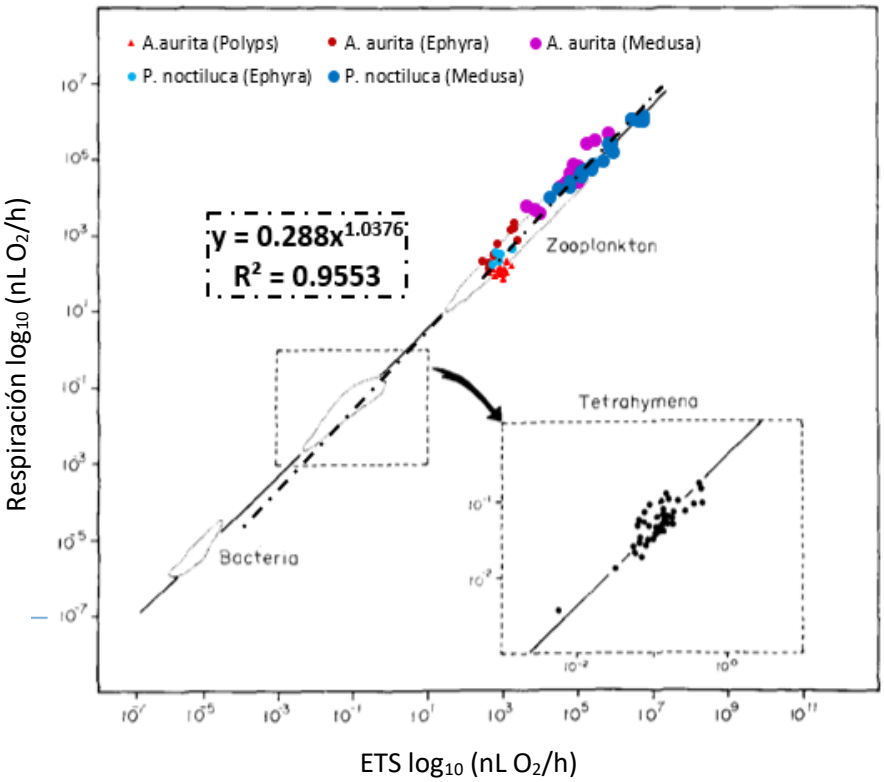


Figura 6. Relación entre las tasas de respiración fisiológicas (respiración) y enzimáticas (ETS) en diferentes grupos de organismos. Los datos de bacterias se obtuvieron de Christensen et al. (1980), datos de zooplancton de King y Packard (1975), datos de protozoos de Finlay et al. (1983) y los datos de medusas de EOMAR (*Proyecto Perseo*). (Modificado de Finlay et al. 1983)

6.2. Hipótesis, objetivos y desarrollo de los mismos

En esta tesis se ha abordado el estudio del metabolismo en organismos marinos desde diferentes enfoques y diferentes objetivos. Esta investigación da un paso en la definición de las respuestas bioquímicas y fisiológicas de la respiración en el bacterioplancton y medusas. Dichos organismos se han expuesto a factores bióticos (metamorfosis) y abióticos (disponibilidad de alimento y MHW), de manera que con los resultados se pretende facilitar la interpretación de cómo estos organismos responden a su entorno.

Capítulo 2

Hipótesis: Un RQ constante no puede definir las tasas fisiológicas respiratorias, porque el RQ cambia según las condiciones de suficiencia nutricional o de limitación de nutrientes.

Objetivo: Comparar el metabolismo respiratorio de dos especies de bacterias marinas, bajo dos sustratos de carbono y dos duraciones experimentales, a corto y largo plazo, desde condiciones de alimentación favorable hasta la inanición.

Experimentación: Se ha monitorizado el metabolismo respiratorio en bacterias desde condiciones de alimento favorables hasta la inanición, y se han calculado los RQ a partir de las mediciones de O_2 respiratorio y CO_2 respiratorio.

Capítulo 3

Hipótesis: Los pólipos de *A. aurita* aumentan su capacidad fisiológica en respuesta a la ola de calor (MHW), pero muestran capacidad de recuperación en el período posterior a la MHW.

Objetivo: Comparar el metabolismo respiratorio de los pólipos de *A. aurita* bajo dos olas de calor marinas con diferente temperatura, durante tres duraciones diferentes, y sus respectivos períodos de recuperación.

Experimentación: Se ha estudiado el metabolismo de los pólipos de la medusa *A. aurita*, simulando una ola de calor marina (MHW).

Capítulo 4

Hipótesis: *A. aurita* mostrará cambios en las actividades enzimáticas y metabólicas respiratorias, reflejando a su vez cambios en su composición bioquímica, durante las transiciones de las etapas de la vida.

Objetivo: Monitorear el metabolismo de *A. aurita* durante la metamorfosis, mientras pasa de la fase de vida de pólipo a medusa y comparar la composición bioquímica de cada fase de vida.

Experimentación: Se ha estudiado el metabolismo respiratorio y excretor durante la metamorfosis de *A. aurita*, pasando por las fases de vida de pólipo a medusa.

6.3. Resumen por capítulo

Capítulo 2

Se comparó el metabolismo respiratorio entre dos estados fisiológicos diferentes de cultivos de *Pseudomonas nautica* y *Vibrio natriegens* cultivados con acetato y piruvato. Los experimentos han tenido una duración de 35 h y 520 h. Se han tomado mediciones de proteínas, piruvato, acetato, producción de CO_2 respiratorio (RCO_2), consumo de O_2 respiratorio (RO_2), actividad de la enzima isocitrato deshidrogenasa (IDH) y la respiración potencial (Φ). Los cocientes respiratorios (RQ), ampliamente utilizados en modelos de ecosistemas oceánicos, en cálculos del flujo de carbono y en evaluaciones del equilibrio metabólico del océano, se calcularon como la relación de las tasas de respiración (RCO_2/RO_2). En todos los cultivos, el RQ tendió a aumentar. Por ende, el estado nutricional de las bacterias afectó al RQ, convirtiendo la constante en una variable a efectos fisiológicos y ecológicos. Estos resultados argumentan que los modelos de ecosistemas, los cálculos oceanográficos del flujo de carbono y las evaluaciones sobre el equilibrio metabólico del océano, que están influenciados por el metabolismo bacteriano, deben reconsiderarse debido a esta variabilidad en el RQ.

Capítulo 3

El aumento de la proliferación de medusas se ha asociado, entre otros factores, al cambio climático antropogénico. El calentamiento global, como parte del cambio climático, genera las olas de calor marinas (MHW), que se han ido observando en las últimas décadas en los océanos. Los MHW son períodos de temperatura superficial del agua de mar cálida anómala, que pueden durar de varios días a meses. Investigaciones recientes han demostrado que los ecosistemas marinos y la pesca son altamente vulnerables a las MHW. Las principales consecuencias para la biota marina se traducen en una mayor mortalidad, extinciones locales, cambios en la estructura de la comunidad y la disminución de la biodiversidad. Desafortunadamente, en un futuro se espera que los MHW sean más frecuentes, más largos y más extremos.

En este capítulo presentamos la respuesta fisiológica de los pólipos de la medusa *Aurelia aurita*, a una MHW simulada. Los pólipos se obtuvieron a partir de medusas recolectadas al sur de Inglaterra, aunque este escifozoo es casi cosmopolita. Hemos medido las tasas metabólicas, el crecimiento, la reproducción y la supervivencia. Las tasas metabólicas se determinaron midiendo la respiración fisiológica con optodos y el sistema de transporte de electrones respiratorio (ETS). Nuestros resultados revelan que estos pólipos de *A. aurita* pueden resistir MHW extremos de hasta 20 días de duración.

Capítulo 4

Muchos científicos afirman que las medusas jugarán un papel clave en los océanos del futuro. En este capítulo estudiaremos la fisiología de la metamorfosis de *A. aurita*, una especie de medusa que cuenta con un ciclo de vida trifásico. Durante la metamorfosis, muchos animales son vulnerables y dependen de la estabilidad ambiental. Para evaluar el metabolismo, se midieron el consumo de O_2 respiratorio (R), la excreción de NH_4^+, la actividad enzimática del sistema respiratorio de transporte de electrones (ETS, Φ), la isocitrato deshidrogenasa (IDH) y la glutamato deshidrogenasa (GDH), durante las transiciones de fase de vida de la medusa *A. aurita*, de pólipo bentónico hasta medusa juvenil. Además, se determinaron los componentes bioquímicos (proteínas, lípidos y carbohidratos). Los hallazgos mostraron que todas las tasas respiratorias y excretoras varían a lo largo de las etapas de la vida. Los pólipos bentónicos y estróbilas presentaron tasas metabólicas más altas. Las estróbilas, acumularon reservas de energía, que fueron disminuyendo hacia el proceso de la estrobilación. La baja relación R/Φ (0,23 ± 0,09) se mantuvo constante durante la metamorfosis, lo que podría indicar que *A. aurita* presenta una gran flexibilidad para cambiar su metabolismo durante su metamorfosis bajo condiciones ambientales variables.

6.4. Conclusiones

Capítulo 2

(1) Se han observado cambios en las tasas de respiración (consumo de O_2 y producción de CO_2) debido al efecto nutricional sobre el estado fisiológico. Las bacterias con una concentración de sustrato limitado, tienen un RQ más alto que cuando crecen en condiciones de suficiencia nutricional. Esto sugiere que las condiciones nutricionales de las bacterias deberían tenerse en cuenta si se quiere emplear un RQ.

(2) Si la variabilidad de RQ se encuentra en otros niveles tróficos, entonces los modelos de ecosistemas, los cálculos del flujo de carbono oceánico y las predicciones del equilibrio entre la autotrofía y la heterotrofía oceánica podrían verse afectados.

Capítulo 3

(1) Se encontró una relación $R/\Phi \geq 1$ durante la ola de calor marina (MHW), pero disminuye hasta alcanzar las condiciones de control en el periodo de recuperación, lo que sugiere que los pólipos de *A. aurita* pudieron controlar su metabolismo en una MHW de 20 días.

(2) Sin embargo, $R/\Phi > 1$ no sigue la teoría constitutiva del método respiratorio ETS. Se necesitan investigaciones futuras para encontrar posibles explicaciones de cómo las tasas de respiración fisiológica pueden exceder la actividad enzimática del ETS.

(3) Los pólipos de *A. aurita* disminuyeron en tamaño pero representaron mayor reproducción asexual que en el grupo control. Durante la MHW el 100% de los pólipos de *A. aurita* sobrevivieron Por lo que

los pólipos de *A. aurita* en un escenario futuro con un aumento de MHW, podrían no verse afectados.

Capítulo 4

(1) En este último capítulo hemos identificado cambios metabólicos y bioquímicos, asociados a la respiración en *A. aurita*, durante las transiciones entre fases de vida. Las tasas más altas se han observado en las etapas de la vida bentónica. Se ha podido observar que la estróbila, acumula reservas de energía, que luego disminuyen hacia la estrobilación.

(2) La actividad respiratoria y la excretora presentaron caídas rápidas durante los últimos días de la metamorfosis de la estróbila. Por lo tanto, las últimas etapas de la estróbila y las primeras éfiras podrían ser más vulnerables a la variabilidad ambiental.

(3) El ratio R/Φ (0.2), se mostró estable durante las transiciones de vida, de pólipo a éfira y de éfira a medusa. Lo cual podría indicar que la metamorfosis en *A. aurita* es un proceso metabólico consolidado, si las condiciones ambientales son estables. Esta estabilidad en el ratio R/Φ, se ha visto anteriormente en pólipos de *A. aurita* bajo diferentes regímenes comida e inanición (Purcell et al., 2019). Por lo tanto, el ratio R/Φ parece ser un buen indicador de respiración para *A.aurita* durante la metamorfosis.

6.5. Bibliografía

Aguiar-González, B., Packard, T. T., Berdalet, E., Roy, S., and Gómez, M., 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, 412, 1-12.

Arístegui, J., and Montero, M. F., 1995. The relationship between community respiration and ETS activity in the ocean. Journal of plankton research, 17(7), 1563-1571.

Ates, R.M.L., 2017. Benthic scavengers and predators of jellyfish, material for a review. Plankt. Benthos Res. 12, 71–77. https://doi.org/10.3800/PBR.12.71.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., and Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Marine ecology progress series, 257-263.

Baltar, F., Bayer, B., Bednarsek, N., Deppeler, S., Escribano, R., Gonzalez, C. E., Hansman, R., Mishra, R. K., Moran, M. A., Repeta, D. J., Robinson, C., Sintes, E., Tamburini, C., Valentin, L. E and Herndl, G. J., 2019. Towards integrating evolution, metabolism, and climate change studies of marine ecosystems. Trends in ecology and evolution, 34(11), 1022-1033.

Båmstedt, U., 1979. Seasonal variation in the respiratory rate and ETS activity of deep-water zooplankton from the Swedish west coast. In Cyclic phenomena in marine plants and animals (pp. 267-274). Pergamon.

Becerra, A., 2021. The Semi-Enzymatic Origin of Metabolic Pathways: Inferring a Very Early Stage of the Evolution of Life. J. Mol. Evol. 2021 893 89, 183–188. https://doi.org/10.1007/S00239-021-09994-0.

Behrends, G., and Schneider, G., 1995. Impact of *Aurelia aurita* medusae (Cnidaria, Scyphozoa) on the standing stock and community composition of mesozooplankton in the Kiel Bight (western Baltic Sea). Marine Ecology Progress Series, 127, 39-45.

Billett, D. S. M., Bett, B. J., Jacobs, C. L., Rouse, I. P., and Wigham, B. D., 2006. Mass deposition of jellyfish in the deep Arabian Sea. Limnology and Oceanography, 51(5), 2077–2083. https://doi.org/10.4319/lo.2006.51.5.2077.

Boero, F., Brotz, L., Gibbons, M. J., Piraino, S., and Zampardi, S., 2016. 3.10 Impacts and effects of ocean warming on jellyfish. Explaining Ocean Warming: Causes, Scale, Effects and Consequences. Gland: IUCN, 213-237.

Bondyale-Juez, D. R., Packard, T. T., Viera-Rodríguez, M. A., and Gómez, M., 2017. Respiration: comparison of the Winkler technique, O_2 electrodes, O_2 optodes and the respiratory electron transport system assay. Marine Biology, 164(12), 1-11.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., and West, G. B., 2004. Toward a metabolic theory of ecology. Ecology, 85(7), 1771-1789.

Bühring SI, Lampadariou N, Moodley L, Tselepides A, Witte U., 2006. Benthic microbial and whole-community responses to different amounts of ^{13}C-enriched algae:*in situ* experiments in the deep Cretan sea (eastern Mediterranean). Limnol Oceanogr 51: 157−165.

Bridge, D., Cunningham, C. W., Schierwater, B., DeSalle, R., Buss, L. W., 1992. Class-level relationships in the phylum Cnidaria: evidence from mitochondrial genome structure. Natl. Acad Sci. 89, 8750–8753.

Brocks, J.J., Jarrett, A.J.M., Sirantoine, E., Hallmann, C., Hoshino, Y., Liyanage, T., 2017. The rise of algae in Cryogenian oceans and the emergence of animals. Nat. 2017 5487669 548, 578–581. https://doi.org/10.1038/nature23457.

Canfield, D.E., Rosing, M.T., Bjerrum, C., 2006. Early anaerobic metabolisms. https://doi.org/10.1098/rstb.2006.1906.

Canfield, D.E., Zuilen, M.A. van, Nabhan, S., Bjerrum, C.J., Zhang, S., Wang, H., Wang, X., 2021. Petrographic carbon in ancient sediments constrains Proterozoic Era atmospheric oxygen levels. Proc. Natl. Acad. Sci. 118. https://doi.org/10.1073/PNAS.2101544118.

Carlson, C. A., Del Giorgio, P. A., and Herndl, G. J., 2007. Microbes and the dissipation of energy and respiration: from cells to ecosystems. Oceanography, 20(2), 89-100.

Cartwright, P., Halgedahl, S. L., Hendricks, J. R., Jarrard, R. D., Marques, A. C., Collins, A. G., and Lieberman, B. S., 2007. Exceptionally preserved jellyfishes from the Middle Cambrian. PloS one, 2(10), e1121.

Chance, B., and Williams, G. R., 1954. Kinetics of cytochrome b5 in rat liver microsomes. Journal of Biological Chemistry, 209(2), 945-951.

Chance, B., and Williams, G. R., 1956. The respiratory chain and oxidative phosphorylation. Adv Enzymol Relat Areas Mol Biol, 17, 65-134.

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

Chi, X., Dierking, J., Hoving, H.-J., Lüskow, F., Denda, A., Christiansen, B., Sommer, U., Hansen, T., Javidpour, J., 2021. Tackling the jelly web: Trophic ecology of gelatinous zooplankton in oceanic food webs of the eastern tropical Atlantic assessed by stable isotope analysis. Limnol. Oceanogr. 66, 289–305. https://doi.org/10.1002/LNO.11605.

Choy, C.A., Haddock, S.H.D., Robison, B.H., 2017. Deep pelagic food web structure as revealed by in situ feeding observations. Proceedings. Biol. Sci. 284. https://doi.org/10.1098/rspb.2017.2116.

Christensen, J. P., Owens, T. G., Devol, A. H., and Packard, T. T., 1980. Respiration and physiological state in marine bacteria. Marine Biology, 55(4), 267-276.

Cloud, P., Glaessner, M.F., 1982. The Ediacarian Period and System: Metazoa Inherit the Earth, science.sciencemag.org.

Condon, R.H., Steinberg, D.K., Del Giorgio, P.A., Bouvier, T.C., Bronk, D.A., Graham, W.M., Ducklow, H.W., Karl, D.M., Analyzed, H.W.D., 2011. Jellyfish blooms result in a major microbial respiratory sink of carbon in marine systems. Natl. Acad Sci. 108, 10225–10230. https://doi.org/10.1073/pnas.1015782108.

Deamer, D., 2021. Where Did Life Begin? Testing Ideas in Prebiotic Analogue Conditions. Life 2021, Vol. 11, Page 134 11, 134. https://doi.org/10.3390/LIFE11020134.

Del Giorgio, P., and Williams, P. (Eds.). 2005. Respiration in aquatic ecosystems. OUP Oxford.

Del Giorgio, P. A., and Duarte, C. M., 2002. Respiration in the open ocean. Nature, 420(6914), 379-384.

Dos Reis, M., Thawornwattana, Y., Angelis, K., Telford, M. J., Donoghue, P. C., and Yang, Z., 2015. Uncertainty in the timing of origin of animals and the limits of precision in molecular timescales. Current biology, 25(22), 2939-2950.
Duarte, C. M., Regaudie-de-Gioux, A., Arrieta, J. M., Delgado-Huertas, A., and Agusti, S., 2013. The oligotrophic ocean is heterotrophic. Annual Review of Marine Science, 5, 551-569.
Ducklow, H. W., Purdie, D. A., Williams, P. J. L., and Davies, J. M., 1986. Bacterioplankton: a sink for carbon in a coastal marine plankton community. Science, 232(4752), 865-867.
Ducklow, H. W., and Doney, S. C., 2013. What is the metabolic state of the oligotrophic ocean? A debate. Annual Review of Marine Science, 5, 525-533.
Eckford-Soper, L.K., Canfield, D.E., 2020. The global explosion of eukaryotic algae: The potential role of phosphorus? PLoS One 15, e0234372. https://doi.org/10.1371/JOURNAL.PONE.0234372.
Falkowski, P. G., Fenchel, T., and Delong, E. F., 2008. The microbial engines that drive Earth's biogeochemical cycles. science, 320(5879), 1034-1039.
Falkowski, P. G., and Wilson, C., 1992. Phytoplankton productivity in the North Pacific ocean since 1900 and implications for absorption of anthropogenic CO 2. Nature, 358(6389), 741-743.
Fernández-Urruzola, I., Packard, T. T., and Gómez, M., 2011. GDH activity and ammonium excretion in the marine mysid, *Leptomysis lingvura*: effects of age and starvation. Journal of experimental marine biology and ecology, 409(1-2), 21-29.
Finlay, B. J., Span, A., and Ochsenbein-Gattlen, C., 1983. Influence of physiological state on indices of respiration rate in protozoa. Comparative Biochemistry and Physiology Part A: Physiology, 74(2), 211-219.
Fleury, B., Carrasco, N., Millan, M., Vettier, L., Szopa, C., 2017. Organic chemistry in a CO_2 rich early Earth atmosphere. Earth Planet. Sci. Lett. 479, 34–42. https://doi.org/10.1016/J.EPSL.2017.09.026.
Gall, M.L., Holmes, S.P., Campbell, H., Byrne, M., 2021. Effects of marine heatwave conditions across the metamorphic transition to the juvenile sea urchin (*Heliocidaris erythrogramma*). Mar. Pollut. Bull. 163, 111914. https://doi.org/10.1016/J.MARPOLBUL.2020.111914.
Goldford, J.E., Segrè, D., 2018. Modern views of ancient metabolic networks. Curr. Opin. Syst. Biol. 8, 117–124. https://doi.org/10.1016/J.COISB.2018.01.004.
González N, Anadón R, Viesca L., 2003. Carbon flux through the microbial community in a temperate sea during summer:role of bacterial metabolism. Aquat Microb Ecol 33: 117−126.
Graham, J. B., 1988. Ecological and evolutionary aspects of integumentary respiration: body size, diffusion, and the Invertebrata. American Zoologist, 28(3), 1031-1045.
Gold, D. A., Katsuki, T., Li, Y., Yan, X., Regulski, M., Ibberson, D., Holstein, T., Steele, R. E., Jacobs, D. K. and Greenspan, R. J., 2019. The genome of the jellyfish *Aurelia* and the evolution of animal complexity. Nature ecology and evolution, 3(1), 96-104.
Gómez, M., Torres, S., and Hernández-León, S., 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. South African Journal of Marine Science, 17(1), 15-20.
Han, J., Zhang, X., Komiya, T., 2016. Integrated Evolution of Cnidarians and Oceanic Geochemistry Before and During the Cambrian Explosion. Cnidaria, past, Present Futur. World Medusa her Sisters 15–29. https://doi.org/10.1007/978-3-319-31305-4_2.
Harvey, H. W., 1956. The Chemistry and Fertility of Sea Waters, HW Harvey, Sc. D., FRS Cambridge: Cambridge University Press, 1955. Journal of the Marine Biological Association of the United Kingdom, 35(1), 289-289.
Harvey, H.W., 1928. Biological Chemistry and Physics of Sea Water. Cambridge University Press.
Hernández-León, S., and Gómez, M., 1996. Factors affecting the respiration/ETS ratio in marine zooplankton. Journal of Plankton Research, 18(2), 239-255.
Herrera, A., Packard, T., Santana, A., and Gómez, M., 2011. Effect of starvation and feeding on respiratory metabolism in *Leptomysis lingvura* (GO Sars, 1866). Journal of experimental marine biology and ecology, 409(1-2), 154-159.
Herrera, A., Gómez, M., Packard, T. T., and de Puelles, M. F., 2014. Zooplankton biomass and electron transport system activity around the Balearic Islands (western Mediterranean). Journal of Marine Systems, 131, 54-62.
Hayashida, H., Matear, R. J., and Strutton, P. G., 2020. Background nutrient concentration determines phytoplankton bloom response to marine heatwaves. Global change biology, 26(9), 4800-4811.
Hoegh-Guldberg, O., Bruno, J.F., 2010. The impact of climate change on the world's marine ecosystems. Science (80-.). https://doi.org/10.1126/science.1189930.
Holms, W. H., 1986. The central metabolic pathways of Escherichia coli: relationship between flux and control at a branch point, efficiency of conversion to biomass, and excretion of acetate. Current topics in cellular regulation, 28, 69-105.
Holstein, T. W., Watanabe, H., and Özbek, S., 2011. Signaling pathways and axis formation in the lower metazoa. Current topics in developmental biology, *97*, 137-177.
Hughes, T.P., Kerry, J.T., Álvarez-Noriega, M., Álvarez-Romero, J.G., Anderson, K.D., Baird, A.H., Babcock, R.C., Beger, M., Bellwood, D.R., Berkelmans, R., Bridge, T.C., Butler, I.R., Byrne, M., Cantin, N.E., Comeau, S., Connolly, S.R., Cumming, G.S., Dalton, S.J., Diaz-Pulido, G., Eakin, C.M., Figueira, W.F., Gilmour, J.P., Harrison, H.B., Heron, S.F., Hoey, A.S., Hobbs, J.-P.A., Hoogenboom, M.O., Kennedy, E. V., Kuo, C., Lough, J.M., Lowe, R.J., Liu, G., McCulloch, M.T., Malcolm, H.A., McWilliam, M.J., Pandolfi, J.M., Pears, R.J., Pratchett, M.S., Schoepf, V., Simpson, T., Skirving, W.J., Sommer, B., Torda, G., Wachenfeld, D.R., Willis, B.L., Wilson, S.K., 2017. Global warming and recurrent mass bleaching of corals. Nat. 2017 5437645 543, 373–377. https://doi.org/10.1038/nature21707
Kasting, J.F., 1993. Earth's early atmosphere. Science (80-.). 259, 920–926. https://doi.org/10.1126/SCIENCE.11536547
Keilin, D., 1966. The history of cell respiration and cytochrome. CUP Archive.
King, F. D., and Packard, T. T., 1975. Respiration and the activity of the respiratory electron transport system in marine zooplankton 1. Limnology and Oceanography, 20(5), 849-854.

Kleiber, M., 1961. The fire of life. An introduction to animal energetics. The fire of life. An introduction to animal energetics.
Kleiber, M., 1932. Body size and metabolism. Hilgardia, 6(11), 315-353.
Knoll, A. H., 2011. The multiple origins of complex multicellularity. Annual Review of Earth and Planetary Sciences, 39, 217- 239.
Kolokotrones, T., Savage, V., Deeds, E. J., and Fontana, W., 2010. Curvature in metabolic scaling. Nature, 464(7289), 753-756.
Kraus, J.E., Fredman, D., Wang, W., Khalturin, K., Technau, U., 2015. Adoption of conserved developmental genes in development and origin of the medusa body plan. Evodevo 6, 23. https://doi.org/10.1186/s13227-015-0017-3.
Krause, A.J., Mills, B.J.W., Zhang, S., Planavsky, N.J., Lenton, T.M., Poulton, S.W., 2018. Stepwise oxygenation of the Paleozoic atmosphere. Nat. Commun. 2018 91 9, 1–10. https://doi.org/10.1038/s41467-018-06383-y.
Lamb, P. D., E. Hunter, J. K. Pinnegar, S. Creer, R. G. Davies, and M. I. Taylor, 2017. Jellyfish on the menu: MtDNA assay reveals scyphozoan predation in the Irish sea. Royal Society Open Science 4:
Lebrato, M., Molinero, J.C., Cartes, J.E., Lloris, D., Mélin, F., Beni-Casadella, L., 2013. Sinking jelly-carbon unveils potential environmental variability along a continental margin. PLoS One 8. https://doi.org/10.1371/journal.pone.0082070.
Lebrato, M., Pitt, K.A., Sweetman, A.K., Jones, D.O.B., Cartes, J.E., Oschlies, A., Condon, R.H., Molinero, J.C., Adler, L., Gaillard, C., Lloris, D., Billett, D.S.M., 2012. Jelly-falls historic and recent observations: A review to drive future research directions. Hydrobiologia. https://doi.org/10.1007/s10750-012-1046-8.
Lebrato, M., Pahlow, M., Frost, J. R., Küter, M., de Jesus Mendes, P., Molinero, J. C., and Oschlies, A., 2019. Sinking of gelatinous zooplankton biomass increases deep carbon transfer efficiency globally. Global Biogeochemical Cycles, 33(12), 1764-1783.
Leung, J.Y.S., Russell, B.D., Connell, S.D., 2019. Adaptive Responses of Marine Gastropods to Heatwaves. One Earth 1, 374–381. https://doi.org/10.1016/J.ONEEAR.2019.10.025.
López-Urrutia, Á., San Martin, E., Harris, R. P., and Irigoien, X., 2006. Scaling the metabolic balance of the oceans. Proceedings of the National Academy of Sciences, 103(23), 8739-8744.
Luo, J. Y., Condon, R. H., Stock, C. A., Duarte, C. M., Lucas, C. H., Pitt, K. A., and Cowen, R. K., 2020. Gelatinous zooplankton-mediated carbon flows in the global oceans: a data-driven modeling study. Global Biogeochemical Cycles, 34(9), e2020GB006704.
Longhurst, A. R., 2010. Ecological geography of the sea. Elsevier.
Lynam, C. P., M. J. Gibbons, B. E. Axelsen, C. A. J. Sparks, J. Coetzee, B. G. Heywood, and A. S. Brierley, 2007. Jellyfish overtake fish in a heavily fished ecosystem. Current Biology 16: R492.
Maldonado, F., Packard, T. T., and Gómez, M., 2012. Understanding tetrazolium reduction and the importance of substrates in measuring respiratory electron transport activity. Journal of Experimental Marine Biology and Ecology, 434, 110-118.
Martínez, I., Gómez, M., and Packard, T. T., 2010. Potential respiration is a better respiratory predictor than biomass in young *Artemia salina*. Journal of experimental marine biology and ecology, 390(2), 78-83.
Mahler, H. R., and Cordes, E. H., 1971. Biological chemistry.
Manchester, L.C., Coto-Montes, A., Boga, J.A., Andersen, L.P.H., Zhou, Z., Galano, A., Vriend, J., Tan, D.-X., Reiter, R.J., 2015. Melatonin: an ancient molecule that makes oxygen metabolically tolerable. J. Pineal Res. 59, 403–419. https://doi.org/10.1111/JPI.12267.
Margulis, L., and Sagan, D., 1986. Microcosmos. University of California Press.
Marlow, H., and Arendt, D., 2014. Evolution: ctenophore genomes and the origin of neurons. Current Biology, 24(16), R757-R761.
Marques, A.C., Collins, A.G., 2005. Cladistic analysis of Medusozoa and cnidarian evolution. Invertebr. Biol. 123, 23–42. https://doi.org/10.1111/j.1744-7410.2004.tb00139.x.
McCauley, D.J., Pinsky, M.L., Palumbi, S.R., Estes, J.A., Joyce, F.H., Warner, R.R., 2015. Marine defaunation: Animal loss in the global ocean. Science (80-.). https://doi.org/10.1126/science.1255641.
Michaelis, L., and Menten, M. L., 1913. Die Kinetik der Invertinwirkung. Biochem. z, 49(333-369), 352.
Mitchell, P., 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature, 191(4784), 144-148.
Mitterwallner, V., Suci, A.N.N., Zamani, N., Lenz, M., 2021. Exposure to moderately elevated temperatures changes food preferences in the tropical marine herbivore *Haliotis squamata*. Mar. Biol. 2021 1687 168, 1–12. https://doi.org/10.1007/S00227-021-03922-Y.
Mojzsis, S.J., Harrison, T.M., Pidgeon, R.T., 2001. Oxygen-isotope evidence from ancient zircons for liquid water at the Earth's surface 4,300 Myr ago. Nat. 2001 4096817 409, 178–181. https://doi.org/10.1038/35051557.
Mori, S., Yamaji, K., Ishida, A., Prokushkin, S. G., Masyagina, O. V., Hagihara, A., and Umari, M., 2010. Mixed-power scaling of whole-plant respiration from seedlings to giant trees. Proceedings of the National Academy of Sciences, 107(4), 1447-1451.
Moroz, L. L., 2021. Multiple origins of neurons from secretory cells. Frontiers in Cell and Developmental Biology, 9.
Narbonne, G.M., 2005. The Ediacara biota: Neoproterozoic origin of animals and their ecosystems. Annu. Rev. Earth Planet. Sci. 33, 421–442. https://doi.org/10.1146/ANNUREV.EARTH.33.092203.122519.
Nelson, D.L., Cox, M.M., 2008. Lehninger principles of biochemistry, Fifth Edition. W.H. Freeman and Company.
Noda-Garcia, L., Liebermeister, W., Tawfik, D.S., 2018. Metabolite-Enzyme Coevolution: From Single Enzymes to Metabolic Pathways and Networks. Annu. Rev. Biochem. 87, 187–216. https://doi.org/10.1146/ANNUREV-BIOCHEM-062917-012023.

Ochoa, S.,1943. Efficiency of aerobic phosphorylation in cell-free heart extracts. Journal of Biological Chemistry, 151(2), 493-505.

Olejarz, J., Iwasa, Y., Knoll, A.H., Nowak, M.A., 2021. The Great Oxygenation Event as a consequence of ecological dynamics modulated by planetary change. Nat. Commun. 2021 121 12, 1–9. https://doi.org/10.1038/s41467-021-23286-7.

Osma, N., Fernández-Urruzola, I., Gómez, M., Montesdeoca-Esponda, S., and Packard, T. T., 2016. Predicting in vivo oxygen consumption rate from ETS activity and bisubstrate enzyme kinetics in cultured marine zooplankton. Marine Biology, 163(7), 1-14.

Owens, T. G., and King, F. D., 1975. The measurement of respiratory electron-transport-system activity in marine zooplankton. Marine Biology, 30(1), 27-36.

Ozaki, K., Reinhard, C.T., 2021. The future lifespan of Earth's oxygenated atmosphere. https://doi.org/10.1038/s41561-021-00693-5.

Packard, T. T., 1985. Measurement of electron transport activity of microplankton. Advances in aquatic microbiology, 3, 207-261.

Packard, T. T., and Gómez, M., 2008. Exploring a first-principles-based model for zooplankton respiration. ICES Journal of Marine Science, 65(3), 371-378.

Packard, T. T., 1971. The Measurement of Respiratory Electron-transport Activity in Marine Phytoplankton.

Packard, T., Berdalet, E., Blasco, D., Roy, S. O., St-Amand, L., Lagacé, B.,Lee, K., and Gagne, J. P., 1996. CO_2 production predicted from isocitrate dehydrogenase activity and bisubstrate enzyme kinetics in the marine bacterium *Pseudomonas nautica*. Aquatic Microbial Ecology, 11(1), 11-19.

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

Pauly, D., Graham, W., Libralato, S., Morissette, L., Deng Palomares, M.L., 2009. Jellyfish in ecosystems, online databases, and ecosystem models. Hydrobiologia 616, 67–85. https://doi.org/10.1007/s10750-008-9583-x.

Perez, T., Garrabou, J., Sartoretto, S., Harmelin, J. G., Francour, P., and Vacelet, J., 2000. Massive mortality of marine invertebrates: an unprecedented event in northwestern Mediterranean. Comptes rendus de l'Academie des sciences. Serie III, Sciences de la vie, 323(10), 853-865.

Pincebourde, S., Sanford, E., Helmuth, B., 2009. An intertidal sea star adjusts thermal inertia to avoid extreme body temperatures. Am. Nat. 174, 890–897. https://doi.org/10.1086/648065.

Pitt, K.A., Duarte, C.M., Lucas, C.H., Sutherland, K.R., Condon, R.H., Mianzan, H., Purcell, J.E., Robinson, K.L., Uye, S.I., 2013. Jellyfish Body Plans Provide Allometric Advantages beyond Low Carbon Content. PLoS One 8, 1–10. https://doi.org/10.1371/journal.pone.0072683.

Pitt, K. A., Welsh, D. T., and Condon, R. H., 2009. Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production. Hydrobiologia, 616(1), 133-149.

Planavsky, N.J., Asael, D., Hofmann, A., Reinhard, C.T., Lalonde, S. V., Knudsen, A., Wang, X., Ossa Ossa, F., Pecoits, E., Smith, A.J.B., Beukes, N.J., Bekker, A., Johnson, T.M., Konhauser, K.O., Lyons, T.W., Rouxel, O.J., 2014. Evidence for oxygenic photosynthesis half a billion years before the Great Oxidation Event. Nat. Geosci. 2014 74 7, 283–286. https://doi.org/10.1038/ngeo2122.

Planavsky, N. J., Crowe, S. A., Fakhraee, M., Beaty, B., Reinhard, C. T., Mills, B. J., Holstege, C. and Konhauser, K. O., 2021. Evolution of the structure and impact of Earth's biosphere. Nature Reviews Earth and Environment, 2(2), 123-139.

Pomeroy, L. R., 1974. The ocean's food web, a changing paradigm. Bioscience, 24(9), 499-504.

Purcell, J. E., Bondyale-Juez, D. R., Romero-Kutzner, V., Martínez, I., Caprioli, R., Tames-Espinosa, M., Almunia, J., Alonso, E., Packard, T.T., and Gómez, M., 2019. Food supply effects on the asexual reproduction and respiratory metabolism of *Aurelia aurita* polyps. Hydrobiologia, 846(1), 135-146.

Redfield, A. C., Ketchum, B. H., and Richards, F. A., 1963. The influence of organisms on the composition of seawater. The sea, 2, 26-77.

Reich, P. B., Hungate, B. A., and Luo, Y., 2006. Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. Annu. Rev. Ecol. Evol. Syst., 37, 611-636.

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

Richards, F. A., 1957. Oxygen in the ocean. Treatise on Marine Ecology and Paleoecology, edited by: Hedgpeth, JW, 1, 185-238.

Richardson, A.J., Bakun, A., Hays, G.C., Gibbons, M.J., 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2009.01.010.

Riley, G. A., 1951. Oxygen, phosphate, and nitrate in the Atlantic Ocean. Bull. Bingham Oceanogr. Coll., 13(1), 1-124.

Robinson, C., and Williams, P. L. B., 2005. Respiration and its measurement in surface marine waters. Respiration in aquatic ecosystems, 147-180.

Robinson, C., 2019. Microbial Respiration, the Engine of Ocean Deoxygenation. Front. Mar. Sci. 0, 533. https://doi.org/10.3389/FMARS.2018.00533.

Romero-Kutzner, V., Packard, T. T., Berdalet, E., Roy, S. O., Gagné, J. P., and Gómez, M., 2015. Respiration quotient variability: bacterial evidence. Marine Ecology Progress Series, 519, 47-59.

Roy, S., and Packard, T., 2001. CO_2 production rate predicted from isocitrate dehydrogenase activity, intracellular substrate concentrations and kinetic constants in the marine bacterium *Pseudomonas nautica*. Marine Biology, 138(6), 1251-1258.

Savenkoff, C., Packard, T. T., Rodier, M., Gérinno, M., Lefèvre, D., and Denis, M., 1995. Relative confribution of dehydrogenases to overall respiratory ETh activity in some marine organisms. Journal of plankton research, 17(8), 1593-1604.

Scanes, E., Parker, L., O'Connor, W., Dove, M., Ross, P. M., 2020. Heatwaves alter survival of the Sydney rock oyster, Saccostrea glomerata. Marine pollution bulletin, 158, 111389.

Scanes, E., Scanes, P., communications, Ross, P.M., 2020. Climate change rapidly warms and acidifies Australian estuaries. Nature communications, 11(1), 1-11.

Shanks, A.L., Rasmuson, L.K., Valley, J.R., Jarvis, M.A., Salant, C., Sutherland, D.A., Lamont, E.I., Hainey, M.A.H., Emlet, R.B., 2020. Marine heat waves, climate change, and failed spawning by coastal invertebrates. Limnol. Oceanogr. 65, 627–636. https://doi.org/10.1002/LNO.11331.

Siekevitz, P., 1957. Powerhouse of the cell. Scientific American, 197(1), 131-144.

Smale, D.A., Wernberg, T., Oliver, E.C.J., Thomsen, M., Harvey, B.P., Straub, S.C., Burrows, M.T., Alexander, L. V., Benthuysen, J.A., Donat, M.G., Feng, M., Hobday, A.J., Holbrook, N.J., Perkins-Kirkpatrick, S.E., Scannell, H.A., Sen Gupta, A., Payne, B.L., Moore, P.J., 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat. Clim. Chang. 1. https://doi.org/10.1038/s41558-019-0412-1.

Steinmetz, P. R., Kraus, J. E., Larroux, C., Hammel, J. U., Amon-Hassenzahl, A., Houliston, E., Wörheide, G., Nickel, M., Degnan, Bernard and Technau, U. (2012). Independent evolution of striated muscles in cnidarians and bilaterians. Nature, 487(7406), 231-234.

Suryan, R.M., Arimitsu, M.L., Coletti, H.A., Hopcroft, R.R., Lindeberg, M.R., Barbeaux, S.J., Batten, S.D., Burt, W.J., Bishop, M.A., Bodkin, J.L., Brenner, R., Campbell, R.W., Cushing, D.A., Danielson, S.L., Dorn, M.W., Drummond, B., Esler, D., Gelatt, T., Hanselman, D.H., Hatch, S.A., Haught, S., Holderied, K., Iken, K., Irons, D.B., Kettle, A.B., Kimmel, D.G., Konar, B., Kuletz, K.J., Laurel, B.J., Maniscalco, J.M., Matkin, C., McKinstry, C.A.E., Monson, D.H., Moran, J.R., Olsen, D., Palsson, W.A., Pegau, W.S., Piatt, J.F., Rogers, L.A., Rojek, N.A., Schaefer, A., Spies, I.B., Straley, J.M., Strom, S.L., Sweeney, K.L., Szymkowiak, M., Weitzman, B.P., Yasumiishi, E.M., Zador, S.G., 2021. Ecosystem response persists after a prolonged marine heatwave. Sci. Reports 2021 111 11, 1–17. https://doi.org/10.1038/s41598-021-83818-5.

Sverdrup, H. U., Johnson, M. W., and Fleming, R. H., 1942. The Oceans: Their physics, chemistry, and general biology (Vol. 1087). New York: Prentice-Hall.

Takao, M., Okawachi, H., Uye, S.I., Okawachi, H., 2014. Natural predators of polyps of *Aurelia aurita* s.l. (Cnidaria: Scyphozoa: Semaeostomeae) and their predation rates. Plankt. Benthos Res. 9, 105–113. https://doi.org/10.3800/pbr.9.105.

Tames-Espinosa, M., Martínez, I., Romero-Kutzner, V., Bondyale-Juez, D. R., Packard, T. T., and Gomez, M., 2018. $NADP^+$-dependent isocitrate dehydrogenase activity in marine plankton. Marine Chemistry, 204, 86-94.

Thuesen, E. V., 2005. Intragel oxygen promotes hypoxia tolerance of scyphomedusae. J. Exp. Biol. 208, 2475–2482. https://doi.org/10.1242/jeb.01655.

Tinta, T., Klun, K., and Herndl, G. J., 2021. The importance of jellyfish–microbe interactions for biogeochemical cycles in the ocean. Limnology and Oceanography, 66(5), 2011-2032.

Williams, P. J. L., Flows of Energy and Materials in Marine Ecosystems: 271, 1984.

Vosjan, J. H.,1982. Respiratory electron transport system activities in marine environments. Hydrobiological Bulletin, 16(1), 61-68.

Vaquer-Sunyer, R., and Duarte, C. M., 2008. Thresholds of hypoxia for marine biodiversity. Proceedings of the National Academy of Sciences, 105(40), 15452-15457.

Vernadsky V., 1926. The Biosphere. Trans. Langmuir, D McMenamin, Springer, New York.

Walsh, K., and Koshland, D. E., 1984. Determination of flux through the branch point of two metabolic cycles. The tricarboxylic acid cycle and the glyoxylate shunt. Journal of Biological Chemistry, 259(15), 9646-9654.

Whelan, N. V., Kocot, K. M., Moroz, T. P., Mukherjee, K., Williams, P., Paulay, G., Moroz, L. and Halanych, K. M., 2017. Ctenophore relationships and their placement as the sister group to all other animals. Nature ecology and evolution, 1(11), 1737-1746.

Whicher, A., Camprubi, E., Pinna, S., Herschy, B., Lane, N., 2018. Acetyl Phosphate as a Primordial Energy Currency at the Origin of Life. Orig. Life Evol. Biosph. 2018 482 48, 159–179. https://doi.org/10.1007/S11084-018-9555-8

Wignall, P. B., and Twitchett, R. J., 1996. Oceanic anoxia and the end Permian mass extinction. Science, 272(5265), 1155-1158.

Zimorski, V., Mentel, M., Tielens, A.G.M., Martin, W.F., 2019. Energy metabolism in anaerobic eukaryotes and Earth's late oxygenation. Free Radic. Biol. Med. 140, 279–294. https://doi.org/10.1016/J.FREERADBIOMED.2019.03.030.

APPENDIX A.

SCIENTIFIC CONTRIBUTIONS

a. Publications:

1. **Title**: Ingestion of polyethylene microspheres occur only in presence of prey in the jellyfish *Aurelia aurita*
   Authors: **Vanesa Romero-Kutzner**, Javier Tarí, Alicia Herrera, Ico Martínez, Daniel R. Bondyale-Juez and May Gómez
   Journal: Marine Pollution Bulletin
   Year: 2022

2. **Title:** Wind drifting vs. pulsating swimming jellyfish: Respiratory metabolism and composition differences in *Physalia physalis*, *Velella vellela*, *Aurelia aurita*, and *Pelagia noctiluca*
   Authors: Daniel R. Bondyale-Juez, **Vanesa Romero-Kutzner**, Jennifer E. Purcell, Ico Martínez, Theodore T. Packard, May Gómez
   Journal: Frontiers in Marine Science – Aquatic Physiology
   Year: 2022

3. **Title**: Ocean acidification induces distinct metabolic responses in subtropical zooplankton under oligotrophic conditions and after simulated upwelling
   Authors: Natalia Osma, Cristian A. Vargas, **Vanesa Romero-Kutzner**, Theodore T. Packard, May Gómez, Ulf Riebesell, Jan Taucher, Lennart Bach, Andrea Ludwig, and Igor Fernández-Urruzola
   Journal: Science of the Total Environment
   Year: 2022

4. **Title**: Metabolic responses of subtropical microplankton after a simulated deep-water upwelling event suggest a possible dominance of mixotrophy under increasing CO_2 levels
   Authors: Mayte Tames-Espinosa, Ico Martínez, **Vanesa Romero-Kutzner**, Josep Coca, María Algueró-Muñiz, Henriette G Horn, Andrea Ludwig, Jan Taucher, Lennart Bach, Ulf Riebesell, Theodore T. Packard and May Gómez
   Journal: Frontiers in Marine Science
   Year: 2020

5. **Title**: La práctica de juegos cooperativos y educativos en grupos disruptivos de Biología y Geología de 1º de la ESO
   Authors: **Vanesa Romero-Kutzner**, May Gómez, Antonio Ramón Ricarte-Sabater
   Book: Tendencias Metodológicas en Innovación Educativa (ISBN: 978-84-9042-372-1)
   Year: 2020

6. **Title**: Protein in marine plankton: a comparison of spectrophotometric methods.
   Authors: Ico Martínez, Alicia Herrera, Mayte Tames-Espinosa, Daniel R. Bondyale-Juez, **Vanesa Romero-Kutzner**, Theodore T. Packard and May Gómez
   Journal: Journal of Experimental Marine Biology and Ecology
   Year: 2020

7. **Title**: Food supply effects on the asexual reproduction and respiratory metabolism of *A. aurita* polyps
   Authors: Jennifer E. Purcell, Daniel R. Bondyale-Juez, **Vanesa Romero-Kutzner**, Ico Martínez, Rosa Caprioli, Mayte Tames-Espinosa, Javier Almunia, Ester Alonso, Theodore T. Packard and May Gómez
   Journal: Hydrobiologia
   Year: 2019

8. **Title**: $NADP^+$- dependent isocitrate dehydrogenase activity in marine plankton
   Authors: Mayte Tames-Espinosa, Ico Martínez, **Vanesa Romero-Kutzner**, Daniel R. Bondyale-Juez, Theodore T. Packard, May Gómez
   Journal: Marine Chemistry
   Year: 2018

b. Conferences (oral communication):

Title: Metabolism in Jellyfish: An enzymatic approach
Authors: Vanesa Romero-Kutzner, Daniel R. Bondyale Juez, Jennifer E. Purcell, Mayte Tames-Espinosa, Ico Martínez, Rosa Caprioli, Javier Almunia, Ester Alonso, Theodore T. Packard and May Gómez
Conference: 2018 Ocean Sciences Meeting**.** Portland (USA), 11-16 February 2018

Title: *Aurelia aurita* polyp response to simulated Marine Heatwaves
Authors: Vanesa Romero-Kutzner, Daniel R. Bondyale Juez, Theodore T. Packard, May Gómez, Cathy Lucas, Kathryn Cook and Dan Mayor
Conference: 6th International Jellyfish Bloom Symposium, Cape Town (South Africa), 3-6 November 2019