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CHANGES IN DISSOLVED OXYGEN DUE TO ANTHROPOGENIC DISTURBANCES AND CONSEQUENCES FOR COASTAL MARINE LIFE

TESI DOCTORAL

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March 2011

Memòria presentada per Raquel Vaquer Sunyer per optar al títol de Doctora en el Programa de Ciències Marines organitzat per la Universitat de les Illes Balears (UIB), departament de Biologia.

Universitat de les Illes Balears

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Departament de Biologia, UIB
Àrea de coneixement: Ecologia marina, canvi global.
Paraules clau: Canvi global, oxigen dissolt, escalfament global, pertorbacions antropogèniques, metabolisme, organismes bentònics, eutrofització
Keywords: Global change, dissolved oxygen, global warming, anthropogenic disturbances, metabolism, benthic organisms, eutrophication
Palabras clave: cambio global, oxígeno disuelto, calentamiento global, perturbaciones antropogénicas, metabolismo, organismos bentónicos, eutrofización
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Data de defensa de la tesi doctoral: 20 de maig de 2011

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Abstract

Increased anthropogenic pressures to coastal ecosystems in the last Century are threatening coastal ecosystems, their biodiversity and ecosystems functioning. The two main stressors affecting coastal systems are increases in nutrients loadings and global warming. How coastal ecosystems will response to the combined effects of these two pressures remain uncertain. In this Ph.D. dissertation I explore the consequences of global warming on planktonic and benthic metabolism and on oxygen dynamics. I also explore the responses of benthic communities to the main consequence of eutrophication, oxygen depletion, and the environmental modulation of the responses of benthic organisms to hypoxia. Results confirm a steeper increase in respiration rates than in production rates with warming in experimental systems, whereas no differences were found between the responses of these metabolic rates to temperature, within the current thermal range, in a natural system. Results suggest an increase in the likelihood of hypoxia with warming. We also show that hypoxia thresholds vary greatly across marine benthic organisms and that the conventional definition of 2 mg O_2/liter to designate waters as hypoxic is below the empirical sublethal and lethal oxygen thresholds for half of the species tested, and explore the environmental modulation of these thresholds.

All studied processes and results obtained within this work reveal, in summary, that anthropogenic disturbances are significantly affecting coastal metabolism, and therefore, oxygen dynamics, leading to oxygen declines due to the combined effects of eutrophication and warming, threatening coastal biodiversity and ecosystems functioning.

Resum

L'increment de les pressions antropogèniques als ecosistemes costaners durant el darrer segle estan posant en perill la seva biodiversitat i el seu funcionament. L'increment en l'aport de nutrients a les costes i l'escalfament global són les dues pressions més importants que afecten als sistemes costaners. Cóm respondran aquests sistemes a l'efecte combinat d'aquestes dues pressions és incert. En aquesta tesi doctoral exploro les conseqüències de l'escalfament global en el metabolisme de les comunitats planctòniques i bentòniques i en les dinàmiques d'oxigen. També exploro les respostes de les comunitats bentòniques a la major conseqüència de l'eutrofització, la disminució de la concentració d'oxigen dissolt, i la modulació ambiental de les respostes dels organismes bentònics a l hipòxia. Els resultats confirmen un major increment en les taxes respiratòries que en les de producció amb l'escalfament en sistemes experimentals, mentre no es troben diferències entre les respostes d'aquestes taxes metabòliques a la temperatura, dins el seu rang tèrmic actual, en sistemes naturals. Els resultats suggereixen que l'escalfament global augmentarà la probabilitat d'episodis hipòxics. També mostram que els llindars d'hipòxia varien àmpliament en els diferents organismes bentònics marins i que la definició tradicional d'hipòxia de 2 mg O_2/litre per designar les aigües com hipòxiques està per davall dels llindars de les concentracions letals i subletals empíriques per a la meitat de les espècies testades. També exploram la modulació ambiental d'aquests llindars.

Tots els processos estudiats i els resultats obtinguts en aquest treball revelen, en resum, que les pertorbacions antropogèniques afecten significativament el metabolisme de les comunitats costaneres i, per tant, les dinàmiques d'oxigen, produint una disminució en la concentració d'oxigen a causa dels efectes combinats de l'eutrofització i l'escalfament, posant en perill la biodiversitat costanera i el funcionament dels ecosistemes.

General Introduction

The global coastal ocean plays a significant role in the global biogeochemical cycles of carbon, nitrogen, oxygen and nutrient elements (Rabouille et al., 2001). The coastal zone, with a surface area of only 10% of the global ocean surface and about the 7% of Earth's surface, supports about the 20% of the oceanic primary production, and about 10% of planet's primary production (del Giorgio & Duarte, 2002)

During last century, the coastal ocean has been exposed to large perturbations, mostly related to human activities on land. Prolonged and intensive use of inorganic fertilizer in agriculture, changes in land use patterns, deforestation, erosion, and discharge of industrial and municipal wastewaters have all contributed to the increase in nutrients in rivers and in the coastal ocean on a global scale (Rabouille et al., 2001). Increased nutrient loading has led to accelerated primary production, or eutrophication; symptoms include increased algal bloom activity (including harmful taxa), accumulation of organic matter, and excessive oxygen consumption leading to hypoxia (less than 2 mg O_2/l) or anoxia (undetectable levels of oxygen). While nutrient-enhanced eutrophication is a "driver" of hypoxia and anoxia, physical-chemical alterations due to climatic events, such as storm-water discharge, flooding, droughts, stagnancy, and elevated temperatures are also involved. The complex interactions of anthropogenic and climatic factors determine the magnitude, duration and aerial extent of productivity, algal blooms, hypoxia and anoxia (Paerl, 2006).

Other important human induced perturbation is the increase of dioxide carbon atmospheric concentration and other "greenhouse gases" released by human activities such as fossil fuel combustion and large-scale deforestation. This increase in CO_2 atmospheric concentration has two important consequences on the ocean metabolism: produces an increase of CO_2 partial pressure in oceans to reach equilibrium between partial pressures in ocean and atmosphere; and produces global warming as consequence of the retention of Infra Red radiation for the CO_2 and the other "greenhouse gases".

Global warming will probably impact biological processes, as temperature plays a fundamental role regulating metabolic processes (Iriberri et al. 1985, White et al. 1991, Brown et al. 2004). The implications of warming for biological activity and ecosystems

functions remain uncertain (Walther et al. 2002). Warming is expected to increase metabolic rates, both respiration and photosynthetic rates. The Metabolic Theory of Ecology (MTE) predicts respiration rates should increase faster with warming than primary production rates do (Brown et al. 2004, Harris et al. 2006). Harris et al. (2006) argued that, because the activation energies for autotrophs are half that of heterotrophs, heterotrophic respiration should increase at twice the rate of net primary production rates for every degree increase in temperature.

Müren et al. (2005) reported that the heterotrophic to autotrophic biomass ratio increased 5 times and the carbon fixation to respiration ratio decreased six times when temperature was raised from 5 to 10 ºC in mesocosm experiments. A shift from autotrophic to heterotrophic biomass and processes may have important consequences by reverting marine biota from acting as carbon sinks to CO_2 sources, delivering feed backs to the climate system. Lopez-Urrutia et al. (2006) predicted that the differential response of heterotrophic and autotrophic processes to warming will result in a negative feedback to climate warming as ocean communities will capture less CO_2. This feedback will further aggravate the anthropogenic effects on global warming.

Dissolved oxygen is the most commonly measured property of seawater that is sensitive to biological cycling and is therefore the first place to look for changes in ocean biogeochemistry in a warming world (Keeling *et al.* 2010). Asses the processes that are involved in the changes of the content of CO_2 and O_2 in the sea-water is a powerful tool to understand the global changes in the biogeochemical cycles of Carbon and oxygen and determine the biological response of the marine organisms to the climatic change.

Geological History of oxygen

The Earth´s original atmospheric components have largely escaped the planet´s gravitational field. The modern atmosphere has evolved from material originated in the Earth´s interior (Rubey 1951; Holland 1963). The atmosphere derived from degassing of the Earth´s interior probably consisted mainly of H_2O, NH_3, N_2, CH_4 and H_2S; oxygen began to appear as a result of newly emerged photosynthetic systems that use water as the source of free oxygen (Commoner 1965).

The timing of the origin of photosynthesis on the early Earth is greatly debated. It is generally agreed that oxygenic photosynthesis had evolved 2.7 thousand million years (Gyr) ago. However, whether photosynthesis occurred before this time remains controversial. A recent study published by Hoashi *et al.* (2009) conclude, on the basis of the presence of primary haematite crystals and associated minerals within the marine sedimentary rocks preserved in a jasper formation of the Pilbara Craton (Australia), that organisms capable of oxygenic photosynthesis evolved more than 700 million years earlier than previously recognized, 3460 million years ago.

The appearance of photosynthesis did not lead to a rapid increase in the molecular oxygen content of the atmosphere because the presence of vast reserves of reduced elements in the Earth's crust acted as molecular oxygen sinks. When all of these reduced elements had been fully oxidized, molecular oxygen could stably exist in the Earth's atmosphere. The appearance of an oxygenic atmosphere was when the rate of production exceeded O_2 photodissociation and loss. Therefore, the development of necessary defences by organisms against the toxic consequences of prolonged exposure to molecular oxygen could have been a gradual process. The actual aerobic organisms are descendents of organisms that evolved defence mechanisms against molecular oxygen. Paradoxically the actual live cannot be supported in absence of oxygen and oxygen depletion in the coastal waters is an increasingly problem that causes serious ecological problems.

The appearance of photosynthesis is connected with the appearance of respiration. These two processes are linked. Whereas, photosynthesis produces organic matter from simpler organic compounds such as carbon dioxide (CO2), water (H2O) and inorganic nutrients (Field *et al*., 1998; Behrenfeld *et al*., 2001) and produces oxygen as an end product; respiration uses oxygen to oxidize the organic matter and produce its inorganic constituents (CO2, and inorganic nutrients) and free energy. These two processes can be resumed by the simplified equation:

$$CO_2 + H_2O + \text{energy} \rightleftharpoons CH_2O + O_2$$

Photosynthesis needs electromagnetic energy (light) and CO_2 and water to synthesis organic

matter, whereas oxygenic respiration needs oxygen and produces CO_2, water and free energy. There are also alternative respiratory pathways that use oxidized molecules other than oxygen as the electron donors, when oxygen is not available in the environment, confined to anaerobic bacteria in hypoxic and anoxic environments.

Photosynthesis and respiration processes regulate oxygen concentration as well as CO_2 concentration in the oceans, with subsequent consequences on climate regulation. Ocean biota plays a major role in controlling the CO_2 partial pressure in the ocean surface, driving the air-sea CO_2 exchange, though photosynthetic and respiration processes (Calleja *et al.* 2005).

CO_2 atmospheric concentration has been modified by human activities since the beginning of agriculture and animal farming that lead to enhanced emissions of CO_2 and CH_4 to the atmosphere. But these emissions have rapidly increased with the burning of fossil fuels that were generated over several hundred million years and will be exhausted by a few generations of humans (Crutzen and Stoermer 2000). As a consequence, the records of atmospheric CO_2, CH_4 and N_2O show a clear acceleration in trends since the end of the 18th Century, following the invention of the steam engine in 1784. Considering these changes in atmosphere concentrations of CO_2, CH_4 and N_2O along with many other major and still growing impacts caused by human activities on earth and atmosphere, Crutzen and Stoermer (2000) proposed to use the term 'Anthropocene' for the current geological epoch.

The term 'Anthropocene' was first coined by Crutzen and Stoermer (2000) to emphasize the central role human activities currently play in affecting the functioning of the Earth System. Increased burning of fossil fuels, deforestation, agricultural activities, and intensive animal farming have released climatically-important greenhouse gases, which have substantially increase in the atmosphere over the past two centuries. The atmospheric concentration of CO_2 has increased by over 30% and that of CH_4 by more than 100% over the past two centuries, contributing substantially to the observed global average temperature rise during the past century (Crutzen and Stoermer 2000; Crutzen 2002; Crutzen and Steffen 2003, Meehl et al. 2007).

Between one-third and one-half of the world's land surface has been transformed by

human action (Vitousek *et al.* 1997), including a doubling of the cropped land during the past century at the expense of forests, which declined by 20% over the same period (McNeill 2000). As a result, human impacts on the structure (e.g., land cover, coastal zone structure) and functioning (e.g., biogeochemical cycling) of the Earth System now equal or exceed in magnitude many forces of nature at the global scale (Crutzen and Steffen 2003).

Nitrogen cycling is one of the most key processes most impacted in the Anthropocene. Nitrogen is now fixed synthetically through the Haber-Bosch reaction, largely to be applied as fertilizer in agriculture, at rates (120 Tg/year) that exceed that naturally fixed in all Earth ecosystems (90 Tg/year, Galloway and Cowling 2002). Excessive application of Nitrogen in agriculture as fertilizer and its release with livestock manure have led to eutrophication of surface and groundwater around the world (Crutzen and Steffen 2003). Increased anthropogenic disturbances to the coastal ocean over the last Century are threatening coastal ecosystems, biodiversity and ecosystems functioning. Two of the main stressors especially affecting coastal systems are increased nutrients inputs and global warming. Coastal eutrophication is manifested through accelerated primary production and increased algal blooms, favoring the accumulation of organic matter, and excessive oxygen consumption when this is decomposed, leading to hypoxia. As a consequence, eutrophication-driven hypoxia is emerging as one of the major threats to coastal biodiversity.

When the oxygen levels in the sea waters drop below 2 mg O_2/l this is the conventional definition of hypoxia, because this is believed to be the oxygen concentration below which benthic dwelling organisms are strongly affected (Diaz & Rosenberg, 1995). However, the empirical basis of this definition is uncertain. This oxygen level was established as the thresholds of oxygen concentration at which fisheries collapse (Renaud, 1986), because bottom-draggling trawls fail to capture fish and shrimps below this oxygen concentration (Rabalais et al., 2002). Although the traditional definition of 2 mg O_2 L^{-1} has been used extensively in literature, there is ample experimental evidence that it may be inadequate to describe the onset of hypoxia impacts for many organisms that are impacted by hypoxia at higher oxygen concentrations (Gray et al., 2002).

Interactions between global warming and hypoxia

Global warming is an increase in the atmospheric and oceanic temperatures as a consequence of the increase in the atmospheric concentration of carbon dioxide and other "greenhouse gases" released by human activities such as fossil fuel combustion and large-scale deforestation. This is also known as climatic change because it refers to the effect on the climate of anthropogenic disturbances (Houghton, 2005). The consequences of global warming in biogeochemical cycles are still largely unknown. However, it is possible to predict a number of impacts of climate change on the concentration and dynamics of O2 and, therefore, the likelihood of hypoxia.

Increasing global temperature has multiple of consequences on climate, hydrology, currents, element cycles, biodiversity, and extent and development of hypoxia. Temperature is one of the key factors controlling the extent of hypoxia (Conley *et al.* 2007), acting through a multitude of interacting processes. Some of these processes include increasing stratification, decrease in oxygen solubility, sea level rise, changes in currents, intensification of coastal upwelling, and increment in frequency of tropical storms and hurricanes, among others.

As a consequence of the warming, there is an increase of water stagnation. Stratification is favoured by increasing temperature and surface freshening (Sarmiento *et al.* 1998), acting as a physical barrier to gas transfer between surface oxygenated waters and bottom waters depleted in oxygen. The increased stratification reduces the downward carbon fluxes and the loss of heat to the atmosphere. Both processes decrease the oceanic uptake of anthropogenic CO_2, contributing to increase warming (Sarmiento *et al.* 1998). The possibility of strengthened stratification alone, from increased surface water temperature, is enough to worsen hypoxia where it presently exists and will trigger its formation in other coastal areas (Rabalais *et al.* 2009).

Oxygen solubility decreases with temperature (Carpenter 1966; Garcia and Gordon 1992). In a recent paper, (Conley *et al.* 2009) demonstrated that hypoxic area in Danish waters will double with a 4ºC increase, as a consequence of changes in oxygen solubility alone, maintaining all other factors unchanged. Changes in oxygen solubility with warming will increase areas suffering hypoxia, especially in temperate and tropical areas.

Global warming is contributing to sea level rise produced by thermal expansion and melting of glaciers. One of the negative effects of the rising of the sea level is the endangerment of coastal wetlands, such as saltmarshes or mangroves. Wetlands significantly improve water quality acting as a nutrient filter, retaining sediments, nutrients, organic carbon and other pollutants (Jordan *et al.* 2003; Jordan *et al.* 2007). The loss of wetlands will probably result in a higher nutrient and organic matter export to coastal areas, contributing to eutrophication.

Climate change can also influence wind patterns with subsequent changes in surface currents, circulation and mixing processes. Severe inner-shelf hypoxia off Oregon coast was documented in 2002 (Service 2004). The causes of the formation of this new hypoxic area were attributed to deviations in the circulation of the California Current System (Grantham *et al.* 2004) that further reflect large-scale wind stress anomalies present over the northeast Pacific in 2002 (Murphree *et al.* 2003).

Increasing temperature can produce intensification of coastal upwelling (Bakun 1990; Bakun and Weeks 2004) with a subsequent oxygen decline in bottom waters bellow the upwelling system when lack of grazers produce accumulation and sinking of primary producers (Bakun and Weeks 2004). Increasing warming produces an enhancement of land heating, resulting in a higher thermal difference between land and sea, favouring the formation of strong pressure gradients between the low pressure cell developed over the heated land surface and the higher pressure existing over the ocean waters. This pressure gradient supports a geostrophic wind along the shore that drives an offshore-directed Eckman transport of the ocean surface layer. These surface layers moved offshore are replaced with cold upwelled waters. Ocean surface cooling, produced by the intensified upwelling, further enhances the land–sea temperature contrast, the associated cross-shore pressure gradient and the upwelling-favourable wind that produces a positive feedback that favours upwelling conditions (Bakun and Weeks 2004).

Trenberth 2005) suggested that the intensity of and rainfalls from hurricanes are increasing, that tropical ocean basins appear to compete to be most favourable for hurricanes to develop and that changes are expected to affect hurricane intensity and rainfall, but the

effect on hurricane numbers remains unclear. A recent paper from (Kerr 2010) confirms that models predict more intense hurricanes under greenhouse warming but an overall lower number. Although the number in hurricanes is expected to decrease, their effects are predicted to be more destructive. The effects of hurricanes in stratification and developing hypoxia can vary depending on several factors. The increase in rainfall is expected to contribute to a higher nutrient loading from land and increasing stratification with freshwater inputs. In contrast, hurricanes in an early hypoxic stage will contribute to dissipation of hypoxia (Rabalais *et al.* 2009) because of mixing.

Warming will also influence biological processes. Temperature plays a fundamental role regulating metabolic processes (Iriberri *et al.* 1985; White *et al.* 1991). Temperature will likely biological activity because accelerates metabolic processes as metabolic rates increase exponentially with temperature (Brown *et al.* 2004). The Metabolic Theory of Ecology (MTE, Brown et al. 2004) models consider the role of temperature in regulating ecosystems metabolism. In basis of allometric equations proposed for Harris et al. 2006, because the activation energies for autotrophs are half that of heterotrophs, for every degree increase in temperature we can expect the heterotrophic respiration to increase at twice the rate of net primary production rates leading to a potential decrease in heterotrophic biomass (Harris *et al.* 2006). Recent studies suggest that climate change may affect the ecosystem function by altering the balance between autotrophy and heterotrophy, favouring heterotrophic organisms: Harris et al. 2006, reason that an hypothetically four degree increase in the summer water of a north-eastern Atlantic estuary will result in a 20% increase in net primary production and a 43% increase in heterotrophic metabolism, resulting in a 16% decrease of the P:R ratios and an increasing likelihood of system heterotrophy. (Müren *et al.* 2005) performed three temperature mesocosm experiments where the results showed that heterotrophic to autotrophic biomass ratio (H/A) increased 5 times when temperature was raised from 5 to 10ºC. In agreement, the carbon fixation to respiration ratio indicated a decrease of six times over the same temperature range. The consequence of this decrease of the P:R ratios, a change in community composition (favouring heterotrophic communities) jointly with temperature increase probably will be an increase in the number of hypoxic events and in their frequency and severity because a decrease in Primary Production/ Respiration ratios result in a decrease in oxygen production in front of an increase in oxygen consumption that will produce a net decrease in oxygen.

Ocean models predict declines in global average dissolved oxygen, owing to global warming, over the next century ranging between the 1 and the 7% (Keeling *et al.* 2010). (Shaffer *et al.* 2009) model predicts long-term ocean oxygen depletion and a great expansion of ocean oxygen-minimum zones for scenarios with high emissions or high climate sensitivity. Whereas Keeling et al. 2010 provided evidence for a global oxygen decline in ocean waters, rates of oxygen decline tend to be greater in coastal waters compared with open ocean ones (Gilbert *et al.* 2010). Observations around the world (Gilbert *et al.* 2005; Bograd *et al.* 2008; Diaz and Rosenberg 2008) confirm that coastal waters are more susceptible to suffer oxygen decline and the subsequent negative consequences on marine life.

Events of low oxygen can cause serious problems in coastal areas of the world. Some of the severe consequences of hypoxic events include changes in populations of marine organisms such as large-scale mortality, as well as changes in biodiversity, changes in species distributions, physiological stress, and other sublethal effects, such as reduced growth and reproduction (Service, 2004).

Hypoxia in coastal areas is governed by physical and biogeochemical processes. Some of the potential causes of hypoxia in the coastal ocean include: enhanced delivery of nutrients and organic matter in areas with limited circulation and vertical mixing (strong water stratification and long water residence time); upwelling of deep oxygen-depleted waters near-coastal areas and subsequent warming; intrusions of deep waters rich nutrients (than can cause phytoplankton blooms). The combined effect of natural upwelling of low oxygen oceanic water and enhanced availability of nutrients and organic matter can accelerate and intensify coastal hypoxia.

Low oxygen conditions have important consequences in biogeochemical cycles and functioning of biological communities.

The combination of eutrophication and an increase in temperature induces algal blooms and, as a consequence, hypoxic events. Increases in temperature also produce an increase in metabolic rates, causing an upset between primary production and respiration. Temperature increase will produce a higher rise in respiration rates than in production rates

(Harris et al, 2006, López-Urrutia et al, 2006). The consequence of this upset between production and respiration probably will cause oxygen depletion.

The decrease in dissolved oxygen owing to global warming will contribute to increase the likelihood of hypoxia worldwide. Dissolved oxygen is the property that has changed more drastically in a shorter period of time in the marine environment (Diaz and Rosenberg 1995). Oxygen deficiencies have increased in frequency, duration, and severity in the world´s coastal areas during the last decades (Diaz and Rosenberg 2008), warming will contribute to exacerbate hypoxia and its consequences for marine life.

Understanding the linkage between biological processes, air-sea CO2 and O2 exchange is essential for understand the dynamics of this two biogenic gases and can predict the responses of the marine life to the anthropogenic increase of “greenhouse gases” (i.e. to an increase of temperature).

General goal and objectives:

The general goal of this thesis is to assess the changes in marine dissolved oxygen owing to anthropogenic disturbances and their consequences on marine life.

The specific objectives contributing to the general goal are:

1. To assess the increase in planktonic and benthic community respiration rates when temperature increases in the range of the predictions for climate change (i.e. ~ 4-6ºC)

2. To search for patterns in the range of diel oxygen variations in coastal ecosystems and elucidate the driving factors and, particularly, the drivers of episodic hypoxia. To experimentally resolve these patters in highly productive ecosystems, such as macroalgae meadows, and to extract information on metabolic processes from diel oxygen variation in these productive ecosystems

3. To search for patterns on oxygen thresholds for macrofauna and how individual species of macrofauna and communities react to hypoxia in experimental systems.

4. To examine the environmental modulation of the O_2 thresholds (Temperature and sulphide).

Methods

Bibliographic research has been the basis for the objectives 3 and 4. A big compilation of papers referred to hypoxic events and the responses of benthic marine communities have served as basis for elucidate the responses of benthic marine organisms to hypoxic conditions, the patterns on oxygen thresholds for macrofauna and how individual species of macrofauna and communities react to hypoxia in experimental systems, examine the environmental modulation of these O_2 thresholds (Temperature and sulphide).

Experimental work

Performance of temperature controlled experiments have served for assess the responses of primary production and respiration to an increase of temperature. Series of different temperature incubations were performed at different periods of year in the coastal Mediterranean Sea. The temperature increases were on the ranges of the predictions for climatic change. For do that we have used Winkler technique, measuring the initial oxygen concentration in sampled water and the oxygen content after incubation under different temperature regimens.

Similar experiments on benthic community have been performed, because benthic community is more sensitive to hypoxic events.

Research vessels on the Arctic Ocean serve for made similar increased temperature experiments and assess the spatial differences in planktonic community respiration responses to a temperature increase.

To search for patterns in the range of diel oxygen variations in coastal ecosystems and elucidate the driving factors and, particularly, the drivers of episodic hypoxia, a multiparameter water quality sensor have been placed at in a coastal Mediterranean zone, with presence of macroalgae (*Caulerpa prolifera*) meadows. The oxygen time series obtained with this sondes have been used to assess the drivers of diel and annual oxygen variations and, particularly, the possible drivers of episodic hypoxia.

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Chapter 1

Experimental evaluation of planktonic respiration response to warming in the European Arctic sector

Raquel Vaquer-Suner, Carlos M. Duarte, Rocío Santiago, Paul Wassmann and Marit Reigstad. *Polar Biology* **33**(12): 1661-1671. **DOI**: 10.1007/s00300-010-0788-x

Abstract

The Arctic Ocean is the region on Earth supporting the steepest warming rate and is also particularly vulnerable due to the vanishing ice cover. Intense warming in the Arctic has strong implications for biological activity and the functioning of an Arctic Ocean deprived of ice cover in summer. We evaluated the impact of increasing temperature on respiration rates of surface marine planktonic communities in the European Arctic sector, a property constraining the future role of the Arctic Ocean in the CO_2 balance of the atmosphere. We performed experiments under four different temperature elevation regimes (*in situ,* +2, +4 and +6ºC above the temperature of the sampled water) during cruises conducted in the Fram Strait region and off Svalbard during late fall - early winter, spring and summer. During late fall-early winter, where only three different temperatures were used, no response to warming was observed whereas respiration rates increased in response to warming in spring and summer, although with variable strength.

Keywords: Q_{10}, respiration, warming, Arctic, Activation Energy.

Introduction

The Arctic region is experiencing the steepest warming rate on Earth, three times faster than the global mean warming rate (ACIA 2004; Trenberth et al. 2007), resulting in an abrupt reduction in ice cover, exceeding the range of natural variability over the past millennia (Walsh 2008). The ice cover over the Arctic Ocean registered a historical minimum, with a reduction of 43% relative to the ice cover in 1979, in September 2007, a loss equivalent to more than twice the area of Alaska (Kerr 2007). Rapid warming is expected to continue in the future, with up to 6 º C warming expected throughout the 21st century (ACIA 2004). Reduced Arctic ice cover is expected to lead to improved growth conditions for phytoplankton (Carmack and Wassmann 2006; Pabi et al. 2008) and result in an 8% to 30% increase in primary production above current rates in regions such as the Barents Sea (Wassmann et al. 2006b; Ellingsen et al. 2008; Wassmann et al. 2008), consistent with trends observed in the Pacific sector of the Arctic Ocean (Arrigo et al. 2008).

Temperature plays a fundamental role regulating metabolic processes (Iriberri et al. 1985; White et al. 1991). The Metabolic Theory of Ecology (MTE; Brown et al. 2004) predicts that primary production should increase with increasing temperature. However, the MTE also predicts that respiration rates should show a stronger response to increased

temperature than photosynthetic rates, resulting in a reduction in the production to respiration ratio with increasing warming (Harris et al. 2006; Lopez-Urrutia et al. 2006). Harris et al. (2006) calculated, considering that the activation energy for photosynthesis is lower than that for respiration, that increasing temperature should lead to an increase in respiration rates twice as fast as that in net primary production rates.

Whereas predictions on the response of primary production to warming are available for the Arctic (Wassmann et al. 2006b; Arrigo et al. 2008; Ellingsen et al. 2008), similar predictions for community respiration rates are not available for the Arctic. The applicability of predictions derived from general metabolic theory (Brown et al. 2004), such as those produced by Harris et al. (2006) and López-Urrutia et al. (2006), to predict the response of respiration rates of Arctic plankton to warming is not guaranteed. There is evidence that bacterial respiration – the largest contributors to plankton respiration (del Giorgio and Cole 1997; Rivkin and Legendre 2001; del Giorgio and Duarte 2002) – show very steep responses to increased temperature at low ambient temperatures (Pomeroy and Wiebe 2001; Middelboe and Lundsgaard 2003), such as those found in Arctic waters. Moreover, the response of bacteria to temperature is also dependent on substrate availability (Pomeroy et al. 1991), which is likely to be low in the Arctic winter, when no primary production or riverine inputs from terrestrial sources occur, in contrast to high rates of primary production and large inputs of organic matter from terrestrial sources to the Arctic in summer (Wassmann et al. 2006a).

Here we examine experimentally the response of respiration rates of Arctic plankton communities to increased water temperature. We do so through experiments conducted in different seasons, each involving increased temperature by up to 6 ºC above the *in situ* sea-surface temperature, thereby encompassing the range of warming possible in the region by year 2050 (ACIA 2004). Evaluating the response of respiration rates of Arctic plankton communities to increased water temperature is essential to predict the consequences on warming for the role of Arctic biota in CO_2 fluxes, as increased respiration rates may weaken, or even revert, the role of Arctic plankton communities as CO_2 sinks.

Materials and Methods

Experiments were collected in three different cruises across contrasting seasons: late fall-early winter, spring and summer, on December 2006, April 2007 and July 2007, respectively, at stations located in the Fram Strait and the Kongsfjorden-Krossfjorden fjord system. The Fram Strait, located between Greenland and the Svalbard Islands, represents a

connection between the North Atlantic and the Arctic Ocean, with an important heat and mass exchange. Large quantities of heat are transported poleward across the Fram Strait by the West Spitsbergen Current (WSC), influencing the climate in the Arctic region as a whole (Hop et al. 2006). The Kongsfjorden-Krossfjorden fjord system is situated on the west coast of Spitsbergen (Svalbard), at the eastern margin of the Fram Strait. It is mainly affected by the northbound transport of water in the WSC and the mixing processes on the shelf result in the presence of Transformed Atlantic Water in the fjords (Hop et al. 2006). The West Spitsbergen Current largely influences the west coast of Svalbard, and directly influences open fjords. Advection of warm water masses during late autum and winter, together with prevailing wind patterns and air temperatures, may prevent ice formation in the fjords (Hop et al. 2006). During December 2006 (our sampling time) Kongsfjorden was almost completely ice free.

Five experiments were conducted on December 2006 on the ARCTOS cruise onboard R/V *Jan Mayen*, one experiment on the Barents Sea and four experiments in the Kongsfjorden (Svalbard). Five experiments were conducted on the Fram Strait on April 2007 during the iAOOS-Norway cruise on board the icebreaker K/B *Svalbard*; and three experiments were conducted at three stations sampled in the Fram Strait on July 2007 during the ATOS cruise onboard the R/V *Hespérides* (Fig 1).

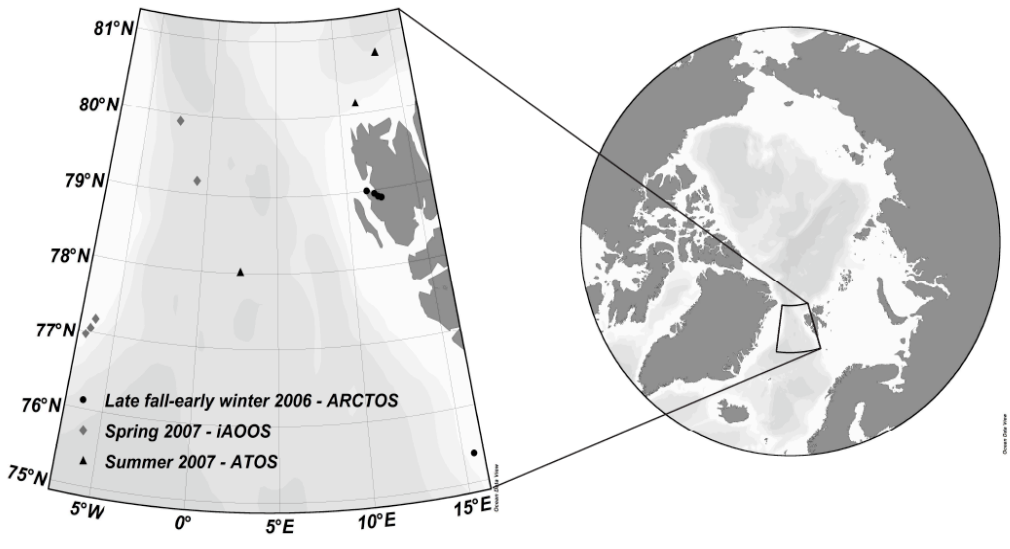

Figure 1. Distribution of the stations along the three cruises

Water samples were collected at 1 meter depth using a 12 L Niskin bottle attached to a Rosette sampler system fitted with a CTD, during the late fall-early winter and spring

cruises, and using a 30L Niskin bottle during the summer cruise. Surface water samples, collected from a total of 13 stations along the Barents Sea (one station), Kongsfjorden (four stations) and Fram Strait (eight stations), were incubated for 48 hours in the dark at three different temperatures in December and four different temperatures in April and July, ranging from the *in situ* temperatures to 4 º C or 6 º C warmer, depending on cruises. Water samples to measure respiration rates were carefully siphoned into a variable number of 75 ml narrow-mouth Winkler bottles. Between 23-39 bottles were filled for each experiment, resulting in 6-11 replicates per treatment. The samples were incubated for 48 hours at each of the experimental temperatures. The incubation at *in situ* temperature on the April 2007 cruise was conducted by suspending the water bottles at the depth where they were sampled from a buoy attached to the ice edge, as the ship stayed at the stations for 48 hours and *in situ* temperature variability over time was minimal at that time. All other experiments were run in incubators on board the research vessel. The actual incubation temperatures used in each experiment are shown in Table 1.

Dissolved oxygen in the bottles was fixed immediately after the end of the incubation period and analysed by high-precision Winkler titration, following the recommendations of Carritt and Carpenter (1966), using a precise automated titration system with potentiometric (redox electrode) end-point detection (Mettler Toledo, DL28 titrator) (Oudot et al. 1988). The respiration rates were calculated from the decline in oxygen concentration after 48 h relative to the initial concentration, and expressed as µmol O_2 L^{-1} d^{-1}.

An estimation of the Activation Energy for respiration (E_a, units eV) was derived from the slope of an Arrhenius plot of the natural logarithm of respiration rate against the inverse of the temperature (Kelvin) multiplied by the Boltzmann´s constant. The Q_{10} (the relative rate of increase in respiration rate expected for a 10ºC temperature increase) was calculated by fitting, using least squares linear regression, the equation (Raven and Geider 1988):

$Q_{10} = e^{\left(\frac{10E_a}{RT^2}\right)}$, where R is the gas constant (8.314472 mol^{-1} K^{-1}), T is the mean absolute temperature across the range over which Q_{10} was measured (K), and E_a is the activation energy (J mol^{-1}), derived from the slope of the Arrhenius equation relating the natural logarithm of respiration rates (in mmol O_2 m^{-3} d^{-1}) to 1/kT, where k is the Boltzmann's constant (8.62 x10^{-5} eV k^{-1}) and T is the temperature (Kelvin). The slope of this

relationship is the activation energy (E_a) in eV, which can be converted to J mol^{-1} using a conversion factor of 96486.9.

Results

A total of 13 warming experiments were conducted at stations with surface seawater temperature ranging from -1.85 ºC (salinity 33.8 psu) to 5.2 ºC (salinity 34.8 psu), with salinity varying between 32.05 and 35.09 psu across stations. The initial oxygen levels varied between stations. The mean oxygen concentration was at 82.2 ± 0.8 % saturation, the minimum was 78.3% saturation and the maximum 91.7% saturation. Respiration rates at the *in situ* temperature ranged over two orders of magnitude across seasons, with extremely low respiration rates down to 0.18 µmol O_2 L^{-1} d^{-1} derived in April (Table 1) and quite high respiration rates during summer (maximum 20.13 µmol O_2 L^{-1} d^{-1}, Table 1). There was no significant relationship between the *in situ* temperature and the corresponding respiration rates ($R^2 = 0.09$, N = 12, $p > 0.05$). The lack of a significant relationship was attributable to an extremely high respiration rate value obtained during summer. If this point is excluded, the relationship becomes significant ($R^2 = 0.45$, N = 11, $p < 0.03$).

Increased temperature led to enhanced community respiration rates in 85% of the experiments. There was, however, considerable variability in the relationships between community respiration rate and temperature (Fig. 2), including two experiments where respiration rates declined with increasing temperature, both conducted during the dark period in the late fall - early winter of 2006 (Fig. 2, Table 2). The slope of the relationships also changed greatly across experiments, indicative of a broad range of Q_{10} and activation energy values across experiments (Fig. 2, Table 3). Only one experiment showed a statistically significant relationship between the natural logarithm of respiration (Ln R) and the inverse of temperature multiplied by the Boltzmann constant (1/kT). However, this was attributable to the low power of the analysis, as the vast majority of the experiments only had 3 or 4 data points to test this relationship. However, meta-analysis derived from the combined probability of the relationships obtained for individual experiments shows that there is indeed an overall tendency for a significant, negative relationship between Ln R and 1/kT (χ^2 test, $p < 0.05$, cf. combining probabilities from tests of significance, Sokal and Rohlf, 1995).

Table 1. Cruise name, season and dates, position (latitude and longitude), in situ temperature (T in situ) and salinity of the stations sampled, along with the experimental temperature (T) and respiration rates (R, µmol O_2 l^{-1} d^{-1}) with their associated error (SE) for the 3 cruises.

Cruise	Season	Exp.	Date	Latitude	Longitude	T *in situ*	Salinity (psu)	T (ºC)	R (µmol O_2 l^{-1} d^{-1})	SE
	Late fall -									
ARCTOS	early winter	Exp 1	30/11/06	75º 22.80 N	15º 35.44 E	5.1	35.1	6.8	0.37	0.67
	2006	Exp 1	30/11/06	75º 22.80 N	15º 35.44 E	5.1	35.1	8.3	0.52	0.68
		Exp 2	2/12/06	78º 57.26 N	11º 57.57 E	1.4	34.5	4.6	6.80	3.65
	24h dark	Exp 2	2/12/06	78º 57.26 N	11º 57.57 E	1.4	34.5	6.3	4.91	1.17
		Exp 2	2/12/06	78º 57.26 N	11º 57.57 E	1.4	34.5	7.7	0.69	1.81
		Exp 3	2/12/06	78º 53.60 N	12º 26.63 E	0.5	34.3	4.6	4.50	0.69
		Exp 3	2/12/06	78º 53.60 N	12º 26.63 E	0.5	34.3	6.1	4.40	0.83
		Exp 3	2/12/06	78º 53.60 N	12º 26.63 E	0.5	34.3	7.5	5.11	0.73
		Exp 4	2/12/06	78º 59.95 N	11º 25.84 E	1.1	34.5	4.7	5.55	0.68
		Exp 4	2/12/06	78º 59.95 N	11º 25.84 E	1.1	34.5	6.0	4.57	0.46
		Exp 4	2/12/06	78º 59.95 N	11º 25.84 E	1.1	34.5	7.9	6.86	1.01
		Exp 5	3/12/06	78º 54.82N	12º11.975E	1.8	34.6	4.7	3.50	0.66
		Exp 5	3/12/06	78º 54.82N	12º11.975E	1.8	34.6	6.0	4.13	0.72
		Exp 5	3/12/06	78º 54.82N	12º11.975E	1.8	34.6	8.0	2.15	0.68
iAOOS	Spring									
2007	2007	Exp 1	15/4/07	79º 54.40 N	02º 27.89 W	-1.8	33.8	0.1	1.11	0.37
		Exp 1	15/4/07	79º 54.40 N	02º 27.89 W	-1.8	33.8	2.7	1.64	1.17
	24h ligth	Exp 1	15/4/07	79º 54.40 N	02º 27.89 W	-1.8	33.8	4.7	5.74	1.11
		Exp 2	19/4/07	79º 09.97 N	00º 46.62 W	-1.7	32.7	-1.8	0.73	1.07
		Exp 2	19/4/07	79º 09.97 N	00º 46.62 W	-1.7	32.7	0.0	2.02	0.51
		Exp 2	19/4/07	79º 09.97 N	00º 46.62 W	-1.7	32.7	2.7	2.55	0.54
		Exp 2	19/4/07	79º 09.97 N	00º 46.62 W	-1.7	32.7	3.9	2.21	0.70
		Exp 3	23/4/07	77º 11.16 N	06º 16.64 W	-1.8	32.9	-1.8	0.44	0.77
		Exp 3	23/4/07	77º 11.16 N	06º 16.64 W	-1.8	32.9	0.03	0.93	0.77
		Exp 3	23/4/07	77º 11.16 N	06º 16.64 W	-1.8	32.9	2.9	0.61	1.65
		Exp 3	23/4/07	77º 11.16 N	06º 16.64 W	-1.8	32.9	3.8	1.36	0.84
		Exp 4	24/4/07	77º 03.34 N	06º 29.33 W	-1.8	32.9	-1.8	0.18	0.72
		Exp 4	24/4/07	77º 03.34 N	06º 29.33 W	-1.8	32.9	0.06	0.94	0.63
		Exp 4	24/4/07	77º 03.34 N	06º 29.33 W	-1.8	32.9	3.2	1.17	0.82
		Exp 4	24/4/07	77º 03.34 N	06º 29.33 W	-1.8	32.9	4.5	2.12	0.90
		Exp 5	25/4/07	76º 57.81 N	06º 42.86 W	-1.7	32.9	-1.7	0.40	0.88
		Exp 5	25/4/07	76º 57.81 N	06º 42.86 W	-1.7	32.9	0.1	0.85	0.93
		Exp 5	25/4/07	76º 57.81 N	06º 42.86 W	-1.7	32.9	2.7	1.31	0.94
		Exp 5	25/4/07	76º 57.81 N	06º 42.86 W	-1.7	32.9	4.7	1.48	0.85

	Summer									
ATOS	2007	Exp 1	6/7/07	78º 00.77 N	2º 33,49 E	3.5	34.7	2.0	19.65	1.48
		Exp 1	6/7/07	78º 00.77 N	2º 33,49 E	3.5	34.7	4.0	19.19	1.50
	24h ligth	Exp 1	6/7/07	78º 00.77 N	2º 33,49 E	3.5	34.7	6.0	20.30	1.50
		Exp 1	6/7/07	78º 00.77 N	2º 33,49 E	3.5	34.7	8.0	20.92	1.59
		Exp 2	9/7/07	80º 08.36 N	11º 19,33 E	5.2	34.8	2.0	0.38	0.26
		Exp 2	9/7/07	80º 08.36 N	11º 19,33 E	5.2	34.8	6.0	1.42	0.25
		Exp 2	9/7/07	80º 08.36 N	11º 19,33 E	5.2	34.8	8.0	1.31	0.55
		Exp 3	18/7/07	80º 45.08 N	13º 27,17 E	-0.03	32.2	0.0	0.67	0.44
		Exp 3	18/7/07	80º 45.08 N	13º 27,17 E	-0.03	32.2	2.0	2.31	0.42
		Exp 3	18/7/07	80º 45.08 N	13º 27,17 E	-0.03	32.2	4.0	2.26	0.41
		Exp 3	18/7/07	80º 45.08 N	13º 27,17 E	-0.03	32.2	6.0	2.73	0.42

Table 2. Parameters and their standard errors and statistics for the fitted regression equations between community respiration rates (µmol O_2 l^{-1} day^{-1}) and incubation temperature (ºC)

Season	Station	Intercept	SE	Slope	SE	R^2	p	N	T situ
Late fall/early winter	Exp 1	0.35		0.11		1.00		2	5.1
Late fall/early winter	Exp 2	16.19	3.44	-1.94	0.54	0.93	0.17	3	1.4
Late fall/early winter	Exp 3	3.37	1.04	0.21	0.17	0.62	0.42	3	0.5
Late fall/early winter	Exp 4	2.73	3.40	0.47	0.53	0.44	0.54	3	1.1
Late fall/early winter	Exp 5	6.15	2.49	-0.46	0.39	0.58	0.45	3	1.85
Spring	Exp 1	0.39	1.63	0.98	0.52	0.78	0.31	3	-1.8
Spring	Exp 2	1.57	0.30	0.26	0.12	0.70	0.16	4	-1.7
Spring	Exp 3	0.71	0.21	0.10	0.08	0.43	0.35	4	-1.8
Spring	Exp 4	0.71	0.21	0.26	0.07	0.87	0.07	4	-1.8
Spring	Exp 5	0.77	0.06	0.17	0.02	0.97	0.02	4	-1.7
Summer	Exp 1	19.07	0.94	0.47	0.17	0.79	0.11	4	3.5
Summer	Exp 2	0.13	0.46	0.17	0.08	0.83	0.27	4	5.2
Summer	Exp 3	1.07	0.45	0.31	0.12	0.76	0.13	4	-0.03

Fitted Q_{10} values ranged greatly, from 0.0008 to 32.9. Q_{10} values were high in summer, ranging from 1.2 to 9.3, while much higher values were found in spring, when Q_{10} ranged from 3.48 to 32.9 (Table 4). The median (± SE) Q_{10} across all 13 experiments conducted was 8.5 ± 2.9 (Fig. 3, Table 5). Mean Q_{10} values averaged 14.3 ± 5.2 at ice-covered stations and 3.6 ± 1.7 in open waters, but these differences were at the edge of statistical significance ($F = 4.37$, $p = 0.06$). Q_{10} values did not differ between seasons ($F = 2.36$, $p > 0.05$, Fig.3), but the mean Q_{10} values were higher during spring than in summer and late fall-early winter (Table 5).

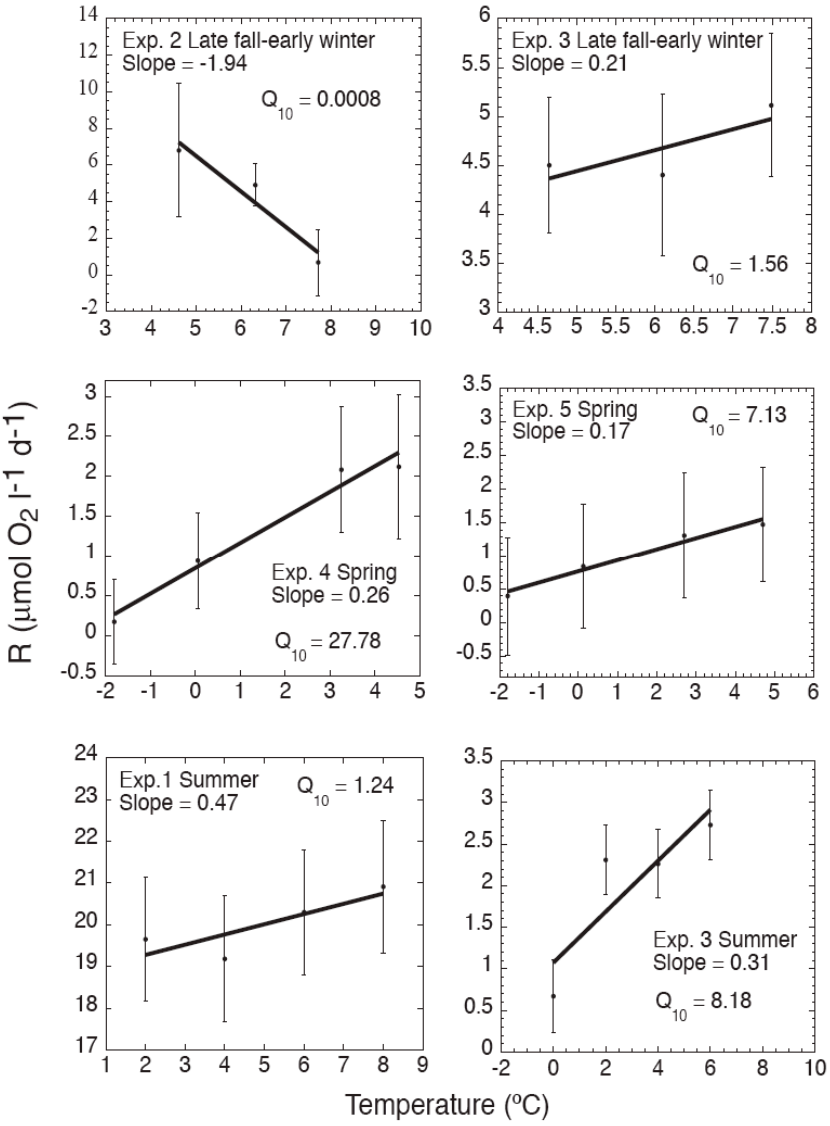

Figure 2. Plots showing the relation between respiration rate (R, µmol O_2 l^{-1} d^{-1}) and the temperature in Celsius degrees for two experiments from each cruise.

The activation energy ranged from 13.8 to 220.6 kJ mol^{-1} K^{-1} (Table 4), with a mean ± SE value of 115.5 ± 20.5 kJ mol^{-1} K^{-1} across experiments (Table 5). There were no significant differences in Ea between seasons ($F = 1.2$, $p > 0.05$) or between stations with ice or free waters ($F = 3.3$, $p > 0.05$), but Ea showed lower values in late fall-early winter (mean ± SE = 78.54 ± 39.46), followed by summer (96.86 ± 41.63) and spring (148.79 ± 27.92) with a high variability across stations (Fig. 3, Table 5).

The overall activation energy for respiration can be derived from the slope of the Arrhenius plot when all experiments are pooled together (Fig. 4). The activation energy derived is this manner was 1.05 ± 0.3 eV or 101.2 ± 28.9 kJ mol^{-1} K^{-1}. An overall Q_{10} can be calculated from this general activation energy, resulting in a Q_{10} value of 5.0.

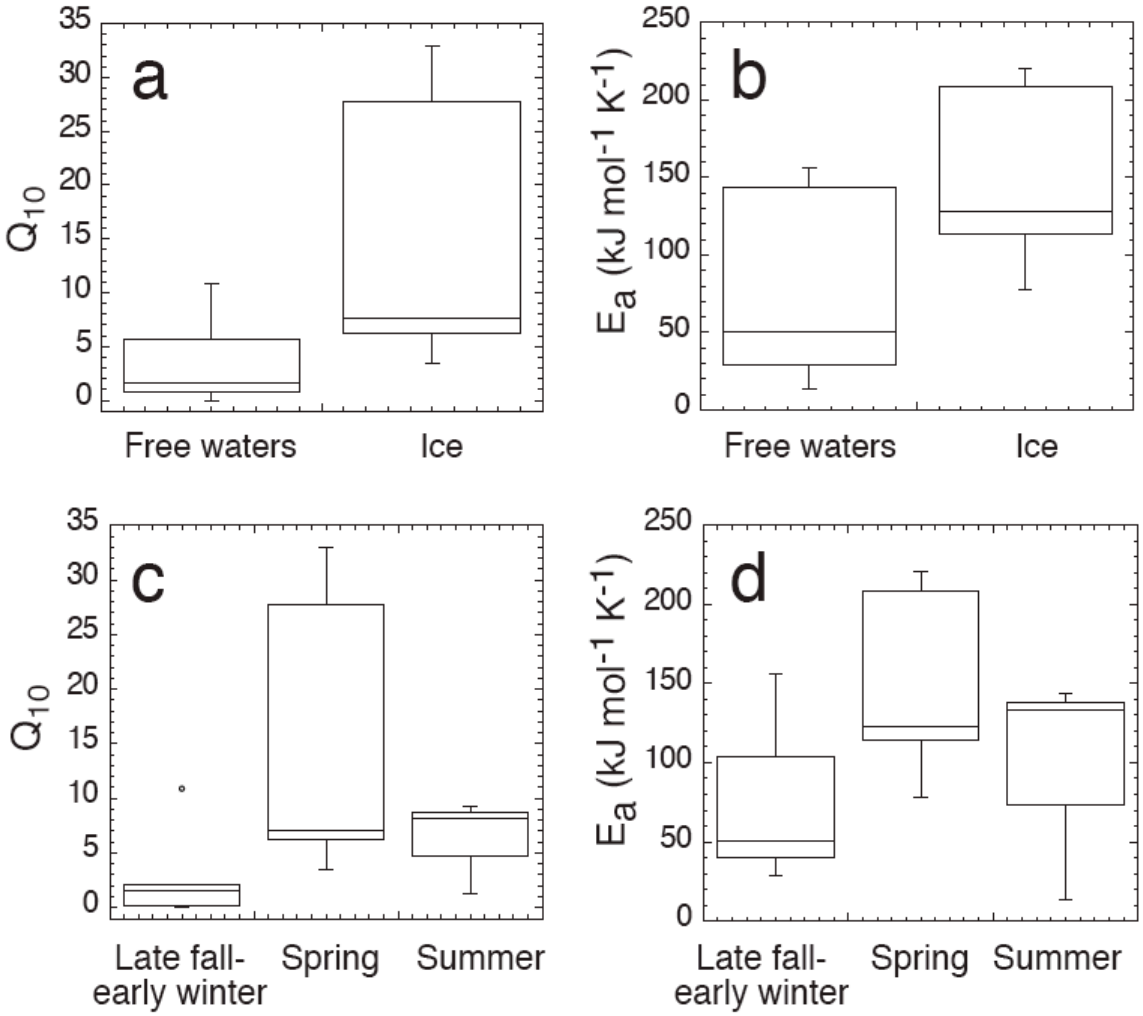

Figure 3. Box plot showing the differences in Q10 (a, c) and activation energy (Ea) (b, d) in the different seasons (c, d) and in stations with presence of ice or in free waters (a, b). The boxes encompass the lower (25%) and upper (75%) quartiles, and the central line shows the median, and the wishkers extend to 1.5 times the interquartile range (IQR)

Discussion

During spring and summer cruises, all experiments showed a positive relationship between respiration rates and incubation temperature. Only two experiments showed declining respiration rates with warming. These experiments were conducted during the 24 h dark period in late fall-early winter, when experimental results showed the greatest variability in responses, with some experiments showing increased respiration rates and some showing reduced respiration rates with warming. During the dark period plankton community respiration rates are probably limited by substrate availability. Bacteria in polar waters operate far below their temperature optima at all times, and in the absence of available

substrate no increase in respiration rates is possible even if the temperature rises (Pomeroy and Wiebe 2001).

Table 3. Parameters and their standard errors and statistics for the fitted Arrhenius equation between the natural logarithm of respiration rate and the inverse of the incubation temperature (Kelvin) multiplied by the Boltzmann's constant ($k = 0.862 \times 10^{-4}$)

Season	Experiment	intercept	SE	Slope (-Ea, eV)	SE	R^2	p	N
Late fall/early winter	Exp 1	66.21		-1.62		1.00		2
Late fall/early winter	Exp 2	-199.26	96.89	4.82	2.33	1.00	0.30	3
Late fall/early winter	Exp 3	13.97	10.01	-0.30	0.24	0.81	0.43	3
Late fall/early winter	Exp 4	23.35	27.76	-0.52	0.67	0.61	0.58	3
Late fall/early winter	Exp 5	-44.64	33.57	1.10	0.81	0.38	0.40	3
Spring	Exp 1	97.02	18.32	-2.29	0.89	0.65	0.24	3
Spring	Exp 2	50.48	24.12	-1.18	0.57	0.87	0.17	4
Spring	Exp 3	33.89	30.69	-0.81	0.64	0.68	0.34	4
Spring	Exp 4	90.97	29.50	-2.16	0.71	0.44	0.09	4
Spring	Exp 5	53.83	12.48	-1.28	0.29	0.82	0.05	4
Summer	Exp 1	9.03	1.60	-0.14	0.05	0.90	0.12	4
Summer	Exp 2	61.85	24.96	-1.49	0.60	0.78	0.24	3
Summer	Exp 3	58.59	26.76	-1.38	0.64	0.86	0.16	4

The experimental Q_{10} values for the current Arctic plankton communities varied greatly, from 0.0008 to 32.9 across experiments, with variability among seasons and locations (Table 4). We found no published Q_{10} values for respiration of Arctic plankton communities, only for specific taxonomic groups of nano-, micro- and mesozooplankton using very high temperatures to assess their Q_{10} values (Hansen et al. 1997). Q_{10} values for Leucine and Thymidine incorporation for Arctic bacteria have been reported with values of 3.1 ± 2.6 and 1.9 ± 0.56, respectively (Kirchman et al. 2005), but not for respiration rates. Our results can be compared to Q_{10} values estimated for Antarctic communities, reported to range between 1.45 and 23.99 (Robinson and Williams 1993), from 2.34 to 11.9 (Tilzer and Dubinsky 1987) and a single value of 3.2 reported by (Vosjan and Olanczukneyman 1991). The median Q_{10} value reported here for Arctic plankton respiration of 6.19 is within the range reported for planktonic communities in the Southern Ocean (1.45 to 23.99, Robinson and Williams 1993), suggesting that the response of respiration rates to warming is comparable for Arctic and Southern Ocean plankton communities. However, the range of Q_{10} values reported for Arctic communities here is much broader, and tends to include higher values,

than those reported for the Southern Ocean (Mean ± SE = 4.93 ± 1.10, Robinson and Williams 1993), probably because all experiments reported for the Southern Ocean were conducted in austral summer.

Table 4. Q_{10} and activation energy (kJ mol^{-1}) values for plankton community respiration with their associated standard error (SE) calculated using error propagation equations and the SE of the fitted regression equation, respectively

Season	Experiment		T *in situ* (ºC)	Q_{10}	SE	Ea (kJ mol^{-1})	SE
Late fall/early winter	Exp 1	Free waters	5.1	10.90		156.49	
Late fall/early winter	Exp 2	Free waters	1.4	0.8 x 10^{-3}	0.003		
Late fall/early winter	Exp 3	Free waters	0.5	1.56	0.56	28.87	23.16
Late fall/early winter	Exp 4	Free waters	1.1	2.17	2.15	50.25	64.45
Late fall/early winter	Exp 5	Free waters	1.85	0.19	0.23		
Spring	Exp 1	Ice	-1.8	32.94	44.80	220.63	85.87
Spring	Exp 2	Ice	-1.7	6.19	5.45	113.98	55.04
Spring	Exp 3	Ice	-1.8	3.48	3.45	77.95	62.04
Spring	Exp 4	Ice	-1.8	27.78	30.53	208.30	68.86
Spring	Exp 5	Ice	-1.7	7.13	3.18	123.12	27.98
Summer	Exp 1	Free waters	3.5	1.24	0.10	13.81	5.19
Summer	Exp 2	Free waters	5.2	9.31	8.37	143.50	57.81
Summer	Exp 3	Ice	-0.03	8.18	7.97	133.25	61.75

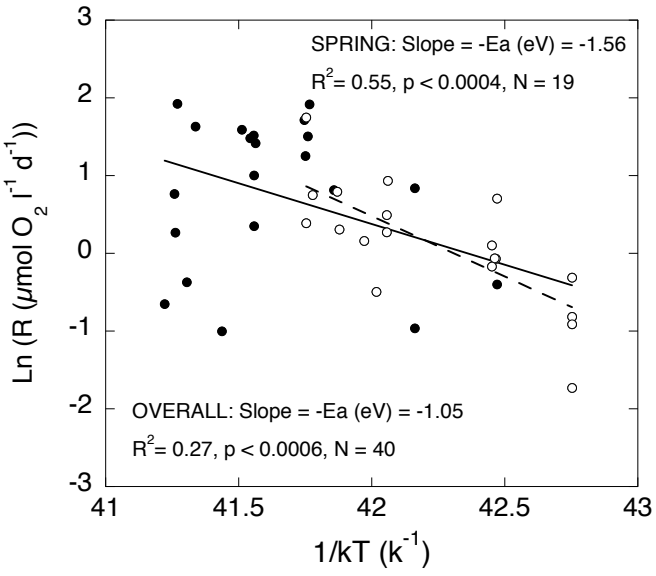

Figure 4. Arrhenius plot showing the relation between natural logarithm of respiration rate (Ln R) and the inverse of the temperature in Kelvin degrees multiplied by the Boltzmann´s constant for all experiments (black line, dark circles) and for experiments conducted in spring (dotted line, open circles)

Table 5. Summary of mean, standard error (SE), median, maximum and minimum values for Q_{10} and the activation energy (kJ mol^{-1}) for the different seasons and all experiments considered together

		Late fall/early winter	Spring	Summer	Overall
Q_{10}	Mean	3.0	15.5	6.2	8.5
	SE	2.0	6.1	2.5	2.9
	Maximum	10.9	32.9	9.3	32.9
	Minimum	0.8×10^{-3}	3.5	1.2	0.8×10^{-3}
	Median	1.6	7.1	8.2	6.2
Ea	Mean	78.5	148.8	96.9	115.5
(kJ mol^{-1})	SE	39.5	27.9	41.6	20.4
	Maximum	156.5	220.6	143.5	220.6
	Minimum	28.9	77.9	13.8	13.8
	Median	50.2	123.2	133.2	123.1

Q_{10} values differ considerably depending on the range of temperatures assessed (Berges et al. 2002), being typically highest at the lower end of the natural temperature range (Pomeroy and Wiebe 2001). Indeed, Kirchman et al. (2005) demonstrated that bacteria are limited by temperature rather than by substrate in polar waters, suggesting that a temperature increase could result in large increases in growth, production and respiration rates. Lomas and Glibert (1999) proved that some diatoms are also limited by temperature, reducing their nitrogen uptake at low temperatures. These studies suggest that small temperature increases at the lower end of the natural temperature range leads to greatly enhanced metabolism, resulting in high Q_{10} values at low temperatures.

The area sampled in this study was quite heterogeneous, as four of the late fall-early winter experiments were sampled in a fjord of Svalbard, samples were collected from a community in heavily ice-covered waters in spring, and most stations were sampled from ice-free waters in summer. Biogeographic processes strongly influence the assemblage structure of picoeukaryotes (Hamilton et al. 2008) and probably also influence the response of the planktonic community to warming, probably accounting for some of the variability in responses. Comparison of Q_{10} values for respiration (this study, mean ± SE = 8.54 ± 2.88, median = 6.19, Fig. 5) with Q_{10} values reported in the literature for photosynthetic rates for natural Arctic planktonic and ice algae communities (mean ± SE = 1.34 ± 0.14, median = 1.13, Fig. 4; Li and Dickie 1984; Li et al. 1984; Michel et al. 1989), which presented no statistically significant differences in photosynthetic Q_{10} values among them ($F = 0.04$, $p =$

0.84), confirms that Q_{10} values for respiration are significantly higher than Q_{10} for photosynthesis in Arctic communities ($F = 9.78$, $p < 0.004$). This finding supports the hypothesis of a greater response of community respiration to warming compared to photosynthesis, suggesting that the CO_2 sink capacity of Arctic planktonic communities will decline with increasing warming.

The Arctic Ocean is surrounded by land and receives large amounts of terrestrial dissolved organic matter (DOM) from riverine inputs, having the highest concentration of terrestrial DOM in any ocean (Benner et al. 2005). Increasing temperature leads to a higher Eurasian rivers discharge (Peterson et al. 2002) and increased runoff leads to higher dissolved organic carbon (DOC) concentration (Cooper et al. 2005). In a warmer Arctic, the input of terrestrial DOM may increase further as tundra soils and permafrost melt. The elevated inputs of terrestrial DOM can be photochemically oxidized in an ice-free Artic Ocean during summer, providing substrates to increase respiration and microbial production. Hence, increased allochthonous organic inputs with warming should enhance Arctic planktonic respiration rates above the levels expected to results from warming.

Climate change will also impact on the species composition of organisms in the Arctic plankton community. Warming will allow organisms to be introduced from both the Atlantic and Pacific into the Arctic, and psychrophilic prokaryotes may, in turn, decline as components of planktonic communities in warmer Arctic waters. Indigenous microorganisms can also evolve and exhibit evolutionary responses to long-term environmental changes (Lynch et al. 1991) and possibly adapt to a warmer Arctic. The power of the results presented here to predict the response of planktonic respiration to a warmer Arctic is, therefore, limited by the effect of parallel changes in the environment, community structure or genetic adaptations, which will compound with the physiological responses to warming.

Our results suggest that a future 6 ºC warming of the Arctic Ocean surface water may yield a mean increment in respirations rates of 62%, doubling the 30% increment expected for primary production (Wassmann et al. 2008), and the 16.1% increase in photosynthetic rates derived from Q_{10} values reported in the literature (Fig. 5). The increase in respiration rates will be further aggravated during spring, when respiration rates may increase by, on average, 76% with a 6 º C warming. The effects of warming on Arctic planktonic respiration rates reported here are relevant to assess possible impacts of warming on carbon cycling in the region, as increased respiration rates imply less. This conclusion is consistent with that of Kirchman et al. (2009), who suggested that planktonic communities in a warmer Arctic

would channel a greater fraction of carbon to the microbial loop, which is characterised by a low carbon use efficiency, reducing the fraction of carbon available to higher trophic levels and that exported to the deep sea and the benthos while increasing the fraction of carbon respired.

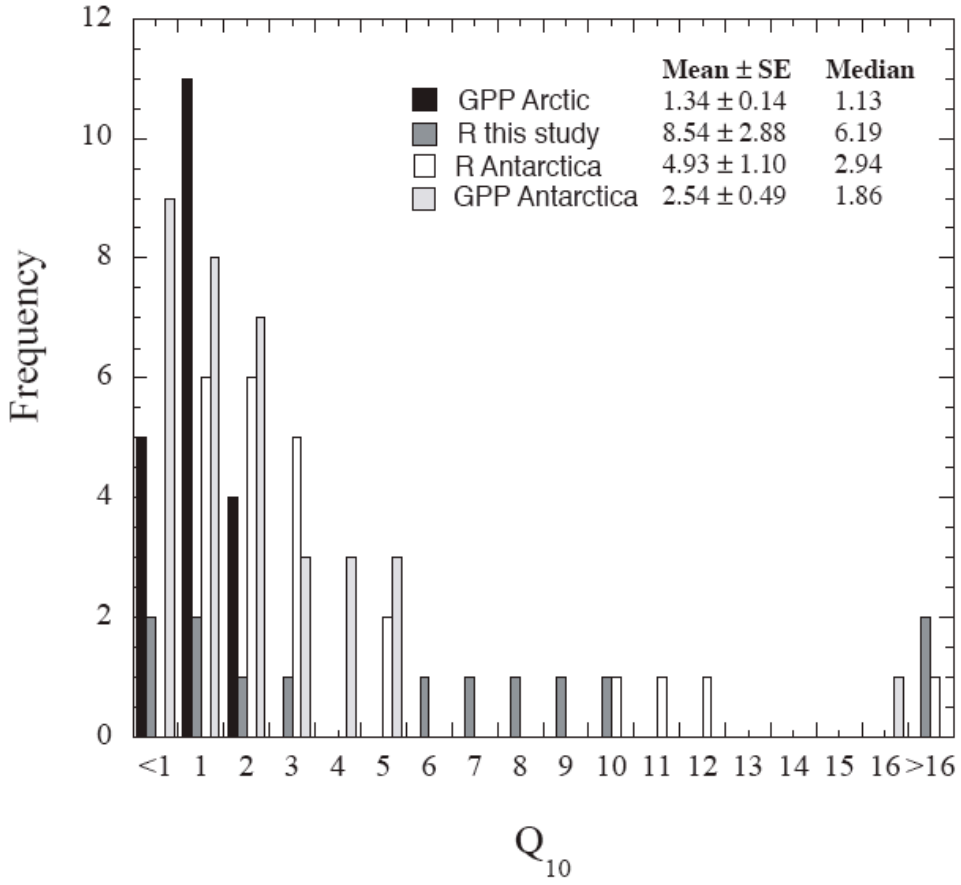

Figure 5. Frequency distribution for Q_{10} values for primary production and community respiration in natural Arctic and Antarctic communities and the corresponding mean ± SE and median values. Data on Q_{10} for respiration for Antarctic communities from (Tilzer and Dubinsky 1987; Vosjan and Olanczukneyman 1991; Robinson and Williams 1993), Q_{10} for photosynthesis of Antarctic communities from (Neori and Holm-Hansen 1982; Tilzer and Dubinsky 1987; Kottmeier and Sullivan 1988; Priscu et al. 1989), Q_{10} for respiration for Arctic communities from this study, and Q_{10} for photosynthesis for Arctic communities from (Li and Dickie 1984; Li et al. 1984; Michel el al. 1989)

Our results suggest that the increase in plankton respiration rates with a 6 ºC warming will far exceed the increase in photosynthetic rates expected from the temperature-dependence of photosynthetic rates reported in the literature. Arctic planktonic communities presently act as an intense sink for atmospheric CO_2 during northern summer (Takahashi et al. 2002). However, the results presented here predict that the role of Arctic communities as significant CO_2 sinks may weaken substantially, and even be reverted to become CO_2 sources to the atmosphere, with global warming.

Acknowledgments

This research was supported by the iAOOS-Norway project (The integrated Arctic Ocean Observing System; project number 176096/S30) through the Research Council of Norway, the ATOS project, funded by the Spanish Ministry of Science and Innovation (POL2006-00550/CTM), both projects integrated in the International Polar Year (IPY), the Floating universities program from EUR-OCEANS (European Network of Excellence for Ocean Ecosystems Analysis), the ARCTOS research network and the Arctic Tipping Points project (www.eu-atp.org), funded by the Framework Program 7 of the European Union (contract number 226248). We thank L. Seuthe, C. Wexels Riser and Camilla Svensen for their help onboard and on the ice, and the crews of R/V *Jan Mayen,* KB *Svalbard* and *R/V Hespérides* for technical support.

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Chapter 2

Evaluation of the Response of coastal Mediterranean Planktonic and Benthic Metabolism to Warming

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Manuscript, 2011

Abstract

The Mediterranean Sea is one of the regions on Earth supporting the steepest warming rate, in addition to the Arctic Sea. Intense warming in the Mediterranean Sea has strong implications for biological activity and ecosystem functioning. To elucidate the effects of warming on planktonic and benthic metabolism we performed experiments under different increasing temperature regimes, ranging from 3 to 6 different temperatures. The lowest range of temperatures assessed was of 2.6 ºC and the maximum of 7.5 ºC. Q_{10} values for respiration rates did not differ significantly from planktonic to benthic communities. Our results suggest that a 6 ºC warming of the Mediterranean waters may yield a mean increment in respiration rates of coastal Mediterranean communities of 73%, higher than the mean increase expected for gross primary production of the 52%. These results confirm earlier theories of a higher increase in respiration rates than in primary production with warming, with the subsequent consequences in the Carbon cycle.

Keywords: Q_{10}, respiration, warming, Mediterranean, Activation Energy.

Introduction

Temperature plays a fundamental role regulating metabolic processes (Iriberri et al. 1985, White et al. 1991, Brown et al. 2004), but the implications of warming for biological activity and ecosystems functions remain uncertain (Walther et al. 2002). Warming is expected to increase metabolic rates, both respiration and photosynthetic rates. The Metabolic Theory of Ecology (MTE) predicts respiration rates to increase faster with warming than primary production rates do (Brown et al. 2004, Harris et al. 2006). Harris et al. (2006) argued that, because the activation energies for autotrophs are half that of heterotrophs, heterotrophic respiration should increase at twice the rate of net primary production rates for every degree increase in temperature. Indeed, Harris et al. (2006), calculated that an hypothetically four degree warming of a north-eastern Atlantic estuary should result in a 20% increase in net primary production and a 43% increase in heterotrophic metabolism, resulting in a 16% decrease in the P/R ratios and an increasing likelihood of system heterotrophy Müren et al. (2005) reported that the heterotrophic to autotrophic biomass ratio increased 5 times and the carbon fixation to respiration ratio decreased six times when temperature was raised from 5 to 10 ºC in mesocosm experiments. Regaudie-de-Gioux and Duarte (in press) concluded, on the basis of an empirical analysis of the relationship between plankton

metabolism and temperature, that the mean (± SE) activation energy describing the temperature-dependence of specific GPP and CR rates of planktonic communities in the ocean were steeper for CR (0.71 ± 0.06 eV) than for GPP (0.43 ± 0.07 eV).

A shift from autotrophic to heterotrophic biomass and processes may have important consequences by reverting marine biota from acting as carbon sinks to CO_2 sources, delivering feed backs to the climate system. Lopez-Urrutia et al. (2006) predicted that the differential response of heterotrophic and autotrophic processes to warming would result in a negative feedback to climate warming as ocean communities would capture less CO_2. This feedback will further aggravate the anthropogenic effects on global warming. This assertion is supported by results from a recent annual manipulated temperature experiment performed by Yvon-Durocher et al. (2010) on freshwater ecosystems. In this experiment warmed and control mesocosms acted as net sinks for CO_2, but the carbon sequestration capacity of the warmed systems was severely compromised, as respiration rates increased faster than primary production, reducing carbon sequestration by 13% (Yvon-Durocher et al. 2010).

Moran et al. (2010) also predicted a shift to phytoplankton communities with smaller cell size with warming, consistent with a documented prevalence of picophytoplankton in warmer waters (Agawin et al. 2000). A shift toward phytoplankton communities of smaller cell size will reduce the efficiency of the biological carbon pump (Moran et al. 2010), as sinking velocity declines with decreasing cell size. This response may trigger an additional feed-back, as reduced ocean carbon sequestration will result in increased CO_2 accumulation in the atmosphere and a faster increase in ocean temperature.

The Mediterranean Sea is warming faster than the global ocean (Vargas-Yáñez et al. 2008), with an increment of 0.5 ºC from 1975 to 2005, twice the mean global temperature increase for that period. The warming expected by the end of this century is forecasted to be highest in summer, with a mean seasonal temperature increase of 6 ºC (for scenarios with high emissions of CO_2) and a lower mean seasonal temperature increase in winter, from 2 to 3º C (Bladé et al. 2010, Vargas-Yáñez et al. 2008). Warming of Mediterranean waters is expected to affect ecosystem metabolism, as predicted from metabolic theory, but this effect has not yet been assessed either for planktonic or benthic communities. We report here the response of the metabolism of Mediterranean planktonic and benthic communities to experimental warming.

Materials and methods

Surface water samples were collected in two different Mediterranean areas, both in Majorca Island, (1) Lighthouse Cap Salines, with pristine, oligotrophic waters (39º15´54´´N, 3º03´16´´E), and (2) Portocolom Bay, a highly impacted semi-enclosed Bay in the Southeast of Majorca Island (39º25´04´´N, 3º15´40´´E, Fig. 1). Sediment cores were extracted from 2.8 m depth at Portocolom by SCUBA diving, containing a random sample of the sediment community, dominated by a meadow of the green alga *Caulerpa prolifera* (Forsskal) and infaunal macroinvertebrates, as well as the bacteria associated with the sediment and living in the overlying water.

A total of 8 experiments were conducted across three contrasting seasons of the year: winter (February 2007); summer (August 2007 and 2009, July 2009 and September 2008); and spring (April 2010, Table 1). Surface seawater temperature ranged from 15.5ºC in February 2007 to 26ºC in August 2006 at the lighthouse Cap Salines and from 16ºC in April 2010 to 26.5ºC in August 2009 at Portocolom. Surface water samples were collected using 20 L carboys.

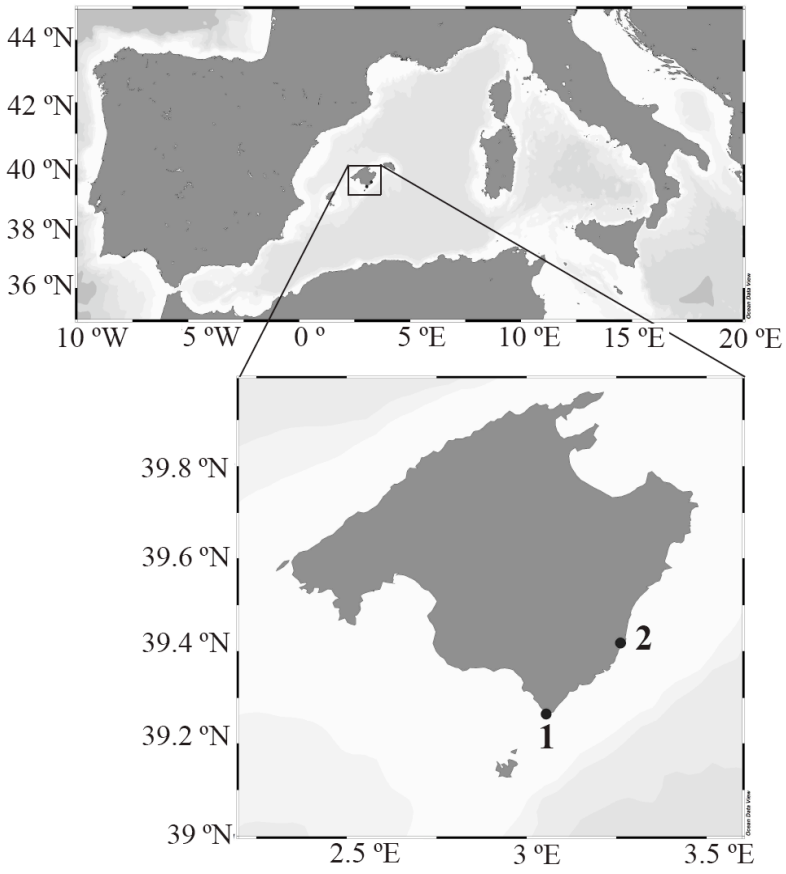

Figure 1. Map of sampling sites. (1) Cap Salines and (2) Portocolom.

Water samples were carefully siphoned into a variable number of 75-ml narrow-mouth Winkler bottles. Between 45 and 81 bottles were filled for each experiment, resulting in 5–10 replicates per treatment, with most treatment having between 6 and 7 replicates. The samples were incubated in 'light' and 'dark' for 24 h at each of the experimental temperatures.

Sediments cores were also incubated in 'light' and 'dark'. Incubation times in sediments cores varied between 5 hours for dark cores and 24 h for light ones. The reason for performing shorter incubation times in the dark was due to a high respiration rates that lead to anoxia in the dark cores after 24 h incubation. Before incubation, rubber stoppers used to extract the sediment cores were replaced by methacrylate stoppers with an o-ring that avoid gas diffusion between sediment ovelying water in the core and incubation water. These methacrylate stoppers had three additional holes with rubber stoppers to sample water after incubation.

To determine initial oxygen content in the sediment cores, after changing rubber stoppers by methacrylate ones, winkler bottles were carefully filled by siphoning overlaying water of the sediment cores using a silicon tube fitted in one of the three holes of the methacrylate stopper. The same procedure was done to determine final oxygen content in the sediment cores after incubations.

Initial samples were fixed immediatelly. All other bottles and sediment cores were incubated in 1500 L tanks with running seawater, previously warmed with solar heat during 3 days without water flow exposed to sunlight in the experiments conducted in August 2007 and September 2008. In February 2007, the incubation of the bottles was made in the laboratory in 50 L aquaria illuminated with artificial light and warmed using thermostats and cooling systems. In July and August 2009 the incubations were conducted in a 500 L tanks with seawater warmed with thermostats and illuminated with artificial light. In April 2010 only dark respiration was evaluated by incubating the bottles in a 50 L tanks with seawater warmed with thermostats.

Dissolved oxygen in the bottles was fixed immediately after the end of the incubation period and analysed by high-precision Winkler titration, following the recommendations of Carritt and Carpenter (1966), using a precise automated titration system with potentiometric (redox electrode) end-point detection (Mettler Toledo, DL28 titrator) (Oudot et al. 1988). In July and August 2009 and April 2010 dissolved oxygen was analysed by spectrophotometric modification of the Winker method, following the recommendations of (Roland et al. 1999).

The planktonic metabolic rates were calculated from the change in oxygen concentration after 24 h relative to the initial concentration, in both 'light' and 'dark' treatments, and expressed as mmol O_2 m^{-3} d^{-1}. The benthic metabolic rates were calculated from the change in oxygen concentration after incubations, relative to the initial concentration and expressed as mmol O_2 m^{-2} d-1.

An estimation of the Activation Energy for respiration (E_a, units eV) was derived from the slope of an Arrhenius plot of the natural logarithm of respiration rate against the inverse of the temperature (Kelvin) multiplied by the Boltzmann´s constant. The Q_{10} (the relative rate of increase in respiration rate expected for a 10ºC temperature increase) was calculated using, the equation (Raven and Geider 1988):

$$Q_{10} = e^{\left(\frac{10E_a}{RT^2}\right)}$$

where R is the gas constant (8.314472 mol^{-1} K^{-1}), T is the mean absolute temperature across the range over which Q_{10} was measured (K), and E_a is the activation energy (J mol^{-1}), derived from the slope of the Arrhenius equation relating the natural logarithm of respiration rates (in mmol O_2 m^{-3} d^{-1}) to 1/kT, where k is the Boltzmann's constant (8.62 x10^{-5} eV k^{-1}) and T is the temperature (Kelvin). The slope of this relationship is the activation energy (E_a) in eV, which was converted to J mol^{-1} using a conversion factor of 96486.9. To calculate Q_{10} values for values including negative NCP, the data were transformed to positive values by subtract the minimum value of NCP measured (NCP min) plus one (NCP-NCP min + 1). The resulting graph had the same slope but different intercept.

Q_{10} values with higher SE than their value were excluded from formal analysis.

Results

Planktonic respiration rates measured at *in situ* temperature ranged over one order of magnitude from 0.70 ± 0.33 mmol O_2 m^{-3} d^{-1} measured in February 2007 in Cap Salines at 15.5ºC to 7.42 ± 0.27 mmol O_2 m^{-3} d^{-1} measured in July 2009 in waters from Portocolom at an *in situ* temperature of 26 ºC. Planktonic net community production (NCP) measured at *in situ* temperature ranged widely from a negative rate of -7.33 ± 0.35 mmol O_2 m^{-3} d^{-1} measured in July 2009 in Portocolom to a positive value of 1.50 ± 0.22 mmol O_2 m^{-3} d^{-1} measured in Cap Salines in August 2007. Planktonic gross primary production (GPP) ranged widely from close to 0 (0.09 ± 0.29 mmol O_2 m^{-3} d^{-1}) measured in July 2009 at Portocolom to a GPP of 3.69 ± 0.27 mmol O_2 m^{-3} d^{-1} measured in August 2007 at Cap Salines (Table 1).

Table 1. Dates, sites, type of community of the different experiments, along with in situ temperature (ºC, T in situ), incubation temperature (T, ºC), Community Respiration rates (CR, mmol O_2 l^{-1} d^{-1}), Net community production (NCP, and Gross Primary Production (GPP) with their associated standard error (SE) for all the experiments conducted. Planktonic NCP, GPP and CR are all reported in mmol O_2 m^{-3} d^{-1} and benthic NCP, GPP and CR in mmol O_2 m^{-2} d^{-1}.

Date	Site	Community	T in situ	T	CR (mmol O_2 m^{-3} d^{-1})	SE	GPP (mmol O_2 m^{-3} d^{-1})	SE	NCP (mmol O_2 m^{-3} d^{-1})	SE
27/2/07	Cap Salines	planktonic	15.5	15.5	0.71	0.33			-1.69	0.82
27/2/07	Cap Salines	planktonic	15.5	17	1.25	0.29	0.92	0.66	-0.33	0.93
27/2/07	Cap Salines	planktonic	15.5	19	2.88	0.51	1.40	0.73	-1.48	0.94
27/2/07	Cap Salines	planktonic	15.5	21	3.79	0.70	0.59	0.77	-3.20	0.84
27/8/07	Cap Salines	planktonic	26	26.2	2.19	0.23	3.69	0.27	1.50	0.22
27/8/07	Cap Salines	planktonic	26	28.5					1.26	0.17
27/8/07	Cap Salines	planktonic	26	30.0	2.07	0.11	2.91	0.18	0.84	0.21
27/8/07	Cap Salines	planktonic	26	30.9	3.48	0.20	3.61	0.23	0.12	0.21
29/8/07	Cap Salines	planktonic	26	26.2	3.53	0.33	2.66	0.42	-0.87	0.10
29/8/07	Cap Salines	planktonic	26	30.4	2.98	0.16	1.95	0.18	-1.03	0.13
29/8/07	Cap Salines	planktonic	26	31.0	2.53	0.14	1.47	0.16	-1.06	0.12
29/8/07	Cap Salines	planktonic	26	31.0	2.45	0.11	1.14	0.16	-1.31	0.14
17/9/08	Portocolom	planktonic	25.5	25.6	1.38	0.12	2.28	0.24	0.59	0.18
17/9/08	Portocolom	planktonic	25.5	27.1	5.77	0.10	6.50	0.30	0.73	0.25
17/9/08	Portocolom	planktonic	25.5	28.2	6.24	0.10	7.82	0.33	1.58	0.29
15/7/09	Portocolom	planktonic	26	26.0	7.42	0.28	0.09	0.29	-7.33	0.35
15/7/09	Portocolom	planktonic	26	27.0	11.15	0.28	0.64	0.42	-10.52	0.44
15/7/09	Portocolom	planktonic	26	29.0	9.47	0.42	0.75	0.40	-8.72	0.26
15/7/09	Portocolom	planktonic	26	29.5	10.91	0.56	0.46	0.55	-10.46	0.29
15/7/09	Portocolom	planktonic	26	31.0	3.42	7.66			-14.10	1.65
15/7/09	Portocolom	planktonic	26	32.6	17.21	0.28			-17.54	0.28
11/8/09	Portocolom	planktonic	26.5	26.5	5.76	0.35	2.05	0.52	-3.71	0.53
11/8/09	Portocolom	planktonic	26.5	28.0	5.10	0.28	3.42	0.34	-1.68	0.30
11/8/09	Portocolom	planktonic	26.5	29.5	7.74	0.38	4.64	0.48	-3.10	0.45
11/8/09	Portocolom	planktonic	26.5	31.5	7.31	0.41	5.24	0.54	-2.07	0.49
11/8/09	Portocolom	planktonic	26.5	32.8	8.83	2.02	7.62	1.79	-1.21	0.91
11/8/09	Portocolom	planktonic	26.5	34.0	0.71	1.02	0.56	0.92	-0.15	0.43
15/4/10	Cap Salines	planktonic	16	15.9	3.52	2.08				
15/4/10	Cap Salines	planktonic	16	19.8	5.40	2.01				
15/4/10	Cap Salines	planktonic	16	19.0	5.30	1.85				
15/4/10	Cap Salines	planktonic	16	17.9	6.16	1.79				
15/4/10	Cap Salines	planktonic	16	21.7	7.44	1.85				
15/4/10	Cap Salines	planktonic	16	22.0	8.62	1.92				
21/4/10	Portocolom	planktonic	16	16.7	5.92	0.27				
21/4/10	Portocolom	planktonic	16	18.3	5.27	0.21				
21/4/10	Portocolom	planktonic	16	19.0	6.48	0.24				
21/4/10	Portocolom	planktonic	16	19.5	7.27	0.19				
21/4/10	Portocolom	planktonic	16	22.1	6.18	0.14				
					R (mmol O2/m2 d)	SE	GPP (mmol O2/m2 d)	SE	NCP (mmol O2/m2 d)	SE
15/7/09	Portocolom	Benthic	27	26.0	45.34	0.45			-48.66	4.08
15/7/09	Portocolom	Benthic	27	27.0	72.71	3.06	53.62	3.11	-19.09	0.56
15/7/09	Portocolom	Benthic	27	29.0	52.60	13.10	13.77	13.16	-38.83	1.26
15/7/09	Portocolom	Benthic	27	29.5	41.85	1.97	6.58	1.99	-35.27	0.28
15/7/09	Portocolom	Benthic	27	31.0	87.60	0.51	38.69	2.21	-48.91	2.15
15/7/09	Portocolom	Benthic	27	32.6	74.61	3.61	22.30	3.63	-52.31	0.37
11/8/09	Portocolom	Benthic	26.5	26.5	43.75	0.42	6.90	0.46	-36.84	0.18
11/8/09	Portocolom	Benthic	26.5	28.0	71.75	0.44	32.21	0.48	-39.54	0.20
11/8/09	Portocolom	Benthic	26.5	29.5	111.13	0.24	74.77	0.32	-36.36	0.21
11/8/09	Portocolom	Benthic	26.5	31.5	90.03	0.17	391.37	0.32	-38.29	0.27
11/8/09	Portocolom	Benthic	26.5	32.8	120.71	0.33	94.63	0.53	-26.07	0.41
11/8/09	Portocolom	Benthic	26.5	34.0	118.00	0.26	82.16	0.38	-35.83	0.28
21/4/10	Portocolom	Benthic	16	16.7	26.22	0.35				
21/4/10	Portocolom	Benthic	16	18.3	57.83	47.08				
21/4/10	Portocolom	Benthic	16	19.0	83.20	10.13				
21/4/10	Portocolom	Benthic	16	19.5	147.08	40.47				
21/4/10	Portocolom	Benthic	16	22.1	89.70	74.34				

Benthic respiration rates measured at *in situ* temperature at Portocolom ranged from 26.22 ± 0.35 mmol O_2 m^{-2} d^{-1} in April 2010 at 16.7 ºC to 45.34 ± 0.45 mmol O_2 m^{-2} d^{-1} in July 2009 (Table 1). The benthic community was heterotrophic, with NCP at *in situ* temperature ranging from -48.66 ± 4.08 mmol O_2 m^{-2} d^{-1} measured in July 2009 to -36.84 ± 0.18 mmol O_2 m^{-2} d^{-1} measured in August 2009. Benthic gross primary production (GPP) measured at *in situ* temperature varied between 6.90 ± 0.46 mmol O_2 m^{-2} d^{-1} measured in August 2009 and 53.62 ± 3.11 mmol O_2 m^{-2} d^{-1} measured in July 2009 (Table 1).

Increased temperature led to enhanced community respiration rates in 91% of experiments. There was considerable variability in the relationships between community respiration rates and temperature (Fig. 2), including one experiment where respiration rates declined with increasing temperature, measured in Cap Salines in August 2009. In some of the experiments the response to warming was ambiguous, as that for planktonic respiration in April 2010 in Portocolom (Fig. 2, Table 2).

Table 2. Parameters and their standard errors and statistics for the fitted Arrhenius equation between the natural logarithm of metabolic rate and the inverse of the incubation temperature (º Kelvin) multiplied by the Boltzmann's constant (k = 0.862 x 10^{-4})

Site	Community	month	year	Season	Metabolic rate	Intercept	SE	Ea (eV, -slope)	SE	R^2	p	N
Cap Salines	Planktonic	February	2007	winter	R	92.60	13.19	2.31	0.33	0.96	0.02	4
Cap Salines	Planktonic	February	2007	winter	NCP	-50.98	33.64	-1.30	0.84	0.54	0.26	4
Cap Salines	Planktonic	February	2007	winter	GPP	-32.49	54.47	-0.82	1.37	0.26	0.66	3
Cap Salines	Planktonic	August	2007	summer	R	20.44	29.04	0.51	0.76	0.31	0.62	3
Cap Salines	Planktonic	August	2007	summer	NCP	-133.10	76.26	-3.45	1.99	0.60	0.22	4
Cap Salines	Planktonic	August	2007	summer	GPP	-5.65	14.43	-0.18	0.38	0.18	0.72	3
Cap Salines	Planktonic	August	2007	summer	R	-18.88	6.41	-0.52	0.17	0.83	0.09	4
Cap Salines	Planktonic	August	2007	summer	NCP	-14.21	9.55	-0.38	0.25	0.53	0.27	4
Cap Salines	Planktonic	August	2007	summer	GPP	-40.07	17.42	-1.06	0.45	0.73	0.14	4
Portocolom	Planktonic	September	2008	autum	R	182.74	77.50	4.69	2.00	0.85	0.26	3
Portocolom	Planktonic	September	2008	autum	GPP	148.70	47.18	3.80	1.22	0.91	0.20	3
Portocolom	Planktonic	September	2008	autum	NCP	112.20	45.28	2.91	1.17	0.86	0.24	3
Portocolom	Planktonic	July	2009	summer	R	34.28	10.17	0.83	0.26	0.77	0.05	5
Portocolom	Planktonic	July	2009	summer	NCP	-91.51	30.61	-2.43	0.80	0.70	0.04	6
Portocolom	Planktonic	July	2009	summer	GPP	123.14	92.13	3.22	2.39	0.48	0.31	4
Portocolom	Benthic	July	2009	summer	R	22.33	16.19	0.48	0.42	0.24	0.32	6
Portocolom	Benthic	July	2009	summer	GPP	-15.03	68.24	-0.47	1.78	0.02	0.81	6
Portocolom	Benthic	July	2009	summer	NCP	-92.89	62.49	-2.47	1.63	0.37	0.20	6
Portocolom	Planktonic	August	2009	summer	R	24.49	8.16	0.59	0.21	0.72	0.07	5
Portocolom	Planktonic	August	2009	summer	GPP	58.30	8.20	1.48	0.21	0.94	0.01	5
Portocolom	Planktonic	August	2009	summer	NCP	48.46	16.90	1.24	0.44	0.66	0.05	6
Portocolom	Benthic	August	2009	summer	R	39.80	10.63	0.92	0.28	0.73	0.03	6
Portocolom	Benthic	August	2009	summer	GPP	106.62	47.95	2.68	1.25	0.53	0.10	6
Portocolom	Benthic	August	2009	summer	NCP	49.32	39.61	1.26	1.03	0.27	0.29	6
Cap Salines	Planktonic	April	2010	spring	R	37.40	8.37	0.90	0.21	0.82	0.01	6
Portocolom	Planktonic	April	2010	spring	R	7.42	9.63	0.14	0.24	0.10	0.60	5
Portocolom	Benthic	April	2010	spring	R	42.78	48.12	0.97	1.21	0.18	0.48	5

Increased temperature led to decreased net community production rates in two thirds (67%) of the experiments. Responses of net community production (NCP) to warming were very variable (Fig. 3), with half of the Q_{10} estimates having standard errors higher than the Q_{10} value, indicating no clear relationship between NCP and warming (Fig. 3, Table 2). Warming led to increased gross community production (GPP) rates in half of the experiments (Fig. 4, Table 2).

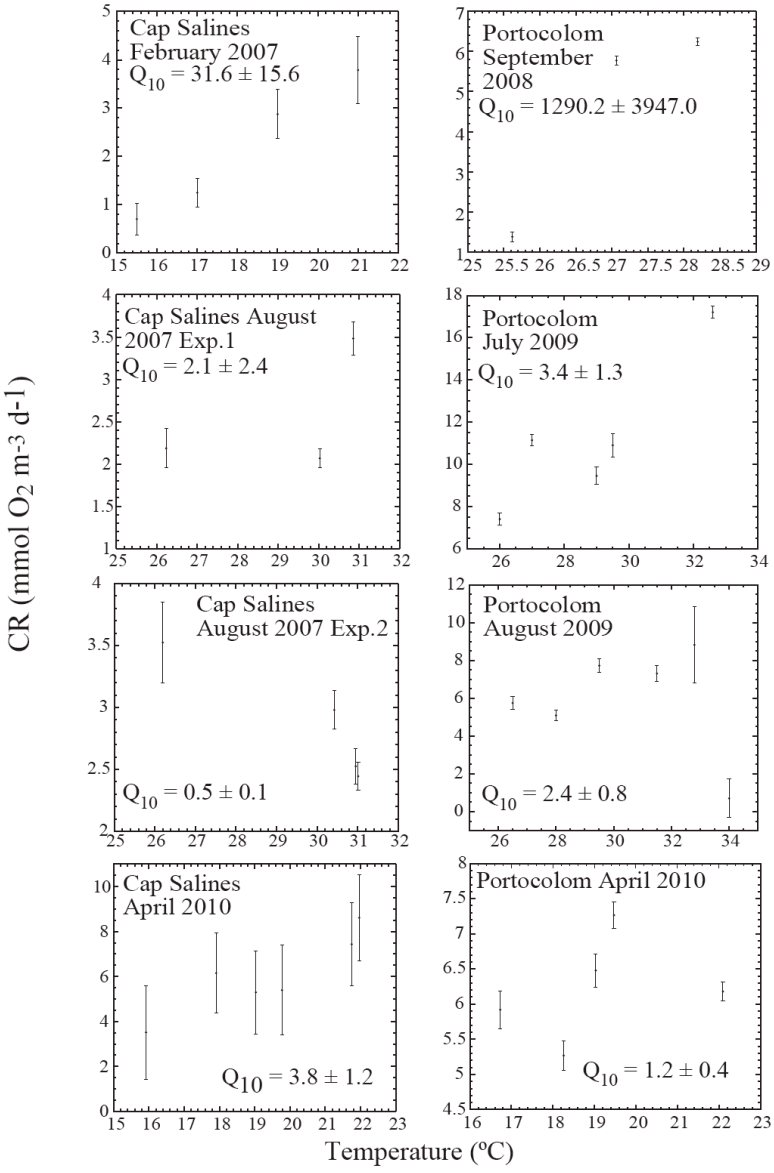

Figure 2. The relationship between planktonic community respiration (CR) rates (mmol O_2 m^{-3} d^{-1}) and temperature (º C) in the various experiments. Error bars represent ± SE.

The slope of the relationships between the natural logarithm and the inverse of the temperature multiplied by the Boltzmann constant (1/kT) also changed greatly across experiments, indicative of a broad range of Q_{10} and activation energy values across experiments (Table 3). Only six experiments from a total of 27 (22.2%) showed a statistically significant relationship between the natural logarithm of respiration (Ln R) and the inverse of temperature multiplied by the Boltzmann constant (1/kT). The low frequency of experiments

with significant Arhenius relationships between Ln R and 1/kT was attributable to the low power of the analysis, as the experiments only had between 3 and 6 data points to test this relationship. However, meta-analysis derived from the combined probability of the relationships obtained for individual experiments showed that there is indeed an overall tendency for a significant, negative relationship between Ln R and 1/kT (χ^2 test, $p < 0.001$, cf. combining probabilities from all individual tests of significance, Sokal and Rohlf 1995).

Table 3. Q_{10} and activation energy (kJ mol^{-1}) values for plankton community respiration with their associated standard error (SE) calculated using error propagation equations and as the SE of the fitted regression equation, respectively. * Indicate SE higher than Q_{10} values, not included in the detailed analysis.

Site	Community	month	year	Season	Metabolic rate	Q_{10}	SE	Ea (KJ mol-1)	SE	
Cap Salines	Planktonic	February	2007	winter	R	31.6	15.6	222.89	31.94	
Cap Salines	Planktonic	February	2007	winter	NCP	0.1	0.2	-125.44	81.43	*
Cap Salines	Planktonic	February	2007	winter	GPP	0.3	0.6	-78.69	132.30	*
Cap Salines	Planktonic	August	2007	summer	R	2.1	2.4	49.05	72.95	*
Cap Salines	Planktonic	August	2007	summer	NCP	0.0	0.0	-333.23	191.53	*
Cap Salines	Planktonic	August	2007	summer	GPP	0.8	0.4	-17.26	36.25	
Cap Salines	Planktonic	August	2007	summer	R	0.5	0.1	-50.16	16.14	
Cap Salines	Planktonic	August	2007	summer	NCP	0.6	0.2	-36.29	24.04	
Cap Salines	Planktonic	August	2007	summer	GPP	0.2	0.1	-102.23	43.84	
Portocolom	Planktonic	September	2008	autum	R	1290.2	3947.0	452.75	193.38	*
Portocolom	Planktonic	September	2008	autum	GPP	332.8	619.9	367.10	117.74	*
Portocolom	Planktonic	September	2008	autum	NCP	84.3	150.7	280.30	112.97	*
Portocolom	Planktonic	July	2009	summer	R	3.4	1.3	80.09	25.54	
Portocolom	Planktonic	July	2009	summer	NCP	0.03	0.03	-234.37	76.95	*
Portocolom	Planktonic	July	2009	summer	GPP	118.5	419.9	310.70	230.59	*
Portocolom	Benthic	July	2009	summer	R	2.0	1.3	45.84	40.70	
Portocolom	Benthic	July	2009	summer	GPP	0.5	1.3	-45.55	171.90	*
Portocolom	Benthic	July	2009	summer	NCP	0.03	0.1	-238.56	157.27	*
Portocolom	Planktonic	August	2009	summer	R	2.4	0.8	56.83	20.54	
Portocolom	Planktonic	August	2009	summer	GPP	9.1	2.9	143.16	20.65	
Portocolom	Planktonic	August	2009	summer	NCP	6.4	4.2	120.06	42.45	
Portocolom	Benthic	August	2009	summer	R	3.9	1.6	89.16	26.81	
Portocolom	Benthic	August	2009	summer	GPP	51.9	96.0	258.69	120.99	*
Portocolom	Benthic	August	2009	summer	NCP	6.4	9.7	121.19	99.38	*
Cap Salines	Planktonic	April	2010	spring	R	19.8	14.9	193.46	48.79	
Portocolom	Planktonic	April	2010	spring	R	1.2	0.4	13.60	23.39	
Portocolom	Benthic	April	2010	spring	R	4.2	7.6	93.69	117.00	*

Fitted Q_{10} values for respiration ranged widely from 0.46 ± 0.11 to 31.58 ± 15.62, with the minimum value corresponding to a summer experiment performed in August 2007 and the higher Q_{10} value to a winter experiment performed in February 2007, both with planktonic communities in Cap Salines (Fig. 2, Table 4). Q_{10} values for respiration were

significantly lower in summer (mean ± SE = 2.4 ± 0.6) than in spring (19.8) or winter (31.6, $F = 233.31$, $p < 0.0001$). Q_{10} values for benthic respiration (mean ± SE = 2.96 ± 0.94) were lower than those for planktonic community respiration (11.53 ± 6.09) but these differences were not statistically significant ($F = 0.71$, $p > 0.05$). There were no significant differences in Q_{10} between sampling sites ($F = 3.55$, $p > 0.05$).

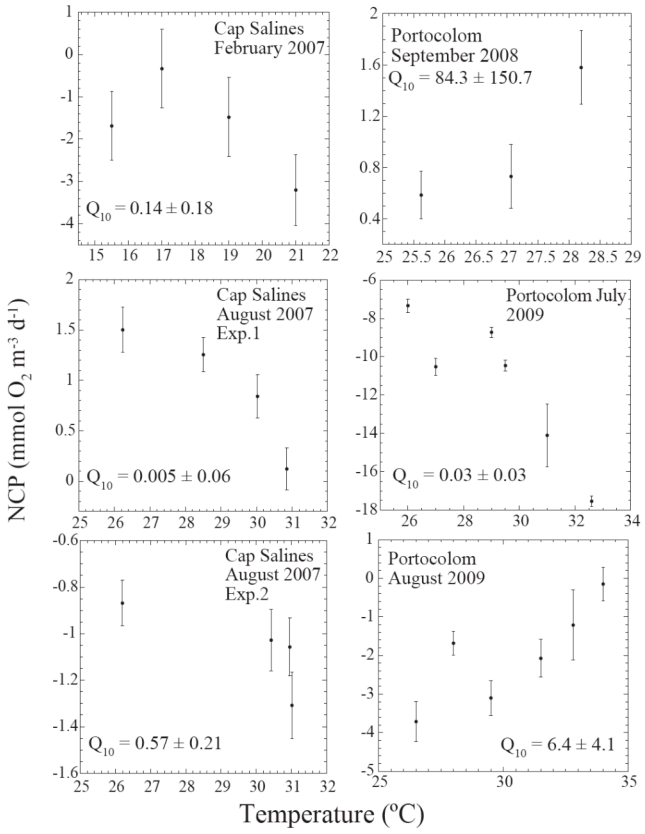

Figure 3. The relationship between planktonic net community production (NCP) rates (mmol O_2 m^{-3} d^{-1}) and temperature (º C) in the various experiments. Error bars represent ± SE.

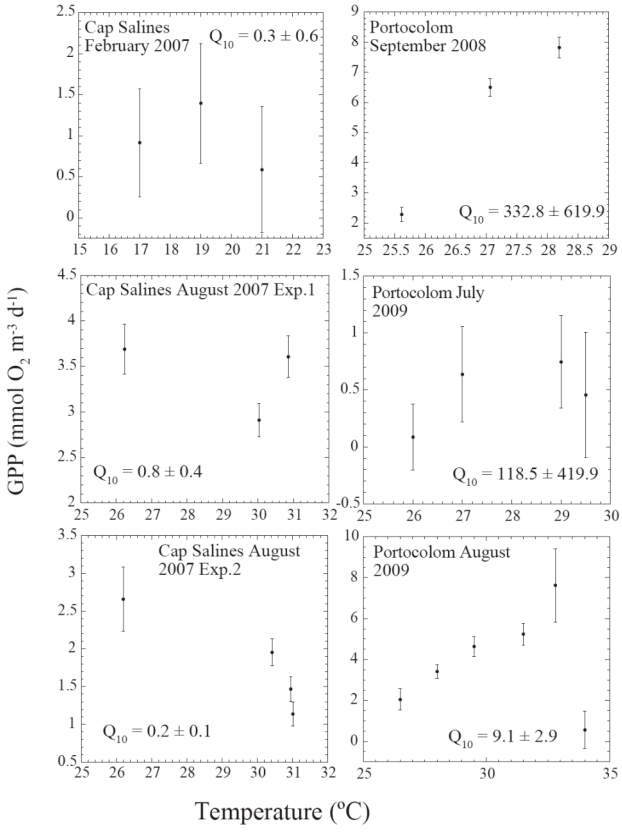

Figure 4. The relationship between planktonic gross primary production (GPP) rates (mmol O_2 m^{-3} d^{-1}) and temperature (º C) in the various experiments. Error bars represent ± SE.

Fitted Q_{10} values for gross primary production ranged broadly from 0.20 ± 0.14 to 9.07 ± 2.88, both values calculated for planktonic community experiments performed in summer, the minimum value at Cap Salines in August 2007 and the maximum Q_{10} value at Portocolom in August 2009. The mean calculated Q_{10} for planktonic GPP was 3.35 ± 2.87 and the median value was 0.76 (Figure 5, Table 4).

The activation energy (Ea) for respiration ranged between 45.84 and 222.89 kJ mol^{-1} K^{-1}, with a mean ± SE value of 114.71 ± 30.47 kJ mol^{-1} K^{-1}, throughout (Table 4). Although

activation energies tended to be higher in planktonic communities (mean ± SE = 138.31 ± 41.05 kJ mol^{-1} K^{-1}) than in benthic communities (mean ± SE = 67.50 ± 21.66 kJ mol^{-1} K^{-1}), there were not significant differences in Ea among community type ($F = 1.26$, $p > 0.05$). Activation energies were significantly higher in spring (193.46 kJ mol^{-1} K^{-1}) and winter (222.89 kJ mol^{-1} K^{-1}) than in summer (67.98 ± 10.04 kJ mol^{-1} K^{-1}, $F = 33.02$, $p < 0.01$). Activation energies were significantly higher in Cap Salines (mean ± SE = 208.17 ± 14.71 kJ mol^{-1} K^{-1}) than in Portocolom (mean ± SE = 67.98 ± 10.04 kJ mol^{-1} K^{-1}, $F = 63.79$, $p < 0.002$).

Table 4. Summary mean, standard error (SE), median, maximum and minimum values for Q_{10} for the different type of community (planktonic and benthic) and all experiments considered together (a), and only for the Q_{10} values with lower SE than its value (b).

a	Mean ± SE	Median	Minimum	Maximum	N
Q_{10} planktonic CR	192.85 ± 182.94	3.4	0.46	1290.2	7
Q_{10} planktonic GPP	76.94 ± 54.58	4.92	0.20	332.83	6
Q_{10} planktonic NCP	15.23 ± 13.85	0.36	0.01	84.3	6
Q_{10} benthic CR	3.38 ± 0.70	3.9	2.02	4.22	3
Q_{10} benthic GPP	26.22 ± 25.72	26.22	0.50	51.94	2
Q_{10} benthic NCP	3.19 ± 3.17	3.19	0.02	6.36	2
Q_{10} CR	136.01 ± 128.28	3.7	0.46	1290.2	10
Q_{10} GPP	64.26 ± 41.09	4.92	0.20	332.83	8
Q_{10} NCP	12.22 ± 10.34	0.35	0.01	84.3	8

b	Mean ± SE	Median	Minimum	Maximum	N
Q_{10} planktonic CR	11.53 ± 6.09	3.42	0.46	31.58	5
Q_{10} planktonic GPP	3.34 ± 2.87	0.76	0.2	9.07	3
Q_{10} planktonic NCP	3.46 ± 2.89	3.46	0.57	6.35	2
Q_{10} benthic CR	2.96 ± 0.94	2.96	2.02	3.9	2
Q_{10} CR	9.08 ± 4.49	3.42	0.46	31.58	7
Q_{10} GPP	3.35 ± 2.87	0.76	0.2	9.07	3
Q_{10} NCP	3.46 ± 2.89	3.46	0.57	6.35	2

Discussion

Warming experiments showed that benthic respiration rates were enhanced with warming in all experiments, whereas in one of the experiments (12.5%) planktonic respiration rates decreased with increasing temperature. In contrast, gross primary production only increased with incubation temperature in half of the experiments, but only 33.3% showed a significant Q_{10} value.

The experimental Q_{10} values for respiration for the current Mediterranean planktonic communities varied greatly from 0.46 to 31.58 across experiments, compared with reported

Q_{10} values for respiration of temperate planktonic communities ranging between 4.0 and 10.55, with a mean of 5.53 ± 0.59 and a median of 4.96 (Lefevre et al. 1994, Fig. 6). The experimental Q_{10} values for respiration for the current Mediterranean benthic communities varied between 2.02 and 3.9 across experiments. We found no published Q_{10} values for respiration of Mediterranean or temperate benthic communities, but these results were comparable to Q_{10} values for respiration of the macroalgae *Caulerpa prolifera*, the macroalgal species dominating the benthic community at Portocolom, reported to range between 1.41 and 2.6 (Terrados and Ros 1992).

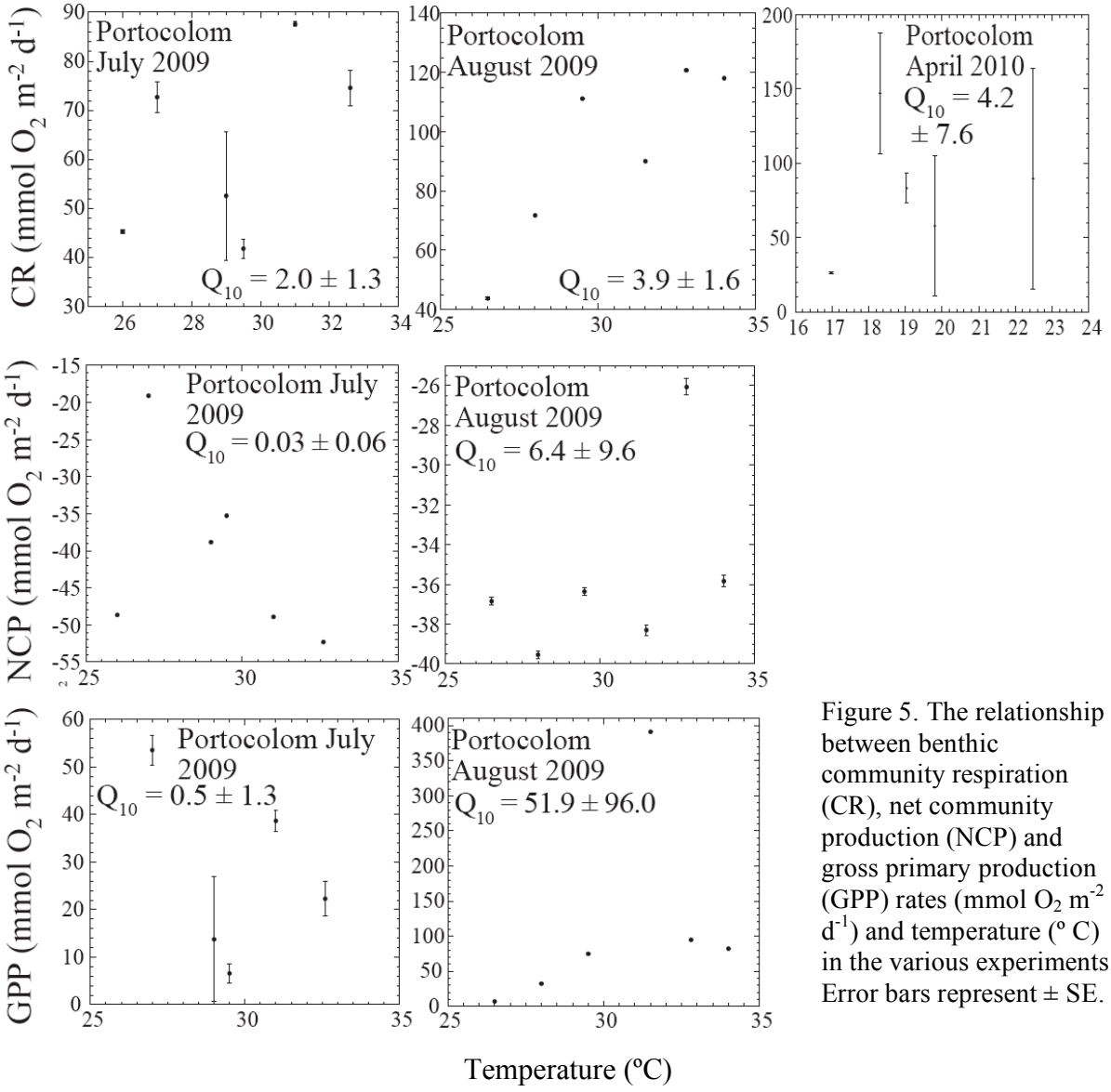

Figure 5. The relationship between benthic community respiration (CR), net community production (NCP) and gross primary production (GPP) rates (mmol O_2 m^{-2} d^{-1}) and temperature (º C) in the various experiments. Error bars represent ± SE.

The experimental Q_{10} values for Mediterranean planktonic gross primary production varied greatly from 0.2 to 9.1 across experiments. Our results can be compared with Q_{10}

values estimated for temperate planktonic communities, reported to range between 0.69 and 3.03 (Lefevre et al. 1994) and with Q10 values estimated for temperate microplankton, reported to be 2.7 (Li and Dickie 1987). Overall, Q_{10} values for respiration were higher than for GPP, consistent with previous observations that Q_{10} values for respiration of temperate planktonic communities tend to be higher than for production (Lefevre et al. 1994, Fig. 6).

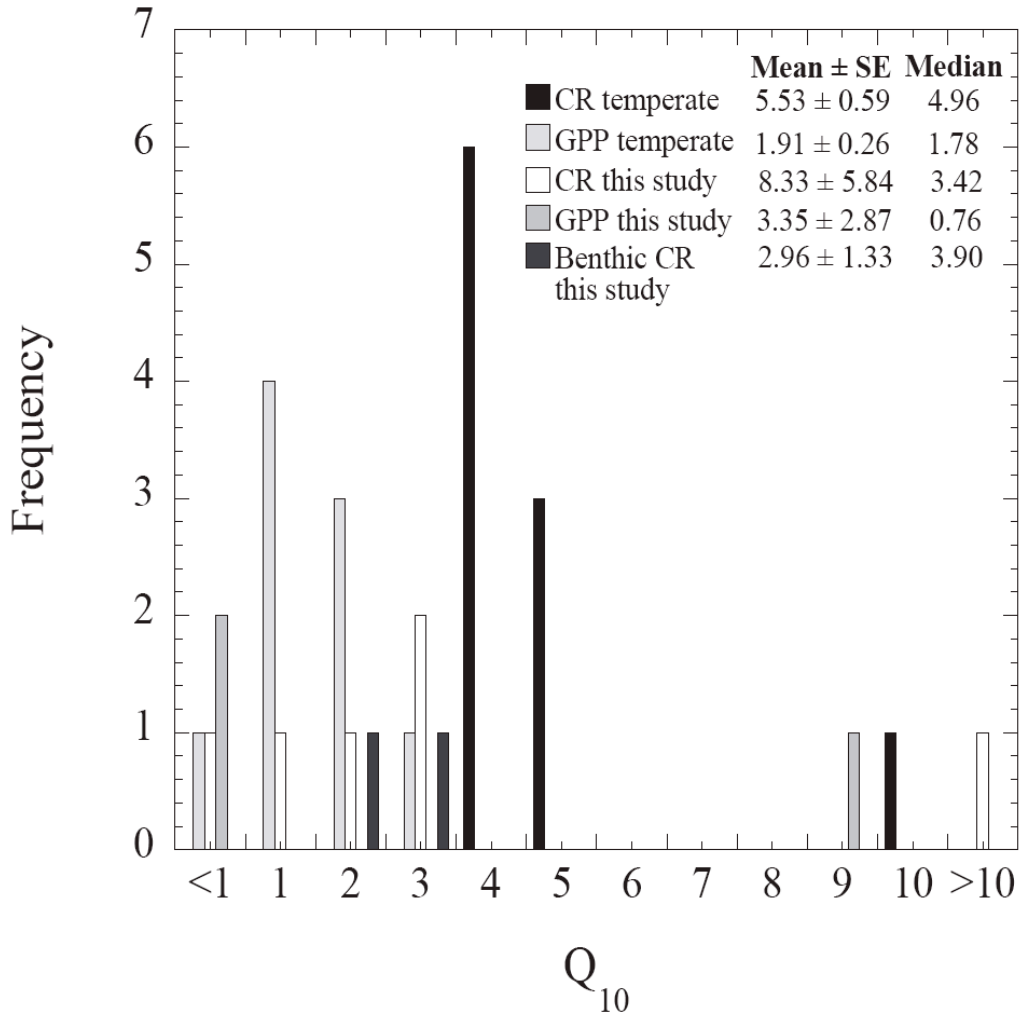

Figure 6. Frequency distribution for Q_{10} values for primary production and community respiration in natural temperate planktonic and benthic communities, and the corresponding mean ± SE and median values for this study and for other temperate communities. Data for other temperate communities derived from Lefreve *et al.* 1994 and Li and Dickie 1987.

The mean activation energy for planktonic respiration found in this study (114.7 ± 30.47 kJ mol^{-1} K^{-1}) is similar to that found for temperate planktonic communities from the Menai Strait (113.0 ± 6.1 kJ mol^{-1} K^{-1},Lefevre et al. 1994) and Arctic planktonic communities (115.5 ± 20.5 kJ mol^{-1} K^{-1}, Vaquer-Sunyer et al. 2010). Although the activation

energy for respiration is consistent with other activation energies for respiration derived from experimental studies, it is higher than the activation energies values calculated from theoretical models, such as that derived by Lopez-Urrutia et al. (2006), where the predicted activation energy for community respiration was 27.02 kJ mol^{-1} K^{-1}, as well as values derived from the relationship between respiration rates, standardised to chlorophyll a concentration, and water temperature for oceanic communities, which derived an activation energy for community respiration of 68.51 ± 5.79 kJ mol^{-1} K^{-1} (Regaudie-de-Gioux and Duarte in press).

The Q_{10} values for respiration did not differ between sample sites ($F = 1.2$, $p > 0.05$) or between planktonic and benthic community ($F = 0.3$, $p > 0.05$). However, there were statistically significant differences between seasons, with communities having higher Q_{10} values in winter than in summer or spring ($F = 199.1$, $p < 0.0001$). An extremely high Q_{10} value (31.6) was derived for planktonic communities of Cap Salines in February 2007, when the in situ temperature was low (15.5ºC) compared to the other experiments. Q_{10} values differ considerably depending on the range of temperatures assessed (Berges et al. 2002), being typically highest at the lower end of the natural temperature range (Pomeroy and Wiebe 2001). Therefore, the high value derived in February 2007, can be attributed to the fact that the community was growing at the minimum annual temperature at the time of the experiment. Organisms adapt to life within a particular thermal window, when organisms are maintained outside their thermal optimal, oxygen levels in body fluids can decrease, as a consequence of excessive oxygen demand at high temperatures or insufficient aerobic capacity of mitochondria at low temperatures (Portner 2001). Indeed a number of experiments showed decreased, rather than increased, metabolic rates when these were assessed above 30 º C, above the current temperature range of the ecosystems examined. For instance, respiration rates decreased in Portocolom at 34ºC (August 2009, Fig. 2), a temperature well above the current temperature range in this ecosystem (maximum temperature, 29.6 º C, Vaquer-Sunyer, unpubl. results).

The experimental results reported here represent the likely physiological impacts of warming on metabolic processes, but ignore the changes that may be derived from a shift in community structure with warming.

As Q_{10} values did not differ significantly for planktonic or benthic respiration rates, a general mean Q_{10} value of 9.1 ± 4.5 for respiration of coastal Mediterranean communities can be calculated, leading to a 73% increase of respiration rates with a 6ºC warming. This 73% increase compares to a 52% increase for planktonic gross primary production.

The results reported here support the prediction that Mediterranean warming will increase respiration rates faster than primary production. Indeed, primary production can even decrease with warming, as observed in over half of the experiments. The ecological consequences of a simultaneous decrease in primary production and increase in respiration rates will be that Mediterranean ecosystems will become net heterotrophic with a tendency to emit CO_2 to the atmosphere and deplete the oxygen pool, possibly rendering Mediterranean coastal ecosystems prone to experience hypoxia.

Acknowledgments

This research is a contribution to the ‘‘Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery (WISER)’’ project, funded by FP7 (contract 226273) and project MEDEICG, funded by the Spanish Ministry of Science and Innovation (number CTM2009-07013). R. Vaquer-Sunyer was funded by the THRESHOLDS integrated project, funded by the European Union 6th Framework Program and WISER project. We thank R. Martínez, P. de la Fuente, L. Royo, M. Noguera, A. Steckbauer and R. García for help on sampling and M. Sánchez-Camacho for help on controlling experimental temperature and setting of experimental tanks.

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Chapter 3

Temperature dependence of oxygen dynamics and community metabolism in a shallow Mediterranean macroalgal meadow (*Caulerpa prolifera*)

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Manuscript, 2011

Abstract

High-frequency dissolved oxygen (DO) measurements have been used to estimate gross primary production (GPP), net ecosystem production (NEP), and community respiration (CR) in a shallow macroalgae (*Caulerpa prolifera*) ecosystem in a highly human influenced Mediterranean closed Bay. Daily averaged GPP and CR ranged from 0 to1488.5 and -88.7 to -1322 mmol O_2 m^{-2} d^{-1}, respectively. The higher GPP and CR were calculated for the same day, when daily averaged water temperature was 28.0 ºC, and resulted in a positive NEP of 166.5 mmol O_2 m^{-2} d^{-1}. The ecosystem was net heterotrophic during the studied period, probably subsidized by allochthonous organic inputs from ground waters and from the surrounding town and boating activity. Dissolved organic Carbon (DOC) dynamics strongly depend on metabolic rates. Oxygen dynamics and metabolic rates strongly depend on water temperature, with lower oxygen content at higher temperatures. The probability of hypoxic conditions increased at a rate of 0.39 (± 0.14) % per Celsius degree. Global warming will increase the likelihood of hypoxia in the bay.

Keywords: Warming, hypoxia, oxygen dynamics, benthic metabolism

Introduction

Dissolved oxygen is an important property in the aquatic ecosystems (Odum 1956, Venkiteswaran et al. 2007) because most metazoans require high dissolved oxygen concentrations (Vaquer-Sunyer and Duarte 2008). Yet, Oxygen cycling is particularly sensitive to eutrophication (Paerl 2006) and climate change (Conley et al. 2009). Indeed, no other environmental important variable has changed so drastically in such a short period of time as dissolved oxygen has (Diaz 2001, Diaz and Rosenberg 1995).

Dissolved oxygen concentration is controlled by multiple factors, such as air-water exchange and aquatic metabolism, among others. Aquatic metabolism is a key factor affecting changes in dissolved oxygen in coastal ecosystems, which are dependent on the net balance between oxygen production through gross primary production (GPP) and consumption through community respiration (CR). Elucidating ecosystem metabolism is, therefore, essential to understand oxygen and carbon dynamics. However, alternatively, the observation of oxygen dynamics can allow metabolic processes to be recovered and estimated (Odum 1958). The use of oxygen probes and loggers to derive ecosystem metabolism is a widespread approach to estimate river and lake ecosystem metabolism (Cole

et al. 2000, Coloso et al. 2008) but has been applied to a much more limited stage in coastal waters (Odum and Hoskin 1958, Odum and Wilson 1962, Ziegler and Benner 1998).

However, this approach has potential beyond the elucidation of ecosystem metabolism, for multiparametric probes record other properties, such as temperature, that are believed to play a key role in controlling oxygen dynamics. Indeed, water temperature affects air-sea fluxes by affecting oxygen solubility and affects oxygen dynamics by affecting ecosystem metabolism (Brown et al. 2004). Indeed, both community respiration and gross primary production are expected to increase with increasing temperature (Brown et al. 2004), but community respiration is expected to show a steeper response to increased temperature than gross primary production does (Harris et al. 2005). Warming, which reduces the solubility of oxygen and increases metabolic oxygen consumption over production, is therefore expected to affect the likelihood of hypoxia in coastal ecosystems (Conley et al. 2009). However, predictions that temperature should affect the metabolism and likelihood of hypoxia of coastal ecosystems rely largely on model calculations, with limited empirical tests. The likelihood of hypoxia is also greater in semi-enclosed bays with limited exchange with coastal waters and eutrophic coastal environments, where increased nutrient inputs lead to enhance organic production and oxygen demand (Karim et al. 2003; Zhang et al. 2010). Here, we use continuous oxygen and temperature records in a shallow, impacted Mediterranean Bay (Portocolom, Majorca Island, W. Mediterranean) to test the predicted temperature-dependence of ecosystem metabolism and the likelihood of hypoxia.

Materials and methods

Study area

The study was conducted in Portocolom, a human-influenced semi-enclosed bay in the Southeast of the island of Majorca (Fig. 1). The study area was located at the Western area of the bay, protected from storms by land. The sampling area is covered by a *Caulerpa prolifera* meadow growing on muddy sediment. Portocolom is a highly human influenced area, receiving important amounts of organic matter though groundwater discharge (Basterretxea et al. 2010) and from deficient sewage systems. The bay is highly impacted by nutrient and organic inputs from the surrounding town and boating activity. Fish farming activities have been conducted in the interior of the Bay during 22 years until 2005 further increasing the organic load of the sediments.

Caulerpa prolifera (Forsskal) Lamouroux, 1809, is an opportunistic Mediterranean native species, widely distributed throughout the Mediterranean Sea, with the exception of the colder areas, such as the Gulf of Lyons and the Adriatic Sea (Sanchez-Moyano et al. 2001). This macroalgae thrives particularly in sheltered, muddy sediments shallower than 20 m (Mateu-Vicens et al. 2010, Sanchez-Moyano et al. 2001), such as those present in Portocolom. *C. prolifera* is a fast-growing macroalgae, which has spread in the study area during the past decades due to increased nutrient loading of the bay (Holmer et al. 2004).

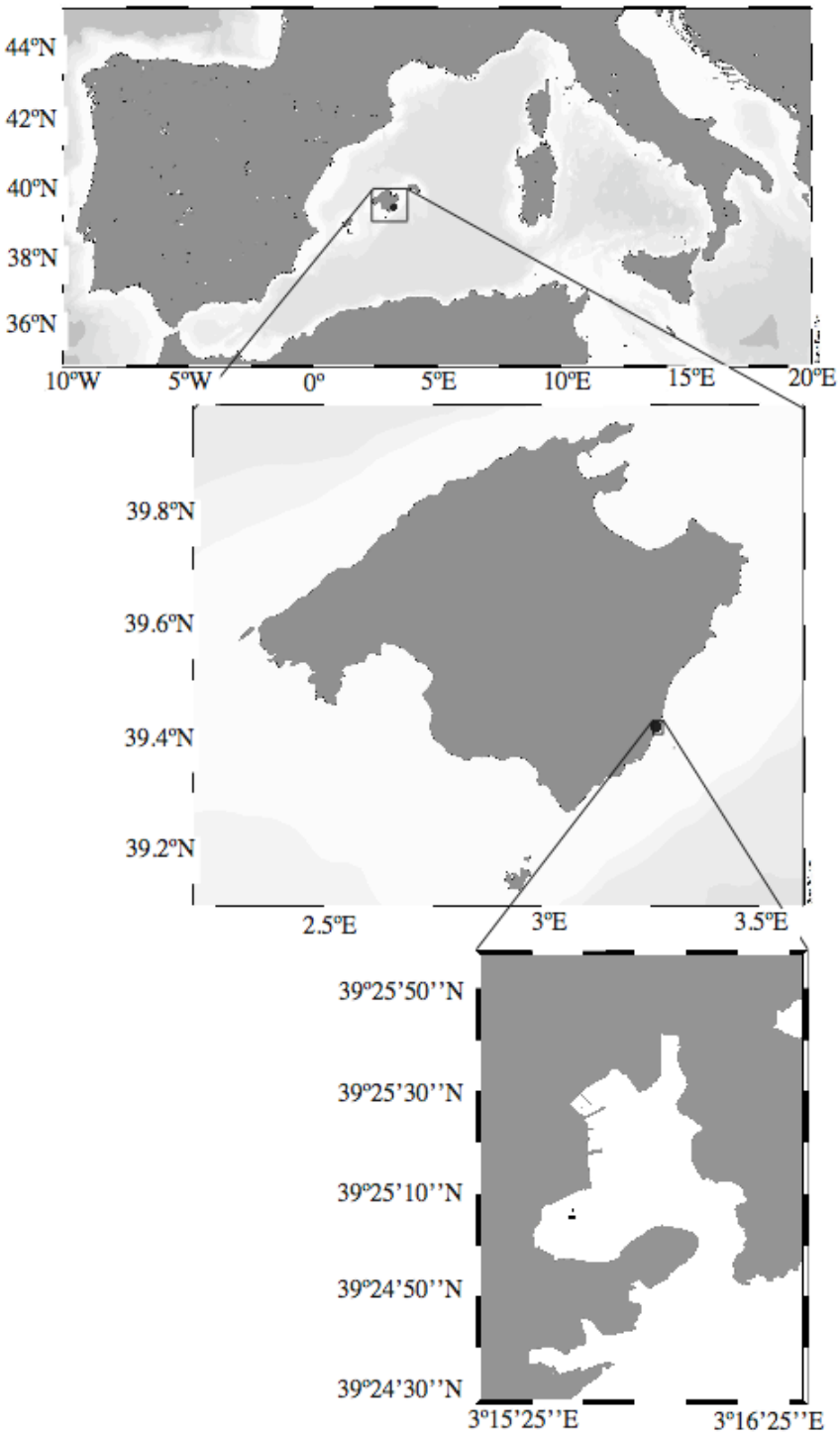

Figure 1. Map showing the sampling area. The flag indicates the place where the probes were moored.

Continuous oxygen and temperature records

Continuous records of oxygen, temperature and salinity were derived using two different types multiparamentric probes an Eureka Manta® multiprobe fitted with a Clark cell dissolved oxygen (DO) sensor, and a Hydrolab DS5X fitted with a Hach Luminescent Dissolved Oxygen sensor (Hach LDO^{TM}) and a central cleaning system to avoid fouling in the DO sensor. All probes were calibrated in water-saturated air prior to deployment. Both probes had temperature sensors, which were calibrated against a handheld Pt 100 Platinum high precision digital thermometer (model 6-109-81.2450, accuracy ± 0.01ºC).

Probes were deployed at a depth of 2.8 m in the Bay close to a mooring block to avoid possible damage by anchoring. Nevertheless, one of the multi-probes was lost due to anchoring and 2 of them were flooded during deployments, leading to some gaps in data coverage. Whereas the study started on November 2008 and finished on February 2010, the data reported here only include data since 15th May 2009, due to the problems reported above.

Samples for dissolved organic carbon (DOC) were taken monthly from surface and bottom waters. Dissolved organic carbon (DOC) measurements were performed on 10 ml water samples sealed in precombusted glass ampoules (450ºC for 5 h) and kept acidified (pH 1–2) until analysis by high temperature catalytic oxidation on a Shimadzu TOC- 5000A. Standards of 44–45 and 2 μmol C l^{-1}, provided by D.A. Hansell and Wenhao Chen (Univ. of Miami), were used to assess the accuracy of the estimates.

Water samples from surface and bottom waters were analysed for nutrient (N, P, Si) concentration monthly. Determination of water surface and air CO_2 was performed monthly using a high-precision (±1ppm) non-dispersive infrared gas analyzer (EGM-4, PP-systems) averaging measurements at 1 min intervals. Before entering the gas analyzer, the gas stream was circulated through a Calcium Sulfate column to avoid interferences from water vapour. To determinate water surface CO_2 we used a peristaltic pump, and a gas exchange column (Mini-Module 1.25x9 Membrane Contactor, Celgard) with an effective surface area of 0.5 m^2, a total volume of 52 ml and a water flow of about 300 ml min^{-1} were utilized for air-surface sea-water equilibration, resulting in a residence time of only 10 s and no temperature difference between *in situ* seawater and water in the equilibrator. The gas phase was continuously circulated through the equilibrator and the infrared gas analyzer. The gas analyzer was calibrated using two dry standards each: pure nitrogen (0.0 ppm CO_2) and a gas

mixture of CO_2 and N_2 containing a CO_2 molar fraction of 541ppm, which revealed an accuracy of ±1ppm in the determinations of CO_2.

Metabolic rates

Metabolic calculations follow Cole et al. (2000), using a program written by Coloso et al. (2008), modified for our application and implemented in MATLAB (version 7.5, the Mathworks Inc.). Changes in DO over 15 minutes intervals were assumed to result from three processes: Net Ecosystem Production (NEP = GPP – CR), diffusive exchange with the atmosphere (D), and other inputs and outputs of DO (A) such as flux between water layers and lateral flows.

(1) $\Delta DO = NEP + D + A$

Diffusion with the atmosphere is regulated by the difference in DO concentration relative to atmospheric equilibrium (DOsat) and the air-sea gas transfer velocity for oxygen (k) at a given temperature.

(2) $D = k\,(DOsat - DO)$

where D can be either negative (removal of DO from system) or positive (addition of DO to system). Wind speed measured on the Bay at 10 minutes intervals was used to predict k_{600} following Cole and Caraco (1998) and k was calculated from k_{600} using the Schmidt number equations of Jahne et al. (1987).

During night, in the absence of sunlight, no photosynthetic production occurs, so that the only metabolic rate operating is respiration, R, which can then be extracted from the rate of change in O_2, corrected for other fluxes, at night (NEP at night = R). R was derived from oxygen changes measured from 1 hour past sunset to 1 hour before the sunrise. During the day, in the presence of solar radiation, NEP is the result of the balance between GPP and R. NEP was, then, calculated from the rate of change in DO from sunrise to sunset, corrected for other processes. During the day, R cannot be measured directly, and we assumed that daytime R equals that calculated during night. GPP was then estimated by the addition of NEP and R calculated during daytime. The assumption of equal R rates during day and night can lead to underestimates of R and, therefore, GPP, because there is evidence that respiration rates are likely to be higher during daylight than during night (Grande et al. 1989, Pace and Y.T. 2005, Pringault et al. 2007), but would not bias NEP estimates (Cole et al. 2000). In order to derive daily metabolic rates, individual estimates of GPP, NEP and R resolved at 15-minute intervals were accumulated over each 24h period during deployments.

Temperature-dependence of oxygen dynamics and metabolic rates

Dissolved oxygen data were grouped in 1ºC temperature bins and the frequency distribution of DO at each 1ºC bin was examined. Quantile regression was used to describe the temperature-dependence of the probability distribution of DO at different temperature bins. The response of DO to temperature was described by fitting the relationship between the 99%, 95%, 50% (median), 5% and 1% quantiles of the distribution of oxygen concentration within 1º C temperature bins and water temperature. Quantile regression estimates multiple rates of change (slopes), from the minimum to maximum response, providing a more thorough description of the relationships between variables, which are missed by other regression methods focused on prediction of the mean value (Cade and Noon 2003). Quantile regression can be considered as an extension of classical least squares estimation of conditional mean models to the estimation of a compilation of models for several conditional quantile functions, considering the median as the central parameter (Koenker 2005).

The probability of hypoxia was calculated as the fraction of DO measurements below hypoxia thresholds, examined both for individual months and 1 ºC water temperature bins. The DO thresholds for marine benthic organisms used to designate waters as hypoxic are temperature-dependent, increased with temperature at an average rate of 0.06 mg O_2 l^{-1} ºC $^{-1}$ (Vaquer-Sunyer and Duarte 2011). We thus calculated, for each day, the threshold of hypoxia by increasing the threshold at a rate of 0.06 mg O_2 l^{-1} ºC $^{-1}$ of mean daily temperature and considering a baseline threshold for hypoxia of 3.5 mg O_2 l^{-1} (Steckbauer *et al., submitted*) for the mean temperature of the bay (22 ºC). We also calculated the maximum duration of periods under continuous hypoxia for each 1 ºC temperature bin of for each month.

The temperature-dependence of metabolic rates was described by the Activation Energies for R and GPP (E_a, units eV), derived from the slope of an Arrhenius plot of the natural logarithm of respiration rate or GPP against the inverse of the temperature (Kelvin) multiplied by the Boltzmann´s constant. The Q_{10} (the relative rate of increase in the metabolic rate expected for a 10ºC temperature increase) was calculated using, the equation (Raven and Geider 1988):

$Q_{10} = e^{\left(\frac{10E_a}{RT^2}\right)}$, where R is the gas constant (8.314472 mol^{-1} K^{-1}), T is the mean absolute temperature across the range over which Q_{10} was measured (K), and E_a is the activation energy (J mol^{-1}), derived from the slope of the Arrhenius equation relating the natural logarithm of respiration rates or GPP (in mmol O_2 m^{-3} d^{-1}) to 1/kT, where k is the Boltzmann's constant (8.62 x10^{-5} eV k^{-1}) and T is the temperature (Kelvin). The slope of this relationship is the activation energy (E_a) in eV, which was converted to J mol^{-1} using a conversion factor of 96486.9. Statistical analyses were performed using JMP 7.02 for simple regression analyses and ANOVA, and R for quantile regression.

Results

Temperature ranged from 12.0 ºC in December 2009 to 29.6 ºC in August 2009, and surface DOC concentrations varied between 69 and 186 µmol C l^{-1}. The lower DOC concentration was found in December 2009, while the highest DOC concentration was measured in September 2009. The DOC concentration in bottom waters varied between 136 and 275 µmol C l^{-1}, measured in December and September 2009, respectively (Table 1). Bottom DOC concentrations were much higher than surface ones (207 ± 15 µmol C l^{-1} and 120 ± 21 µmol C l^{-1}, respectively, $F = 12.12$, $p < 0.005$). Nutrient concentrations were highly variable, but generally high, indicative of eutrophic conditions in the bay (Table 1). Mean (±SE) total phosphorus, total nitrogen, phosphate and silicate in surface waters were 3.5 ± 1.1 µmol P L^{-1}, 49.1 ± 13.0 µmol N L^{-1}, 0.9 ± 0.4 µmol P L^{-1} and 7.3 ± 4.8 µmol Si L^{-1}, respectively, similar to those in bottom waters (3.7 ± 1.3 µmol P L^{-1}, 43.6 ± 8.1 µmol N L^{-1}, 0.7 ± 0.4 µmol N L^{-1} and 6.0 ± 3.5 µmol Si L^{-1}, respectively). Nutrient concentrations tended to be highest in July and August (Table 1). Water surface CO_2 varied between 389 and 490 ppm during the studied period, with the highest values measured in August and September 2009. Air pCO_2 varied between 354 and 375 ppm (Table 1). pCO_2 was always higher in water than in air, suggesting that the Bay acts as a CO_2 source to the atmosphere.

Dissolved oxygen ranged from 28.1 to 208.8 % saturation, corresponding to 1.46 mg O_2 L^{-1} to 13.68 mg O_2 L^{-1} along the study, both extremes measured in summer 2009. The lowest oxygen content was measured before sunset on August 6, 2009, when water temperature was 27.8ºC, whereas the highest dissolved oxygen concentration was measured on July 24, 2009 after midday (3:20 pm) in waters at 27.4ºC.

Table 1. Nutrients concentrations for total Phosphorus (P total), total Nitrogen (N total), phosphate, silicate and the ratio between nitrates and nitrites (NO2/NO3) data for each month (all concentrations are given in µmol l^{-1}). As well as TOC and pCO2 in air (pCO_{2a}) and water (pCO_{2w}) data measured each month. BDL: below detection limit.

Month	Surface P total (µM)	N total (µM)	Phosphate (µM)	Silicate (µM)	NO_2/NO_3	DOC (µM)	pCO_2a (ppm)	pCO_2w (ppm)
May	3.6	43.8	0.8	2.6	14.3		354	389
June							358	481
July	2.0	110.4	0.3	5.3	1.9		359	471
August	8.8	71.2	3.0	33.8	7.3	179	356	490
September	0.4	16.1	0.4	5.2	0.6	186	375	490
October						118	370	464
November	2.7	34.6	BDL	0.6	0.4	93	368	400
December	2.5	32.3	0.3	1.1	1.2	76	371	450
January	4.4	35.1	0.6	2.8	4.4	69		
February								

	Bottom P total (µM)	N total (µM)	Phosphate (µM)	Silicate (µM)	NO_2/NO_3	DOC (µM)
May						
June						201
July	3.7	41.8	0.2	5.5	1.3	246
August	8.9	71.6	2.4	21.3	6.7	226
September	0.4	15.4	0.3	5.7	0.2	275
October						201
November	3.3	48.1	0.0	1.1	0.4	188
December	2.5	37.7	0.8	1.1	0.7	136
January	3.7	47.2	0.6	1.5	1.4	183
February						

Daily averaged GPP ranged between 0 and 1488.5 mmol O_2 m^{-2} d^{-1}, with the lowest gross primary production rates measured on October 21 and 22 and December 22 and 23, 2009, when the daily averaged temperature was 20.42 and 20.44 º C and 13.44 and 14.22ºC, respectively. Daily averaged CR rates in those days were 291.0, 282.8, 214.7 and 208.9 mmol O_2 m^{-2} d^{-1}, respectively, resulting in negative NEP rates indicative of heterotrophic conditions. The highest GPP rate was measured on August 6, 2009, when daily-averaged temperature was 28.0 ºC and CR was 1322.0 mmol O_2 m^{-2} d^{-1}, the highest calculated respiration rate, resulting in a positive net ecosystem production of 166.5 mmol O_2 m^{-2} d^{-1}. The mean (± SE) calculated GPP for the studied period (May 2009 to February 2010) was 455.2 ± 20.0 mmol O_2 m^{-2} d^{-1}, and the median was 423.1 mmol O_2 m^{-2} d^{-1} (Fig. 2, Table 2).

Daily averaged CR ranged between 88.7 and 1322 mmol O_2 m^{-2} d^{-1}. The minimum respiration rate was measured on January 26, 2010 at a water temperature of 13.4ºC when GPP was 58.7 mmol O_2 m^{-2} d^{-1} and NEP was negative at -30.0 mmol O_2 m^{-2} d^{-1}. The highest respiration rate was obtained concurrently with the highest calculated GPP, on August 6. The

mean calculated CR for the studied period was 490.3 ± 17.5 mmol O_2 m^{-2} d^{-1}, and the median was 454 mmol O_2 m^{-2} d^{-1} (Fig. 2, Table 2).

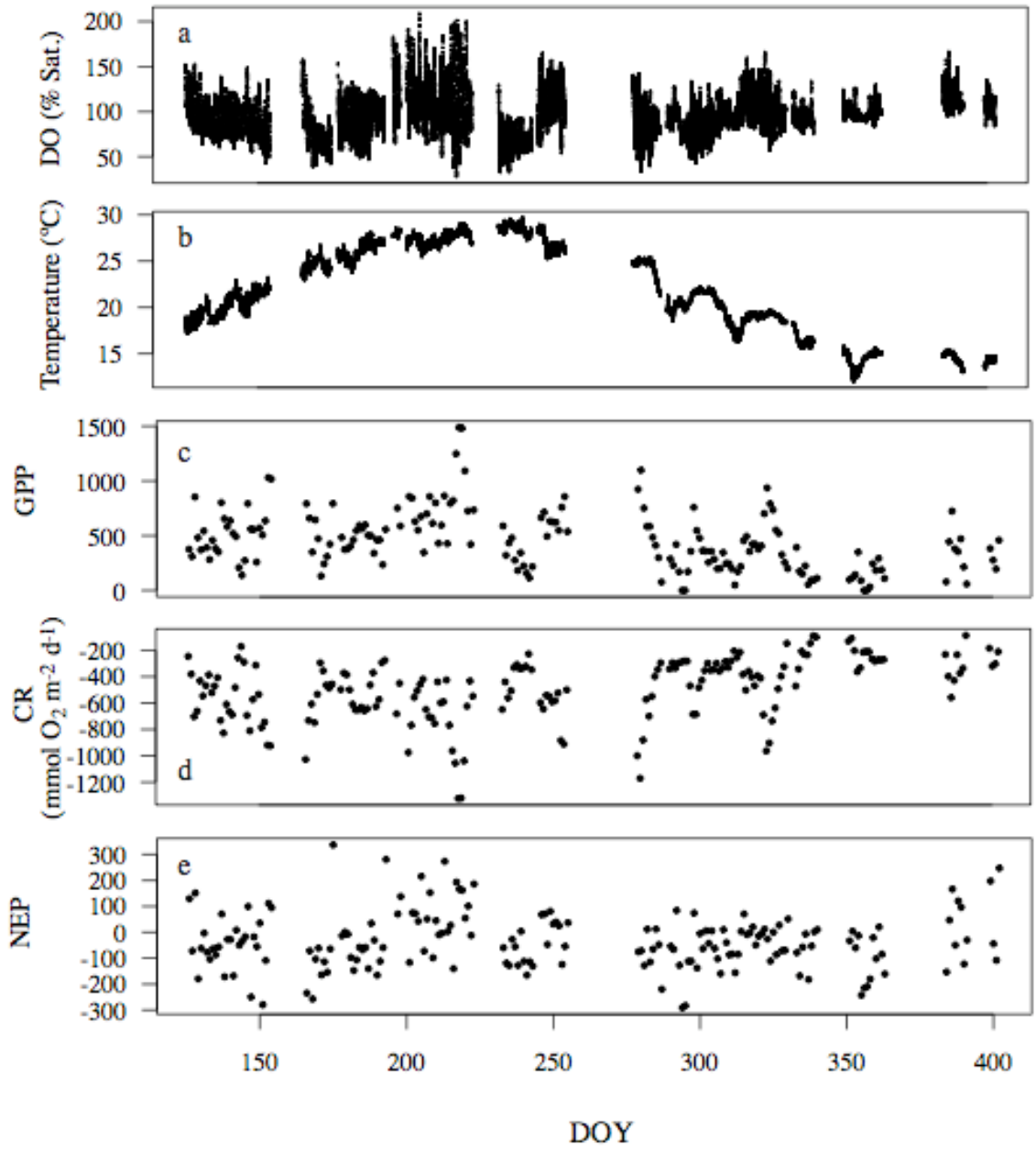

Figure 2. Time series of (a) dissolved oxygen (DO, % saturation) and (b) temperature during the study period. Daily averaged metabolic rates of (c) gross primary production (GPP), (d) community respiration (CR) and (e) net ecosystem production (NEP), all expressed in mmol O_2 m^{-2} d^{-1}.

Daily averaged NEP ranged between heterotrophy with a minimum value of -291.0 mmol O_2 m^{-2} d^{-1} and autotrophic, with a maximum NEP of 337.1 mmol O_2 m^{-2} d^{-1}. The lowest NEP was obtained in October 21 and the maximum NEP was obtained on June 24. The mean (±SE) calculated NEP for the studied period was -35.2 ± 8.1 mmol O_2 m^{-2} d^{-1}, indicating that

the ecosystem was net heterotrophic, with respiration rates exceeding the ecosystem primary production. The median was also negative at -48.9 mmol O_2 m^{-2} d^{-1}, with 66.3% of the days showing negative NEP values (Fig. 2, Table 2). Monthly averaged metabolic rates are showed in Figure 3 and summarized in Table 3. In general, the highest metabolic rates were found in summer (Table 4).

Table 2. Summary of daily averaged metabolic rates. Mean, SE, median, range and number of estimates (N) of areal gross primary production (GPP), community respiration (CR) and net ecosystem production (NCP) rates (mmolO2 m^{-2} d^{-1}).

	GPP	NEP	CR	Temperature (ºC)
Mean	455.19	-35.15	-490.34	21.94
SE	20.03	8.06	17.49	0.34
Median	423.1	-48.9	-454	21.56
Maximum	1488.5	337.1	-89	28.96
Minimum	0	-291	-1322	12.94
N	184	184	184	184

Table 3. Summary of mean and SE monthly averaged metabolic rates: areal gross primary production (GPP), community respiration (CR) and net ecosystem production (NCP) rates (mmolO2 m^{-2} d^{-1}).

Month	GPP	SE	CR	SE	NEP	SE	Mean T	SE	N
May	475.45	36.67	525.64	37.11	-50.19	1.69	19.97	0.24	26
June	538.13	64.65	590.92	54.98	-63.28	29.75	24.34	0.32	17
July	579.52	32.01	582.09	31.25	-2.58	22.90	26.81	0.15	26
Aug.	610.98	91.16	611.87	71.70	-0.89	28.51	28.17	0.11	22
Sep.	645.61	36.55	632.78	48.54	12.82	22.12	26.78	0.31	10
Oct.	414.13	57.60	491.57	51.36	-77.44	20.60	22.13	0.42	24
Nov.	381.43	40.57	426.66	37.93	-45.23	12.07	18.98	0.22	28
Dec.	134.40	22.63	218.90	17.93	-84.50	21.12	14.93	0.27	19
Jan.	341.19	83.46	331.71	54.50	9.48	44.04	14.58	0.23	8
Feb.	328.51	67.00	255.25	38.57	73.25	101.30	14.29	0.08	4

The average DOC in both bottom and surface waters were positively correlated with monthly averaged GPP (R^2= 0.87, $p < 0.0008$ and R^2= 0.83, $p < 0.02$, respectively, Fig. 4a). Monthly averaged CR was also positively correlated with average DOC in bottom waters and in surface waters (R^2= 0.78, $p < 0.004$ and R^2= 0.87, $p < 0.007$, respectively, Fig. 4b).

Table 4. Seasonal average of metabolic rates (mean ± SE). All the rates are expressed in mmol O_2 m^{-2} d^{-1}. The letters indicate the results of the Tukey HSD test, whereby the metabolic rate did not differ significantly from seasons with the same letter.

Season	GPP		CR		NEP		GPP/CR		N
Spring	509.5 ± 40.8	A	-571.0 ± 35.6	A	-61.5 ± 17.8	B	0.89 ± 0.05	A B	36
Summer	586.6 ± 30.4	A	-586.0 ± 26.5	A	0.7 ± 13.2	A	0.99 ± 0.04	A	65
Autum	352.1 ± 30.9	B	-411.1 ± 26.9	B	-59.0 ± 13.4	B	0.82 ± 0.04	B	63
Winter	254.8 ± 54.8	B	-283.8 ± 47.8	B	-29.07 ± 23.8	A B	0.86 ± 0.06	A B	20

Metabolic rates increased significantly with increasing temperature, resulting in similar overall activation energies for GPP and CR of 0.55 ± 0.07 eV (or 53.34 ± 6.89 KJ mol^{-1} K^{-1}) and 0.45 ± 0.05 eV (or 43.58 ± 4.87 KJ mol^{-1} K^{-1}), respectively, derived from the slope of the Arrhenius plot of each metabolic rate (Fig. 5a and 5b). Calculated Q_{10} values for GPP and Community respiration were 2.06 ± 0.39 and 1.98 ± 0.21, respectively.

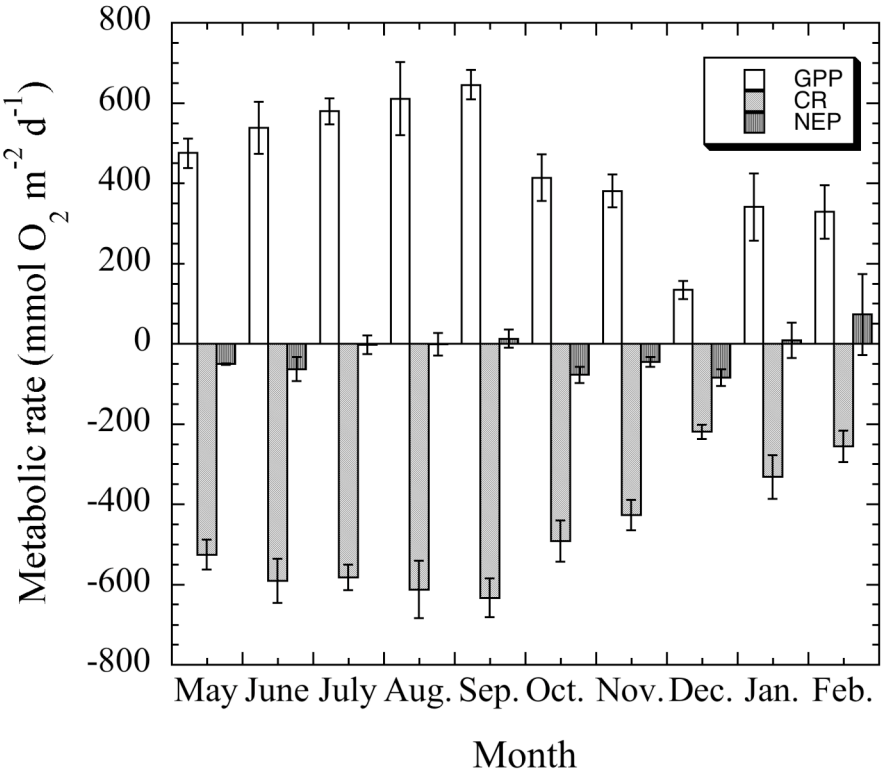

Figure 3. Monthly averaged metabolic rates for gross primary production (GPP), community respiration (CR) and net ecosystem production (NEP), all expressed in mmol O_2 m^{-2} d^{-1}.

The examination of DO frequency distribution for bins of 1ºC revealed that the probability of finding lower oxygen content increased with increasing temperature (Fig. 6). Oxygen content varied with water temperature (Fig. 6 and 7), with low temperatures having a narrower DO range and tending to be higher than for high water temperatures. At the low range of water temperatures the DO range is narrow, with most values around 100% sat. At

higher temperatures DO range increases progressively, and DO values tend to be lower. When water temperature rise to 30ºC the frequency distribution of DO is restricted again, as well as in the lower range of temperatures, but with lower DO values, around 70-80% saturation.

Oxygen concentrations varied with water temperature (Fig. 7), with both the 5 % and 95 % quantiles of DO increasing with increasing temperature, indicating that the range of DO tends to increase with increasing temperature (Fig. 7). Indeed, the increase in the range, as the 90% interquantile, with increasing temperature can be calculated as the difference between the slope of the fitted 95% and the 5 % quantile regression equations (Fig. 7), equivalent to 4.8 % Sat. DO º C^{-1}.

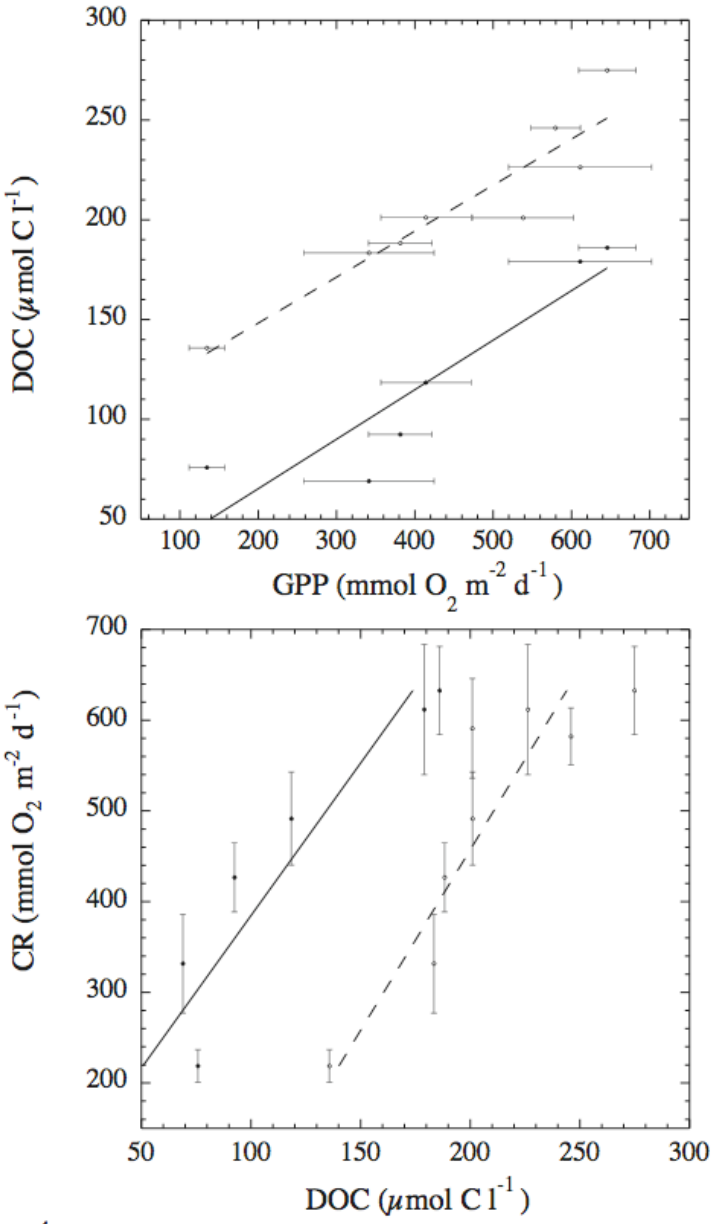

Figure 4. The relationship between (a) dissolved organic carbon (DOC, µmol C l^{-1}) in bottom waters (open circles) and surface waters (black circles) and monthly averaged gross primary production (GPP, mmol O_2 m^{-2} d^{-1}). The solid line represents the fitted regression for DOC in surface waters [DOC (µmol C l^{-1}) surface = 15.51 + 0.25 (± 0.06) GPP (µmol O_2 m^{-2} d^{-1}) in surface waters (R^2 = 0.83, p < 0.02, N = 6)], and the dashed line represents the fitted regression for DOC in bottom waters [DOC (µmol C l^{-1}) bottom = 102.15 + 0.23 (± 0.04) GPP (µmol O_2 m^{-2} d^{-1}) in bottom waters (R^2 = 0.87, p < 0.0008, N = 8)]. The relationship between (b) monthly averaged community respiration (CR) rates (mmol O_2 m^{-2} d^{-1}) and dissolved organic carbon (DOC, µmol C l^{-1}) in bottom waters (open circles) and surface waters (black circles). The solid line represents the fitted regression between CR and DOC in surface waters [CR = 101.15 + 2.92 (± 0.57) DOC (µmol C l^{-1}) in surface waters (R^2 = 0.87, p < 0.007, N = 6)], and the dashed line represents the fitted regression between CR and DOC in bottom waters [CR = -161.84 + 3.13 (± 0.67) DOC (µmol C l^{-1}) in bottom waters (R^2 = 0.78, p < 0.004, N = 8)]. Error bars represent ± SE.

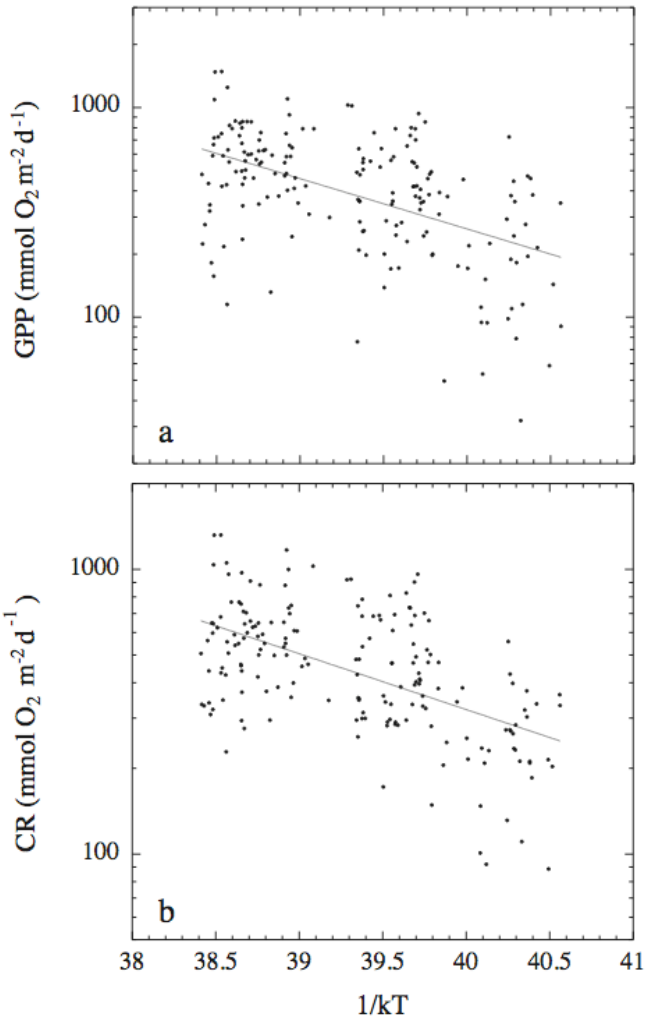

Figure 5. Arrhenius plots showing the relation between the natural logarithm of (a) gross primary production rates (GPP) and (b) community respiration rates (CR) *versus* the inverse of the temperature in Kelvin degrees multiplied by the Boltzmann's constant (1/kT) for the daily averaged metabolic rates and temperatures.

Considering an average threshold of oxygen concentration for hypoxia of 3.5 mg O_2 l^{-1} (Steckbauer *et al*. submitted), the highest probability of hypoxia of 0.07 was found at 29ºC (Table 5). The probability of finding hypoxic conditions increased linearly with increasing temperature following the relationship: Probability of hypoxia (%) = -3.61 + 0.23 (± 0.08) Temperature (ºC) (R^2 = 0.31, $p < 0.02$, N = 18). However, the threshold of oxygen concentration for hypoxia for marine benthic organisms is not constant, but increases linearly with water temperature at a rate of 0.06 (±0.01) mg O_2 l^{-1} (Vaquer-Sunyer and Duarte 2011). Allowing for the DO used to design waters as hypoxic to increase with increasing temperature at that rate, yields a steeper increase in the probability of hypoxic conditions with increasing water temperature at a rate of 0.39 (± 0.14) % per degree Celsius (R^2 = 0.31, $p < 0.02$, N = 18). Using this temperature-dependent definition of thresholds of oxygen for hypoxia results in much higher probability of hypoxia, with the maximum probability being 0.15 at 29 ºC (Fig. 8), for a corresponding DO thresholds for hypoxia < 3.92 mg O_2 l^{-1}. The probability of hypoxia was highest in August, when water temperatures were highest (Figure 8, Table 5), followed by June and October.

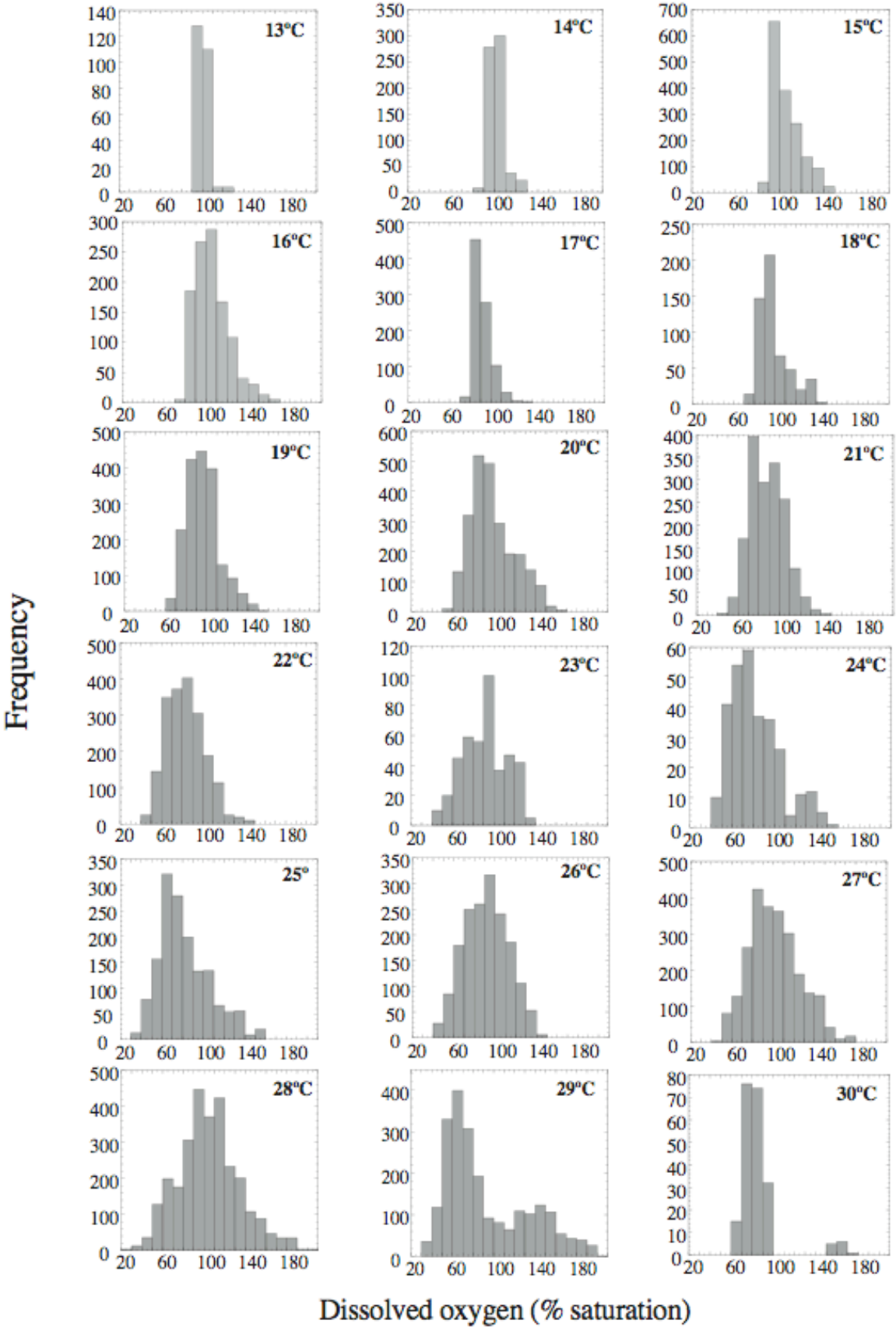

Figure 6. Frequency distribution of dissolved oxygen (DO, % saturation) for 1ºC bins.

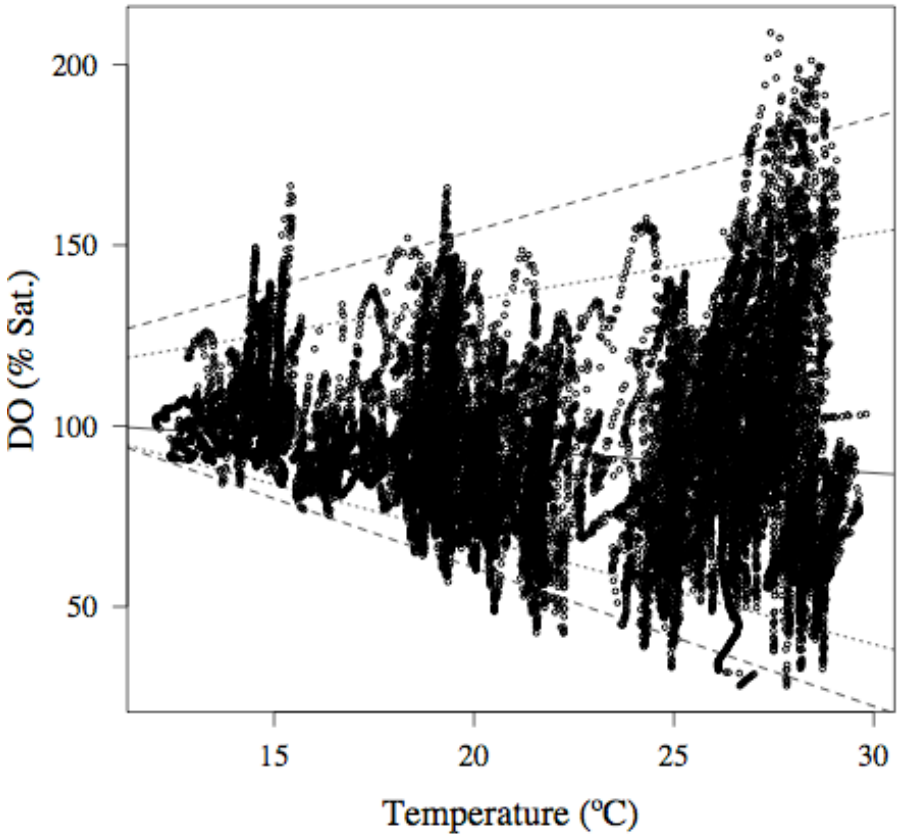

Figure 7. The relationship between dissolved oxygen (DO, % saturation) and water temperature during the studied period. The solid line represents the fitted regression for the median or the 50% quartile [DO (% sat.) = 107.29 (± 0.61) -0.68 (± 0.03) Temperature; N=24857, $p < 0.0001$)]. The dotted lines represent the fitted regression for the upper 95% quantile [DO (% sat.) = 97.96 (± 1.80) + 1.85 (± 0.09) Temperature; N=24857, $p < 0.0001$)] and the lower 5% quartile quantile [DO (% sat.) = 128.05 (± 0.59) – 2.95 (± 0.03) Temperature; N=24857, $p < 0.0001$)]. The dashed lines represent the fitted regression for the upper 99% quantile [DO (% sat.) = 81.49 (± 4.55) +3.13 (± 0.20) Temperature; N=24857, $p < 0.0001$)] and the lower 1% quartile [DO (% sat.) = 137.36 (± 1.59) – 3.83 (± 0.08) Temperature; N=24857, $p < 0.0001$)].

The maximum duration of hypoxic conditions also increased with increasing temperature (Fig. 8) as described by the relationship Maximum hypoxia duration (minutes) = -215.5 + 13.5 (±4.2) Temperature (ºC) ($R^2 = 0.40$, $p < 0.006$). The maximum continuous time under hypoxic conditions was, when using a fixed thresholds of hypoxia of 3.5 mg O_2 l^{-1} (Steckbauer *et al*. submitted), 390 minutes (6.5 hours), observed on August 24, when water temperature was 28.96º C, but the total length of time the community remained exposed to hypoxic condition that day reached 420 minutes (7 hours, Table 5). When using the temperature-dependent threshold the maximum continuous time under hypoxia was calculated to be 600 minutes (10 hours, Figure 8). The maximum continuous time under hypoxia increased linearly with water temperature at a rate of 19.8 (± 5.9) minutes $ºC^{-1}$ (R^2 =

0.42, $p < 0.004$, $N = 18$). The maximum continuous duration of hypoxia was found in August when the community remained under hypoxic conditions from 11:20 pm of August 22 to 09:20 am of August 23.

Discussion

The community studied showed a prevalence of net heterotrophic conditions during the studied period with a negative mean calculated NEP for the studied period (-35.2 ± 8.1 mmol O_2 m^{-2} d^{-1}), indicating that the ecosystem was net heterotrophic, with respiration rates exceeding ecosystem primary production. Although the studied period did not cover the full year, lacking the months of March and April, we suggest that the ecosystem was net heterotrophic at the annual scale. Soon after the studied was concluded an algal bloom occurred in April 2010, leading to a severe hypoxic event, with oxygen concentration in bottom waters ranging from 0.9 to 1.3 % saturation (i.e. from 0.07 to 0.09 mg O_2 l^{-1}) between the April 26 and 27. Large areas of sediments were observed by SCUBA diving to be covered with large amounts of dead decaying algal biomass. Net heterotrophy was probably subsidized by organic carbon inputs from ground waters and/or by organic inputs from the surrounding town and boating activity, as reflected in the strong correlation between DOC concentration, which reached relatively high values in the Bay, and community respiration.

Nutrient and DOC concentrations increased in the summer, when the population resident in Portocolom watershed increases four fold. The high increase in human population in Portocolom during summer months is associated with an increase in the nutrients and organic matter inputs to the Bay. Indeed, all the nutrient concentrations in bottom waters showed maximum values in August and surface DOC concentrations were significantly higher in summer than in other seasons ($F = 19.32$, $p < 0.02$, $N = 6$). GPP and CR rates were also significantly higher in spring and summer than in winter and fall, but summer values were higher than spring ones (Table 4).

DOC increased linearly with GPP (Fig. 4a). Algae and seagrasses are known to release important amounts of dissolved organic carbon (Barron and Duarte 2009, Haas and Wild 2010). Various processes may be responsible for the release of organic compounds. Some of these processes in algae may include excretion, lysis, leaching and shedding of algal debris (Cole 1982, Wada et al. 2007). In the case of seagrass ecosystems may include processes such as exudation by epiphytes, excretion and sloppy feeding by consumers, and diffusive release from sediments (Barron and Duarte 2009). Therefore, the relatively high

DOC values in the bay can be derived from both, anthropogenic inputs and DOC released by benthic algae.

Here, we report increasing community respiration rates with increasing DOC content in both surface and bottom waters (Fig. 4b), suggesting that community respiration is driven by release of bio-labile DOC. This finding is consistent with recent observations of an important role of organic matter released by benthic algae in microbial oxygen consumption (Haas et al. 2010, Wild et al. 2010). Indeed, Holmer et al. (2004) suggested that *C. prolifera*, the dominant benthic primary producer in Portocolom, is an important source of organic carbon in the ecosystem. Holmer et al. (2004) observed enhanced mineralization rates in Portocolom sediments colonised by *C. prolifera*, suggesting that the detritus they produce is highly labile for sediment bacteria (Holmer *et al.* 2004). High sulphate reduction rates have also been reported in *C. prolifera* stands in Portocolom, suggesting that high abundance of C. prolifera stimulates benthic sulfate reduction rates (Holmer *et al.* 2009).

Monthly averaged GPP and CR increased from May to September, reaching their maximum in September, when the monthly averaged water temperature was 26.8ºC. The monthly averaged GPP and CR increased with increasing temperature but reached their maximum when water temperature started to decrease. Calculated Q_{10} values for GPP and CR were very close, but calculated Q_{10} value for GPP was slightly higher, than for CR (2.06 ± 0.39 and 1.98 ± 0.21, respectively). This results contrast with most Q_{10} values reported in literature (e.g. (Lefevre et al. 1994, Robinson and Williams 1993, Vaquer-Sunyer et al. 2010) and do not support the higher Q_{10} values for respiration than for production predicted by the Metabolic Theory of Ecology (MTE, (Brown et al. 2004). However, comparative analysis of pelagic community metabolism in the open ocean do yield results consistent with the MTE theory (Regaudie de Gioux and Duarte submitted), suggesting that the stronger temperature dependence of CR compared to GPP may not apply to benthic-dominated systems.

Dense, active assemblages of benthic macrophytes remove nutrients from the water column or from the sediments, often depleting nutrient concentrations in the overlying waters (Grall and Chauvaud 2002), increasing the resistance of ecosystems to eutrophication (Duarte 1995, Lloret et al. 2008). Decreased *Caulerpa prolifera* photosynthesis in summer, when water temperature exceeds 30 ºC, and the consequent decrease in its nutrient uptake may allow phytoplankton proliferation in the bay as nutrients will become available. In turn, phytoplankton proliferation will decrease water transparency, and, thus, affect negatively *C. prolifera* photosynthesis. Reduced *C. prolifera* activity may, in turn, affect heterotrophic

processes in the benthic compartment, which are greatly enhanced in the presence of active *C. prolifera* communities (Holmer et al. 2004, 2009).

Global warming will probably lead to a higher probability of seawater temperatures above 30 ºC, and thus, increase the periods when *C. prolifera* photosynthesis is inhibited (Lloret et al. 2008), affecting ecosystem GPP responses to warming and nutrient availability in the bay. Provided seawater temperatures above 30 ºC, 3.5 º C than mean maximum temperatures recorded in the late 20th Century, are already observed, the extent of warming of Portocolom Bay has already brought the ecosystem near a tipping point, where slight warming would probably inhibit *C. prolifera* photosynthesis and compromise ecosystem capacity to capture nutrients.

The variability in oxygen concentration was temperature-dependent (Fig. 6 and 7). Oxygen concentrations were high and showed a narrow range of variation at the low range of water temperature in the Bay, with most values around 100% saturation. At these temperatures the biological activity is depressed, resulting in low community respiration rates and gross primary production. The range of oxygen concentration increased progressively and DO values tended to be lower at higher temperatures. Both respiration rates and primary production are enhanced with increasing temperature, leading to low oxygen concentration during night, when only respiration occurs, and high oxygen content during day due to high primary production rates. The range of dissolved oxygen is also narrower, but with a low mean temperature at around 70-80% saturation at the highest temperature, 30ºC, reached in the Bay. This high temperature is a recent phenomenon in the Balearic Islands, where maximum water temperature has increased from a mean value of 26.5 º C over the late 20th Century to temperature approaching 30 º C during the first decade of the 21st Century (Marbá and Duarte 2010). Thus, it is possible that the thermal windows for most of the organisms in the Portocolom community be exceeded at the maximum temperature reached, which has been shown to be associated with enhanced mortality of the Mediterranean seagrass *Posidonia oceanica* (Marbà and Duarte 2010) and Lloret et al. (2008) reported that *C. prolifera* photosynthesis decreases at seawater temperatures above 30 ºC. Thus, metabolic rates would be restricted at the highest temperature reached. Water temperature at 30ºC was only found during daytime, at night temperatures were always lower than 30ºC, so the lack of low oxygen values at 30ºC can be also attributed to the absence of night-time measurements in that temperature bin.

Table 5. Probability of hypoxic conditions (DO < 3.5 mg O_2 l^{-1} and temperature-dependent threshold) in the bay at different water temperatures and for the different months, and Maximum continuous hypoxia duration in the same day (minutes). D.D.A.T.: Depending on daily averaged temperatures.

Temperature (ºC)	Hypoxia threshold (mg O_2 l^{-1})	Probability	Maximum hypoxia duration (minutes)	Temperature-dependent hypoxia threshold (mg O_2 l^{-1})	Probability	Maximum hypoxia duration (minutes)
13	3.5	0	0	2.96	0	0
14	3.5	0	0	3.02	0	0
15	3.5	0	0	3.08	0	0
16	3.5	0	0	3.14	0	0
17	3.5	0	0	3.20	0	0
18	3.5	0	0	3.26	0	0
19	3.5	0	0	3.32	0	0
20	3.5	0	0	3.38	0	0
21	3.5	0	0	3.44	0	0
22	3.5	0.04	90	3.50	0.008	160
23	3.5	0.04	90	3.56	0.017	90
24	3.5	0.04	135	3.62	0.037	135
25	3.5	0.04	285	3.68	0.073	360
26	3.5	0.04	105	3.74	0.026	120
27	3.5	0.04	50	3.80	0.014	190
28	3.5	0.04	210	3.86	0.031	260
29	3.5	0.04	390	3.92	0.147	600
30	3.5	0	0	3.98	0	0

Month	Hypoxia threshold (mg O_2 l^{-1})	Probability	Maximum hypoxia duration (minutes)	Temperature-dependent hypoxia threshold	Probability	Maximum hypoxia duration (minutes)
May	3.5	0	0	D.D.A.T.	0.000	0
June	3.5	0.04	285	D.D.A.T.	0.048	360
July	3.5	0.004	50	D.D.A.T.	0.011	190
August	3.5	0.07	390	D.D.A.T.	0.145	600
September	3.5	0	0	D.D.A.T.	0.001	10
October	3.5	0.02	240	D.D.A.T.	0.021	240
November	3.5	0	0	D.D.A.T.	0.000	0
December	3.5	0	0	D.D.A.T.	0.000	0
January	3.5	0	0	D.D.A.T.	0.000	0
February	3.5	0	0	D.D.A.T.	0.000	0

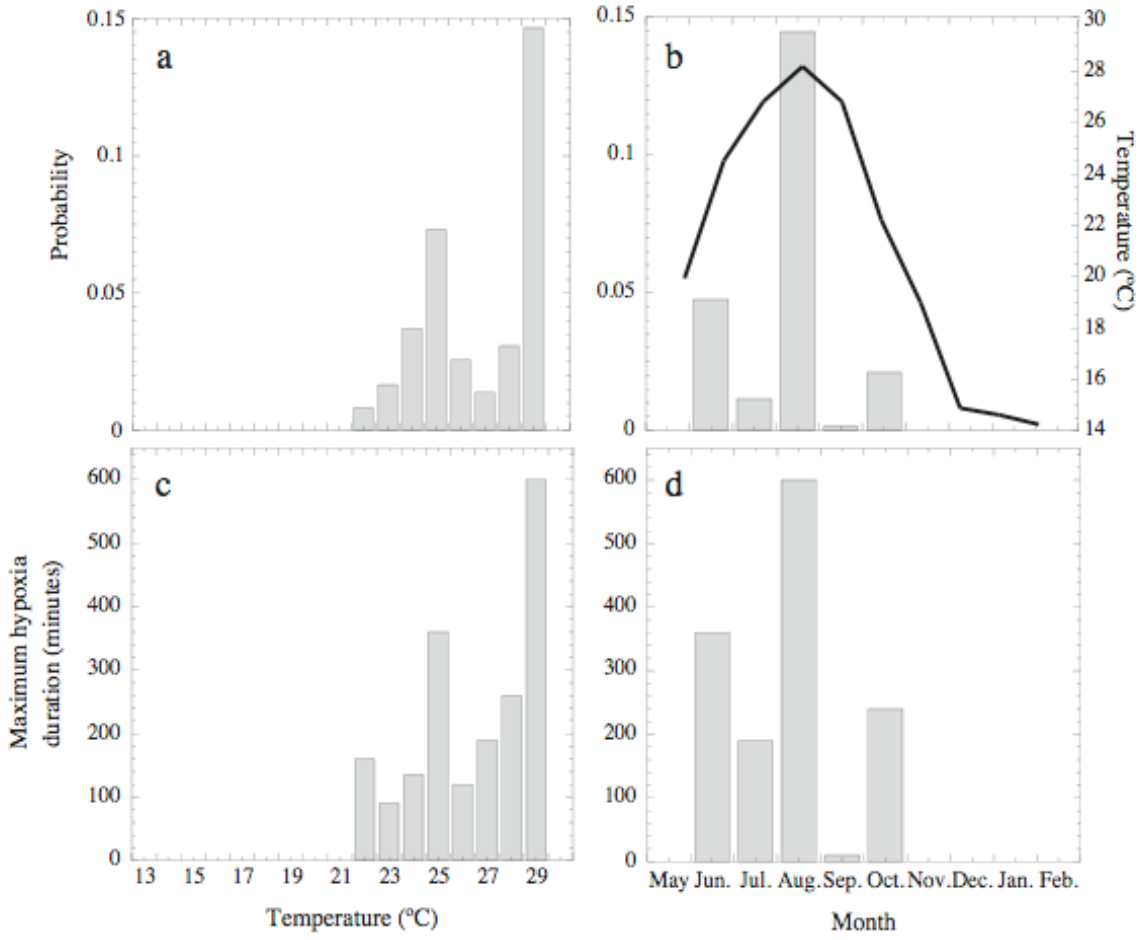

Figure 8. Changes in the probability of hypoxic conditions (a) with temperaturesand (b) across different months. The solid line indicates the monthly averaged temperature. Changes in the maximum continuous duration of hypoxia (c) at different temperatures and (d) over different months. Hypoxia is defined as waters with oxygen content lower than 3.5 mg O_2 l^{-1} at the mean water temperature (22ºC), with this threshold oxygen concentration increasing by 0.06 mg O_2 l^{-1} $ºC^{-1}$ with increasing water temperature (from Vaquer-Sunyer and Duarte 2011).

Hypoxic conditions did not occur at water temperature lower than 22 ºC. The probability of encountering hypoxic conditions (DO < 3.5 mg O_2 L^{-1}) in Portocolom increased linearly with increasing temperature at a rate of 0.23 (± 0.08) % $ºC^{-1}$. However, the probability increased even faster when the temperature-dependence of thresholds of hypoxia for marine benthic organisms, which increases linearly with water temperature at a rate of 0.06 (±0.01) mg O_2 l^{-1} (Vaquer-Sunyer and Duarte 2011) was considered. Use of this relationship to increase the thresholds of hypoxia with increasing temperature over the mean water temperature of the bay (22 ºC), yields an increase in the probability of hypoxic conditions at a rate of 0.39 (± 0.14) % per degree Celsius ($R^2 = 0.31$, $p < 0.02$, $N = 18$). Using

this temperature-dependent definition of thresholds of hypoxia, the highest probability of hypoxia of 0.15 was found at 29 ºC (Fig. 8). Although the probability of hypoxia increases linearly with temperature, this increase is not monotonous, with waters at 26, 27 and 28 ºC having lower probability of hypoxia than waters at 25ºC. The probability of hypoxia was greater in June than July, likely because during June the water temperature increased steeply, from 21.2ºC on the 1st of June to 26.5ºC on the 20th of June. Sudden temperature increase produces stress in the organisms, causing an increase in respiration rates. In July, water temperature did not increase as fast as it did in June, allowing *C. prolifera* to acclimatize, increasing production rates, and avoiding hypoxic conditions. August, with a monthly averaged water temperature of 28.2 ºC, was the warmest month of the year, and had the highest probability and the highest continuous duration of hypoxia, with the community remaining under hypoxia over 10 hours in the nights of the 22nd and 23rd of August. Indeed, the maximum continuous duration of hypoxic events increased linearly with water temperature at a rate of 19.8 (± 5.9) minutes per degree Celsius for temperature-dependent thresholds of hypoxia. During September high precipitation rates lead to floods in the studied area. Most measurements of this month were lost due to the difficulties to dive to recover the instruments in brown waters with large loads of resuspended sediments. In October, water transparency improved allowing diving again, low oxygen conditions were found, with a probability of hypoxia of 0.02 when monthly averaged water temperature was of 22.2 ºC.

Warming decreases oxygen solubility, as solubility of gases is temperature-dependent (Carpenter 1966) and increases respiration rates (Jones 1977), as temperature plays a fundamental role in regulating metabolic processes (Iriberri et al. 1985). Warming also affects the response of marine organisms to hypoxia, reducing survival times of marine benthic macrofauna under hypoxia and increasing the oxygen thresholds for hypoxia-driven mortality (Vaquer-Sunyer and Duarte 2011). Hence, warming triggers a range of temperature-dependent responses affecting oxygen dynamics in the ecosystems as well as organismal demands that result in a steep increase in the likelihood of hypoxia with warming, as documented here. Warming is, thus, expected to have broad negative consequences on benthic fauna, as the organisms will have higher oxygen requirements at higher temperature while less oxygen will be available. Hence, further warming will greatly increase the likelihood and duration of hypoxic and anoxic events. Global warming triggers, therefore, a cascade of responses increasing the likelihood of hypoxia, with their associated negative

consequences for marine life, and may, thus, have catastrophic consequences in the bay, as well as in other similar Mediterranean Bays.

Acknowledgements

This research is a contribution to the ‘‘Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery (WISER)’’ project, funded by FP7 (contract 226273) and project MEDEICG, funded by the Spanish Ministry of Science and Innovation (number CTM2009-07013). R. Vaquer-Sunyer was funded by the WISER project. We thank R. Martínez, L. Royo, M. Noguera and A. Steckbauer for help on sampling and J.C. Alonso for nutrient analysis. We want to thank J.J. Coloso his generosity for sharing his Matlab program with us.

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Chapter 4

Thresholds of hypoxia for marine biodiversity

Raquel Vaquer-Suner and Carlos M. Duarte. *Proceedings of the National Academy of Sciences of the United States of America* **105**:15452-15457, 2008.

Abstract

Hypoxia is a mounting problem affecting the world's coastalwaters, with severe consequences for marine life, including death and catastrophic changes. Hypoxia is forecast to increase owing to the combined effects of the continued spread of coastal eutrophication and global warming. A broad comparative analysis across a range of contrasting marine benthic organisms showed that hypoxia thresholds vary greatly across marine benthic organisms and that the conventional definition of 2 mg O2/liter to designate waters as hypoxic is below the empirical sublethal and lethal O2 thresholds for half of the species tested. These results imply that the number and area of coastal ecosystems affected by hypoxia and the future extent of hypoxia impacts on marine life have been generally underestimated.

Keywords: benthic community , oxygen, coastal ecosystems, eutrophication, impacts

Introduction

Dissolved oxygen in coastal waters has changed drastically over the past decades, arguably more so than any other ecologically important variable (Diaz and Rosenberg 1995; Diaz 2001), leading to the widespread occurrence of hypoxia. An assessment of the literature shows that the number of coastal sites where hypoxia has been reported has increased with an exponential growth rate of 5.54% $year^{-1}$ over time (Fig.1, Annex 1 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/) Although, this growth rate can be partially attributed to an increased observational effort, increasing the number of costal ecosystems monitored and the likelihood of detecting hypoxia therein, this growth also reflects an increase in the prevalence of hypoxia in different types of coastal ecosystems. Multiple reports from careful monitoring time series provide evidence for an unambiguous increase in the number of hypoxic zones, their extension, severity and duration (Conley, Carstensen et al. 2007; Chan, Barth et al. 2008; Stramma, Johnson et al. 2008; Turner, Rabalais et al. 2008). This growth is expected to continue, as the prevalence of hypoxia is forecasted to increase further due to the combined effects of eutrophication, leading to the excessive production of organic matter that increases the oxygen demand of coastal ecosystems (Service 2004), and the increase in temperature caused by climate change, which enhances the respiratory oxygen demand of the organisms (Harris, Duarte et al. 2006), reduces oxygen solubility (Carpenter 1966), and

reduces the ventilation of coastal waters by affecting stratification patterns (Stow, Qian et al. 2005).

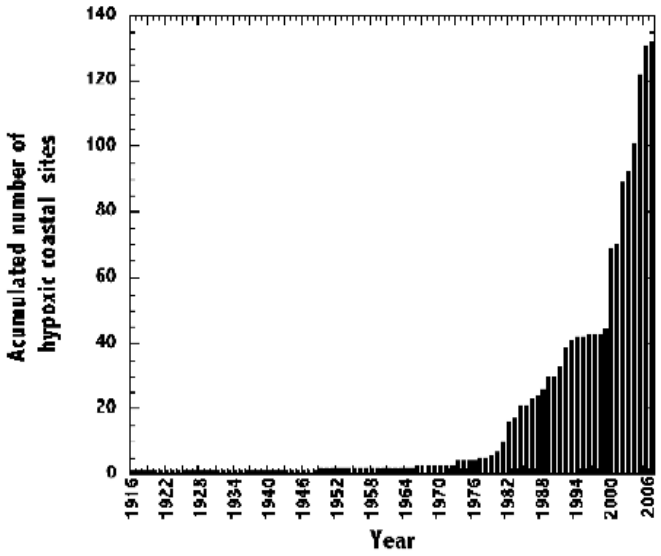

Figure 1. Accumulated number through time of coastal sites where hypoxia has been reported. Exponential growth rate = 5.54 ± 0.23% year^{-1} (R^2 = 0.86, P < 0.01).

Coastal hypoxia is, thus, emerging as a major threat to coastal ecosystems globally. Hypoxia has been shown to trigger mortality events, resulting in a depletion of metazoans in the ecosystems, resulting in so-called "dead zones" devoid of fisheries resources such as fish, shrimp and crabs (Renaud 1986; Rabalais, Turner et al. 2002). Hypoxia leads to major loss in biodiversity and impacts on the surviving organisms through sublethal stresses, such as reduced growth and reproduction, physiological stress, forced migration, reduction of suitable habitat, increased vulnerability to predation, and disruption of life cycles (Rabalais, Turner et al. 2002; Service 2004). Benthic organisms are particularly vulnerable to coastal hypoxia because they live farthest from contact with atmospheric oxygen supply and because coastal sediments tend to be depleted in oxygen relative to the overlying water column.

Assessing the thresholds of oxygen where lethal and sublethal impacts occur is critical to establish the vulnerability of marine organisms to hypoxia and to set management targets to avoid catastrophic mortality. Hundreds of experiments to determine thresholds of hypoxia for a range of benthic organisms have been conducted. However, the oxygen thresholds for hypoxia proposed in the literature (Renaud 1986; Pihl, Baden et al. 1991; Diaz and Rosenberg 1995) are based on limited observations of impacts on organisms (Service 2004), and a thorough empirical assessment of the available experimental evidence is still pending.

Whereas the thresholds of hypoxia proposed in the literature range broadly from 0.28 mg O_2/liter (Fiadeiro and Strickland 1968) to 4 mg O_2/liter (Paerl 2006), most reports (55%) refer to a value of 2 mg O_2/liter or lower (mean ± SE of thresholds proposed in the literature = 2.31 ± 0.10 mg O_2/liter, Annex 2 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/) used in most conventional applications (Turner, Rabalais et al. 2005). This thresholds refers to the oxygen level for fisheries collapse (Renaud 1986) but there is ample experimental evidence that a 2 mg O_2/liter threshold may be inadequate to describe the onset of hypoxia impacts for many organisms, which experience hypoxia impacts at higher oxygen concentrations (e.g. Vargo and Sastry 1977). Moreover, the diversity of behavioral and physiological adaptations to hypoxia (Hagerman 1998) suggests that different taxa are likely to exhibit different vulnerability to hypoxia and may have, therefore, different oxygen thresholds (Gray, Wu et al. 2002), a possibility that is not addressed by the conventional oxygen thresholds in use (cf. Annex 2 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/).

The goal of this paper is to examine the variability in oxygen thresholds for hypoxia across benthic organisms, and to test for the existence of consistent differences among taxa. We do so on the basis of a comparative analysis of experimental-derived oxygen thresholds for lethal and sublethal responses to hypoxia of benthic organisms. We aim at improving our understanding on the levels of hypoxia that cause significant impacts on marine benthic communities. This understanding will offer a more rigorous basis to establish critical thresholds to preserve fishery resources and to effectively conserve coastal biodiversity as hypoxia continues to rise as a threat to coastal ecosystems.

Methods

We searched the literature for reports of hypoxia on the Web of Science ™ and Scholar Google ™ using the keywords “hypoxia, marine, benthic, sea“ and their combinations to guide the search. This search delivered over 6,000 published reports of responses of benthic marine organisms to hypoxia, which were then examined further for the availability of experimental assessments of responses to reduced oxygen content. This search delivered a total of 872 experimental assessments examining the distribution of oxygen thresholds, involving 206 different species of marine benthic organisms.

The outcome of experimental assessments, which follow standard toxicity tests, was summarized using the following indicators of oxygen thresholds: Median Lethal Oxygen Concentration (LC_{50}) and Median Sublethal Oxygen Concentration (SLC_{50}), representing the

statistically-derived O_2 concentration at which 50% of the organisms in a given population die or exhibit sublethal responses, respectively, and the Median Lethal time (LT_{50}), representing the statistically-derived time interval at which 50% of a given population dies following exposure to low O_2 levels. The vast majority (99.1%) of the experiments designed to assess Median Lethal Time chose < 2 mg O_2/liter as experimental conditions, consistent with the widespread acceptance of 2 mg O_2/liter as the threshold for hypoxia in the literature (Table A2 at Appendix). Yet, this choice indicates that the lethal times reported represent lethal times under acute hypoxia. Only a few experiments testing species particularly sensitive to hypoxia (0.87%), used higher experimental O_2 conditions. We analysed these indicators to extract oxygen thresholds conductive to the effective conservation of marine biodiversity.

ANOVA was used to test for differences in oxygen thresholds among taxonomic groups. ANOVA analysis was conducted after checking for normality using the Shapiro-Wilk test and homogeneity of variances using the Levene's test. The Tukey honestly significant difference (HSD) *post-hoc* test was used to determine differences between mean threshold values among taxa ($\alpha = 0.05$) .We also classified the species tested according to their mobility as “fast moving” (fish and a few mollusks, such as octopus), “highly mobile” (most crustaceans), “reduced mobility” (some crustaceans, gastropods, polychaetas, echinoderms, jelly fish, comb fish (ctenophora), priapulidans, flatworms and sipunculida) and “sesile” (anemones, bryozoans and bivalves). ANOVAwas used to test for differences in thresholds with mobility, following the procedures outlined above. Two-way ANOVA was used to test for the combined effect of taxonomic membership and the extent of mobility of the organisms tested on the experimentally-derived thresholds.

Results

We found a total of 872 published experiments reporting oxygen thresholds and/or lethal times for a total of 206 species spanning across the full taxonomic range of benthic metazoans. The examination of thresholds for hypoxia derived experimentally revealed the existence of a broad range of variability, with median lethal and sublethal oxygen thresholds and median lethal times after exposure to hypoxia ranging over an order of magnitude across experiments (Fig. 2, Annex3, Annex 4 and Annex 5 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/).

Table 1. Distribution of thresholds of hypoxia for different groups of benthic organisms. Mean ± SE, 90 % pecentile (10 % percentile for lethal times) and number of observations of the median lethal concentration (LC_{50}), median sublethal concentration (SLC_{50}), and median lethal times (LT_{50}) for the various groups.

	LC_{50} (mg O_2/liter) **Mean ± SE** **90 percentile** **N**	**SLC_{50} (mg O_2/liter)** **Mean ± SE** **90 percentile** **N**	**LTC50 (h)** **Mean ± SE** **10 percentile** **N**
Fish	1.54 ± 0.07 2.51 77	4.41 ± 0.39 8.09 34	59.9 ± 12.3 0.9 39
Crustaceans	2.45± 0.14 5.72 168	3.21 ± 0.28 5.0 30	55.5 ± 12.4 1.0 102
Gastropods	0.89 ± 0.11 1.62 12		
Bivalves	1.42 ± 0.14 3.43 19		
Molluscs		1.99 ± 0.16 2.83 28	412.9 ± 37.3 55.4 239
Annelids		1.20 ± 0.25 1.37 10	132.2 ± 18.7 37.8 43
Echinoderms		1.22 ± 0.22 2.12 8	201.1 ± 44.8 33.6 23
Cnidarians		0.69 ± 0.11 1.43 19	232.5± 114.4 24 8
Priapulidans			1512.0 ± 684.0 820.8 3

The cumulative distributions representing the distribution of oxygen thresholds present a change in slope near the 90 % percentile of the distribution and the 10 % percentile

of median the lethal time (Fig. 2), showing the existence of a small proportion (10%) of experiments yielding extreme sensitivity to hypoxia, reflected in particularly high oxygen thresholds for hypoxic responses (> 5 mg O_2/liter) and short (< 2 h) lethal times. All relevant thresholds varied significantly across taxa (Fig. 3).

Median lethal concentration (LC_{50})

Median lethal oxygen concentrations ranged from 8.6 mg O_2/liter for the first larval zoea stage of the crustacean *Cancer irroratus* (Vargo and Sastry 1977), the most sensitive specie tested, to persistent resistance to complete anoxia of the oyster *Crassostrea virginica* at temperatures of 20 ºC (Stickle, Kapper et al. 1989). The larval stages of *Cancer irroratus* (Vargo and Sastry 1977) were found to be extremely vulnerable to hypoxia, with thresholds exceeding the 95% percentile of the distribution of LC_{50} values across crustaceans. The mean median lethal oxygen concentration (± SE) for all organisms tested was found to be 2.05 ± 0.09 mg O_2/liter, whereas the median was 1.60 ± 0.12 mg O_2/liter, and the coefficient of variation was 78% across experiments, indicative of considerable variability in these thresholds across organisms (Fig. 2a). Ninety percent of the experiments showed LC_{50} values below 4.59 mg O_2/liter (Fig.2a).

Some of the variability in median lethal O_2 thresholds was attributable to differences across groups (ANOVA, F= 10.03, $P < 0.001$, Fig. 3a), as crustaceans showed O_2 thresholds significantly higher than other taxa did (Tukey *post-hoc* HSD test, $p < 0.05$, Fig. 3a). Gastropods showed the lowest median lethal oxygen thresholds, although it did not differ significantly (Tukey *post-hoc* HSD test, $p > 0.05$, Fig. 3a) from that of fish and bivalves (Table 2). We also tested whether the extent of mobility of the organisms accounted for variability in the experimentally-derived median lethal O_2 thresholds. Indeed, we found the median lethal O_2 thresholds differed significantly with the extent of mobility of the organisms tested ANOVA, F= 11.29, $P < 0.001$). Two-way ANOVA showed that the degree of mobility (F-test, $P < 0.001$), which accounted for 20% of the variance in median lethal concentrations among experiments, was superior to differences among taxa (F-test, $P > 0.05$), in accounting for variability across experiments.

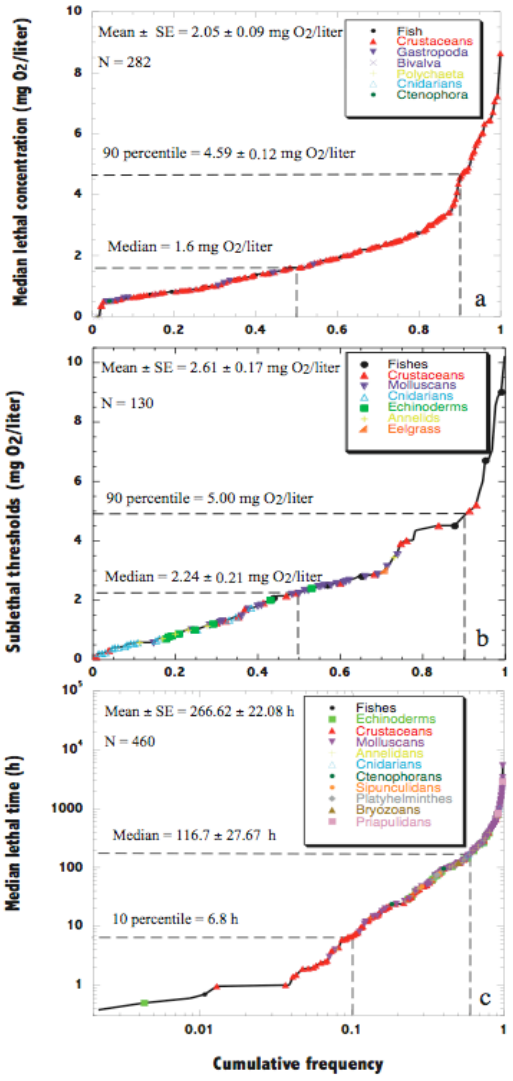

Figure 2. Cumulative distribution of (a) median lethal concentration (mg O_2/liter), (b) sublethal thresholds (mg O_2/liter), and (c) median lethal time (h) for marine benthic communities (Annexes 3, 4 and 5 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/). The mean ± SE, median ± SE, 90% percentile (10% percentile for median lethal time) and the number of experiments are indicated.

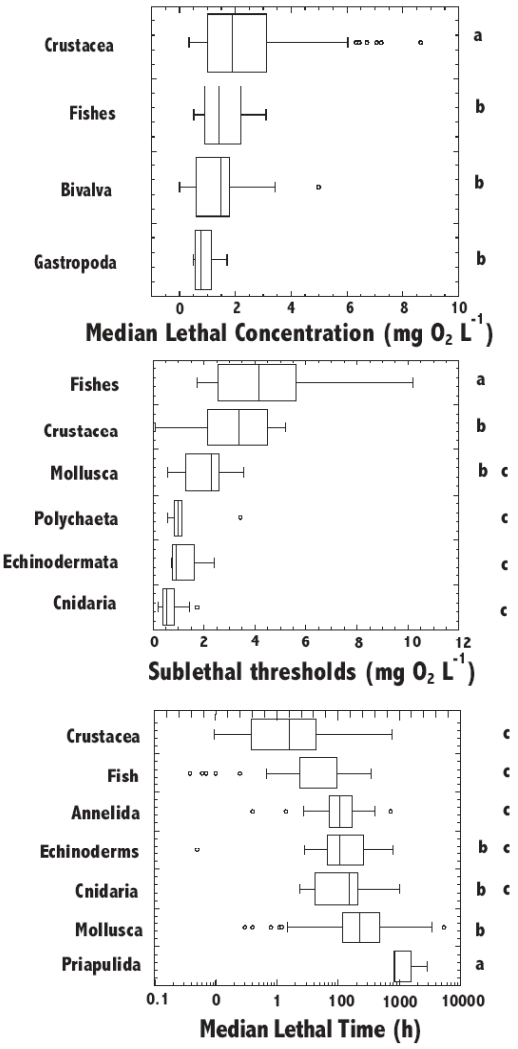

Figure 3. Box plot showing the distributions of oxygen thresholds among taxa for (a) median lethal concentration (mg O_2/liter), (b) lethal time (h). The letters indicate the results of the Tukey HSD test, where the property examined did not differ significantly for taxa with the same letter.

Median sublethal concentration (SLC_{50})

Median sublethal thresholds ranged from 10.2 mg O_2/liter for cod, *Gadus morhua,* which raises its ventilatory water flow below this concentration (Saunders 1963; Davis 1975) to 0.085 mg O_2/liter for the burrowing shrimp *Calocaris macandreae* (Thalassinidea), which switches from aerobic to anaerobic metabolism below this threshold (Anderson, Taylor et al. 1994). The mean ± SE SLC_{50} was 2.61 ± 0.17 mg O_2/liter, the median SLC_{50} was 2.24 ± 0.21

mg O_2/liter and the coefficient of variation was 76%, showing important variability in median sublethal thresholds among experiments (Fig. 2b). Ninety percent of the experiments conducted reported median sublethal oxygen concentrations below 5.00 mg O_2/liter (Fig. 2b).

Table 2.Results for the one way ANOVA test.

	Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Prob > F**
SLC_{50}	**Taxa**	5.00	284.10	56.82	21.75	1.28E-15
	Error	125.00	326.60	2.61	.	.
	C. Total	130.00	610.70	.	.	.
LC_{50}	**Taxa**	3	70.73	23.58	10.03	0.00000272
	Error	275	646.66	2.35	.	.
	C. Total	278	717.40	.	.	.
LT_{50}	**Taxa**	8	17019932	2127491.5	11.1213857	2.22E-14
	Error	449	85892505.8	191297.34	.	.
	C. Total	457	102912438	.	.	.
	Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Prob > F**
SLC_{50}	**Mobility**	3	182.03	60.68	23.19	5.31E-12
	Error	125	327.05	2.62	.	.
	C. Total	128	509.08	.	.	.
LC_{50}	**Mobility**	3	78.87	26.29	11.29	5.21E-07
	Error	278	647.56	2.33	.	.
	C. Total	281	726.43	.	.	.
LT_{50}	**Mobility**	3	12444317.7	4148105.89	21.15	7.22E-13
	Error	471	92385300.1	196147.13	.	.
	C. Total	474	104829618	.	.	.

As for the median lethal O_2 thresholds, some of the variability in median sublethal O_2 thresholds was attributable to differences among taxa, which were stronger for sublethal than for lethal responses (ANOVA, $F = 21.75$, $P < 0.001$, Fig. 3b). Fish had significantly higher oxygen thresholds for sublethal responses (Tukey *post-hoc* HSD test, $p > 0.05$, Fig. 3b), which typically involved avoidance of hypoxic waters, depress activity, shift to oxygen-dependent metabolism or increased ventilatory water flow. Crustaceans also presented significantly higher oxygen thresholds for sublethal responses than polychaetes, echinoderms and cnidarians (Tukey *post-hoc* HSD test, $p > 0.05$, Fig. 3b), which typically involved avoidance of hypoxic waters, reduced growth, reduce predation rates, lethargy or decreased

activity, among others. Fish and crustaceans, which showed the highest median sublethal O_2 thresholds, are also the taxa with the highest mobility, which confers them some capacity to avoid hypoxic waters. Indeed, two-way ANOVA showed that both differences among taxa and the extent of mobility among organisms were significant (F-test, $P < 0.001$), accounting for 52% of the variance in median sublethal concentrations among experiments ($F = 11.6$, $P < 0.001$).

Median lethal time (LT_{50})

The median lethal time upon exposure to acute hypoxia ranged greatly across organisms tested (Fig. 2c), from only 23 minutes for the flounder *Platichthys flesus* (Tallqvist, Sandberg-Kilpi et al. 1999; Gray, Wu et al. 2002), to more than 32 weeks for the bivalve *Astarte borealis* at temperatures below 20 ºC (Dries and Theede 1974). The mean (± SE) median lethal time was 267.9 ± 22.0 h, the median was 116.7 ± 27.67 h and the coefficient of variation was 178% across experiments, indicative of considerable variability in these thresholds across organisms (Fig. 2c). Ten percent of the organisms showed median lethal time upon exposure to acute of less than 6.8 hours (Fig. 2c). There were significant differences in median lethal time under hypoxia among taxa (ANOVA, $F = 11.12$, $P < 0.001$, Fig 3c). In particular, priapulida, the most tolerant group, had significantly longer median lethal time under hypoxia than other taxonomic groups, and molluscs, the second most tolerant group, also showed significantly longer median lethal time under hypoxia than annelidans, fish and crustaceans, which were the most sensitive groups (Fig. 3c). Sesile organisms also had longer median lethal times than mobile organisms did (F-test, $P < 0.01$). However, two-way ANOVA showed that differences among taxa (F-test, $P < 0.001$), which explained 17% of the variance in median lethal times, were superior to differences in mobility (F-test, $P > 0.05$), to account for variability among experiments.

We also found significant ontogenic shifts in survival time, with early stages having survival times, on average, 64 ± 7% (H_0 linear regression slope = 1, *t-test,* $p < 0.05$) of those of more developed stages for anyone species, whereas similar effects were not found for lethal or sublethal concentrations.

Table 3. Results for the Tukey (HSD) *post hoc* test. Groups not conncted with same letters are significantly different.

SLC50				Mean
Fish	A			4.71
Crustacea		B		3.21
Mollusca		B	C	1.99
Echinoderms			C	1.22
Polychaeta			C	1.20
Cnidaria			C	0.69
LC50				Mean
Crustacea	A			2.46
Osteichthyes		B		1.55
Bivalva		B		1.44
Gastropoda		B		0.89
LT50				Mean
Priapulida	A			1512.00
Mollusca		B		411.81
Cnidaria		B	C	232.50
Echinoderms		B	C	201.14
Annelida			C	133.61
Sipunculida		B	C	63.60
Ctenophora		B	C	60.00
Fihes			C	59.96
Crustacea			C	51.69

SLC50			Mean
Fast	A		3.60
High mobile	A		3.39
Sesile		B	1.51
Reduced mobility		B	0.97
LC50			Mean
High mobile	A		2.46
Fast		B	1.55
Sesile		B	1.44
Reduced mobility		B	0.78
LT50			Mean
Sesile	A		423.57
Reduced mobility		B	195.27
Fast		B	59.96
High mobile		B	56.15

Discussion

The results presented here provide evidence of the broad, order-of-magnitude variability in the thresholds of oxygen concentrations for hypoxia among benthic marine organisms, which cannot be adequately captured by a single, universal threshold. This variability partially derived from significant differences in oxygen thresholds across taxa. The most sensitive organisms were crustaceans, which showed the highest median lethal O_2 concentration and the shortest median lethal time, while fish exhibit sublethal responses at the highest O_2 concentration (Fig. 3). On the other hand, molluscs, with the lowest median lethal O_2 concentration, are the organisms most tolerant to hypoxia, together with cnidarians,

which showed the lowest median lethal O_2 concentration for sublethal threshold, and Priapulidans, which showed the longest median lethal time.

The differences in oxygen thresholds for hypoxia across taxa probably reflect the broad differences in adaptations to cope with low oxygen conditions among benthic organisms, which span a broad range of behavioral and metabolic changes. Mobile organisms have the capacity to avoid hypoxic waters and, thus, tend to show comparably high oxygen thresholds. Benthic fish have been reported to move to near-surface waters to breath when bottom waters become hypoxic (Wu 2002), and crustaceans move to shallower areas (Bell, Eggleston et al. 2003), where these organisms are more vulnerable to predation. Yet, fast moving organisms (e.g. fish) do not necessarily show higher lethal thresholds than those with more restrictive mobility (e.g. crustaceans), pointing to differences among taxa independent of their relative mobility.

Many benthic organisms (polychaetes, annelids, crustaceans, bivalves, priapulidans and anemones) leave their burrows or tubes to move to the sediment surface or reduce their burial depth (Pihl, Baden et al. 1992; Nilsson and Rosenberg 1994) in the presence of hypoxia. Some bivalves stretch their siphons upward into the water column to reach waters with higher oxygen concentrations (Jorgensen 1980). Some echinoderms stand immobile on their arm tips with the central disc elevated to avoid the hypoxic bottom water (Baden, Loo et al. 1990) and some gastropods climb structures to reach waters with higher oxygen concentration. Metabolic adaptations to cope with hypoxia include the depression of activity in the presence of hypoxia, as reported for echinoderms (Diehl, Mcedward et al. 1979); reduced feeding activity (e.g. some crustaceans, molluscs and polychaetes, (Tamai 1993; Llanso and Diaz 1994; Bell, Eggleston et al. 2003); reduced metabolic rates (e.g. Cnidarians, (Rutherford and Thuesen 2005) and heart beat rate (some crustaceans, (Harper and Reiber 1999); and shift to anaerobic metabolism over time scales of hours to days, an adaptation widespread among bivalves (Brooks, Dezwaan et al. 1991; de Zwaan, Cattan et al. 1993), polychaetes (Grieshaber and Volkel 1998), oligochaetes (Dubilier, Windoffer et al. 1997), echinoderms (Ellington 1975), and the mud-shrimp *Calocaris macandreae* (Anderson, Taylor et al. 1994),among others.

The broad variability in oxygen thresholds shown here is in contrast with the widespread use of uniform thresholds for hypoxia in the literature (Annex 2 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/). The vast majority of studies and reports continue to use the 2 mg O_2/liter convention, originally derived as the oxygen

threshold for fisheries collapse (Renaud 1986). A total of 43% and 21.5% of the published reports used the 2 mg O_2/liter and the 2 ml/liter (i.e. 2.85 mg O_2/liter) threshold, respectively, and a single study (Paerl 2006) used a threshold of 4 mg O_2/liter (Annex 2 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/). A seminal review by Gray (Gray, Wu et al. 2002), which included experimental studies reporting mortality thresholds well above 2 mg O_2/liter, concluded that "mortality occurs where concentrations are below 2.0 to 0.5 mg O_2/liter" and the USEPA recommends a threshold of 2.3 mg O_2/liter for juvenile and adult aquatic organisms survival (U.S. 2000). In a recent review, Díaz and Rosenberg state that ""hypoxia occurs when DO falls below 2 ml of O2/liter... culminating in mass mortality when DO declines below 0.5 ml of O2/liter" (Diaz and Rosenberg 2008).The results presented here show that the conventionally accepted level of 2 mg O_2/liter falls well below the oxygen thresholds for the more sensitive taxa.

Whereas the conventional 2 mg O_2/liter may signal levels of hypoxia at which fisheries collapses, the results presented here show that it is inadequate as a threshold to conserve coastal biodiversity, as significant mortality would have already been experienced by many species. The frequency distribution of thresholds of hypoxia compiled here (Fig. 2) shows that 42.85% and 61.43% of the species tested here experience substantial (> 50% of the population) mortality and sublethal responses, respectively, at oxygen thresholds above 2 mg O_2/liter. In particular, most fish and crustaceans would be lost before the oxygen content of the waters reaches the threshold of 2 mg O_2/liter for these waters to be considered hypoxic by conventional criteria. Indeed, fish and crustaceans are main fishery resources, so that the 2 mg O_2/liter threshold may be too low not only to effectively conserve biodiversity but to conserve fisheries resources as well.

Currently used thresholds of hypoxia are not conservative enough to avoid widespread mortality losses and need be critically revised. The frequency distribution of thresholds presented here provides a basis to allow the evaluation of the risk of biodiversity losses with decreasing oxygen concentration, thereby considering a range of thresholds for hypoxia, rather than a mean value that does not capture the order-of-magnitude variability across organisms. For instance, waters with oxygen concentrations below 4.6 mg O_2/liter, the 90% pecentile of the distribution of mean lethal concentrations, would be expected to maintain the population for most, except the 10% most sensitive, species. This oxygen level could be, thus, considered as a precautionary limit to avoid catastrophic mortality events, except for the most sensitive crab species, and effectively conserve marine biodiversity. Indeed, it is

possible to carry this analysis further to consider taxon-specific thresholds of hypoxia, at the 90% percentile of the distribution of LC_{50} for the various taxa (Table 1). Taxon-specific approaches help accommodate some of the variability in experimental thresholds and allow the definition of more specific conservation targets in legislative, managerial and restoration plans.

There are important limitations to extrapolate from experimentally-determined thresholds in controlled, laboratory conditions to the field (Alderdice and .Cr 1971; de Zwaan, Cortesi et al. 1995; Diaz and Rosenberg 1995; de Zwaan, Cattani et al. 2001) derived from (a) the fact that the oxygen concentrations in the experiments are held constant, whereas they would show variations in nature due to diel cycles in net community production, including the contribution of the organisms tested themselves, and mixing; (b) hypoxia often occurs in concert with other stresses in nature, and although some experiments addressed thresholds of hypoxia in the presence of additional stressors (high temperature, sulfide, etc.), most experiments used reduced oxygen as the single treatment variable; and (c) the experimental evaluation of the role of mobility in avoiding hypoxia is cumbersome, and was directly addressed only in two of the experimental studies reviewed here (Das and Stickle 1994; Wannamaker and Rice 2000). The alternative approach to estimate oxygen thresholds for mortality of the various species of benthic organisms in the field is, however, elusive, because this would require an accurate estimate of their population sizes and because, as indicated above, oxygen levels fluctuate in ecosystems rendering it difficult to assign observed mortalities to a specific oxygen value. Indeed, the difficulties to resolve oxygen thresholds in the field explain why the bulk of the studies conducted to this end, synthesized here (Annexes 3 and 5 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/), have been conducted under laboratory conditions. These considerations apply not only to oxygen thresholds but to all experiments in toxicology, which cannot be appropriately controlled in the field. Most of the processes indicated above would lead, however, to the laboratory-determined oxygen thresholds being below those in the field, except in the case of avoidance for mobile organisms (which is, however, addressed as a sublethal response in laboratory experiments). Hence, the results derived from laboratory experiments should be considered to be conservative.

Consideration of the different thresholds of hypoxia among taxa derived here (Fig. 3, Table 1) predicts that the sequence of losses of benthic fauna during hypoxic events should be initiated by the loss of fish, followed by crustaceans, then worms, echinoderms and

molluscs as oxygen declines. This prediction is consistent with the observed sequence of losses of benthic fauna during hypoxic events, as reported in Danish Fjords (Jorgensen 1980) and the Baltic Sea (Modig and Olafsson 1998). The agreement between the sequences of losses of various taxa with hypoxia predicted from laboratory experiments and those observed in coastal areas impacted by hypoxia provides additional confidence on the relevance of laboratory experiments. The pattern of recolonization of benthic fauna lost to hypoxia upon subsequent improvement of the oxygen conditions differs, however, from the pattern of loss, as recolonization patterns, which are initiated by polychaetes (Lu and Wu 2000; Rosenberg, Agrenius et al. 2002), are determined by life-history and dispersal properties of the organisms, and not their resistance to hypoxia.

The conclusion that oxygen depletion induces significant mortality at critical oxygen thresholds exceeding by 2.3 times the 2 mg O_2/liter threshold generally used in the literature implies that the present inventory of the number and extent of hypoxic areas in the coastal zone, which uses the occurrence of oxygen levels ≤ 2 mg O_2/liter (Fig. 1), represents an underestimate of the coastal areas experiencing mortality of benthic organisms attributable to hypoxia. Hence, benthic organisms may be suffering substantial mortality in areas not presently designated as hypoxic. The conventional 2 mg O_2/liter limit serves to separate "dead zones", depleted of most of the commercially-harvested species, from waters supporting significant benthic animal communities. However, it fails to reflect the oxygen threshold at which these communities experience hypoxia-derived mortality. The pace of growth of hypoxia as a major threat to coastal biodiversity and associated living resources may be, therefore, greater than hitherto considered. Moreover, there is ample evidence that the oxygen requirements of marine animals are even higher in the presence of concurrent stresses, such as high temperature (Matthews and McMahon 1999) or sulfide concentrations (Vistisen and Vismann 1997), suggesting that areas under stress are particularly prone to experience hypoxia-derived catastrophic mortality. These interactions are not considered in present assessments and classifications, but are likely to play a more prominent role in the future as global warming and other mounting stresses in the coastal ocean increase the sensitivity of benthic organisms to oxygen depletion. Indeed, a recent assessment concluded that the area of hypoxia (defined as ≤ 2 mg O_2/liter) in Danish coastal waters, one of the countries most severely affected by this problem, will more than double under the projected temperature increase over the 21st Century (Conley, Carstensen et al. 2007), an estimate that

need be revised upwards on the light of the higher oxygen thresholds for hypoxia proposed here.

The analysis presented here demonstrates that hypoxia impacts occur at a broad range of oxygen concentrations, including oxygen concentrations well above the oxygen thresholds generally used to diagnose hypoxia at present. The vulnerability of coastal ecosystems to hypoxia is, thus, greater than currently recognized, with fish and crustaceans being the most vulnerable faunal components. The number and extent of the coastal zones affected by hypoxia is, thus, likely to be greater than hitherto realized, and the prospects for future expansion of these areas more disturbing than currently forecasted. Coastal hypoxia is, thus, emerging as a major threat to coastal ecosystems globally. The revised thresholds of hypoxia provided here will help better protect these ecosystems, conserve their biodiversity and set successful management targets to avoid hypoxia-derived biodiversity losses in coastal waters.

Acknowledgments

This research is a contribution to the THRESHOLDS integrated project, funded by the EU 6th Framework Program. We dedicate this paper to the memory of the late John S. Gray.

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Chapter 5

Environmental modulation of thresholds of hypoxia for marine benthic communities.

Includes:

1. Sulfide exposure accelerates hypoxia-driven mortality.

2. Temperature effects on thresholds of hypoxia for marine benthic communities.

Raquel Vaquer-Suner and Carlos M. Duarte

Sulfide exposure accelerates hypoxia-driven mortality.

Abstract

The effect of the presence of sulfide on the survival of benthic organisms under hypoxia was tested using a meta-analysis of published experimental results evaluating the effects of the presence of hydrogen sulfide on the median survival time of benthic macrofauna under hypoxia. The meta-analysis confirmed that survival times under hypoxia are reduced by an average of 30% in marine benthic communities exposed to hydrogen sulfide. The effect of sulfide on survival was higher for eggs forms than for juvenile or adult stages. The aggravation of the negative effects of spreading hypoxia in the presence of sulfide suggests that the threats derived from hypoxia to marine biodiversity are greater than anticipated on the basis of the direct effects of low oxygen concentration alone.

Introduction

Dissolved oxygen is a property that has changed drastically in a short period of time in the marine environment (Diaz and Rosenberg 1995; Diaz 2001). Oxygen deficiencies have increased in frequency, duration, and severity in the world´s coastal areas during the recent decades (Diaz and Rosenberg 2008), and as a consequence hypoxia is emerging as a major threat to marine biodiversity (Vaquer-Sunyer and Duarte 2008). Hypoxia reduces the abundance and diversity of the benthic macrofauna (Josefson and Widbom 1988; Rosenberg et al. 1991; Gray et al. 2002) and affects the structure and function of marine ecosystems (Wu 2002). Changes in the benthic macrofauna community observed after episodes of severe oxygen deficiency indicate differential tolerance to oxygen concentrations (Diaz and Rosenberg 1995), with mollusks and polychaetes being typically more tolerant to oxygen deficiency than echinoderms, fishes, and crustaceans (Theede et al. 1969; Taylor and Spicer 1987; Levitt and Arp 1991).

These differences in tolerance are reflected also in the length of time organisms survive under hypoxia (Vaquer-Sunyer and Duarte 2008). However, the onset of hypoxia is followed by a number of changes in the ecosystem that significantly affect the conditions for further organismal survival (Conley et al. 2009). In particular, as hypoxia progresses, benthic microbial communities shift to sulfate reduction, and sulfide concentrations increase in the

environment (Conley et al. 2009). When oxygen is not available to oxidize organic matter, prokaryotes utilize alternative electron acceptors such as sulfate, nitrate, nitrite, or metal oxides. Sulfate is normally the most abundant, and its reduction has sulfide as an end product (Berner 1984). Sulfide is always present in the anoxic layer of marine sediments (Fenchel and Jorgensen 1977) and its diffusion into bottom water is controlled by the oxic sediment depth, dictated by the rate of oxygen diffusion and the oxygen consumption in the sediment (Vistisen and Vismann 1997). During hypoxic events, the anoxic layer of the sediments migrates upwards and can reach the water column, with sulfide intrusion into the bottom water. Sulfide is very toxic to most aerobic organisms because of its inhibition of cytochrome *c* oxidase activity at micromolar (μmol L^{-1}) concentrations (Nicholls 1975*b*; Petersen 1977; Nicholls and Kim 1982). Sulfide binds with high affinity to the ferric iron in the heme site of cytochrome aa_3 (Nicholls 1975*a*). The interaction of sulfide with blood proteins such as hemoglobin, binding to the hemoglobin porphyrin ring (Berzofsky et al. 1971), reduces oxygen delivery to mitochondria in some species (Evans 1967). As sulfide increases during hypoxia, the macrofauna is also exposed to sulfide, so that benthic mass mortalities during hypoxic events may be a consequence of both sulfide toxicity and hypoxia rather than low oxygen concentration alone (Vistisen and Vismann 1997). Environmental stressors can have additive effects in shortening survival time of marine organisms under hypoxia, such as increasing temperature, increased pCO_2 levels in the ambient waters (Portner and Farrell 2008), and the presence of hydrogen sulfide and contaminants, among others.

Here we test whether the presence of sulfide affects survival of benthic animals under hypoxia. More specifically, we use a meta-analysis of published experimental results examining the survival of benthic organisms under both hypoxia and the presence of sulfide to evaluate the effects of the presence of hydrogen sulfide on the median survival time of benthic macrofauna under hypoxia.

Methods

We searched for reports of hypoxia on the Web of Science and Scholar Google using the keywords ‘hypoxia’, ‘marine’, ‘benthic’, ‘sea’, and ‘sulfide’ and their combinations to guide the search. This search delivered more than 6000 published reports of responses of benthic marine organisms to hypoxia, which were then examined further for the availability of experimental assessments of responses to reduced oxygen concentration that included treatments with addition of sulfide. This more restricted search delivered a total of 68

experimental assessments examining the median lethal time (LT_{50}), representing the statistically derived time interval at which 50% of a given population dies after exposure to low O_2 levels; involving 30 different species of marine benthos.

The vast majority (98.5%) of the experiments used very low oxygen concentrations to assess median lethal time in presence of sulfide (mean ± SE = 0.2 ± 0.1 mg O_2 L^{-1}), and one experiment used 5.75 mg O_2 L^{-1} to compare survival under normoxia in the presence and the absence of sulfide.

The full data set is available in Annex 6 at http://imedea.uib csic.es/users/raquel/phD_Annexes/.

A general linear nested model was used to assess changes in median survival time under hypoxia of animals exposed to sulfide as a function of hydrogen sulfide concentration and the median lethal time in the absence of sulfide, and analysis of covariance was used to test for differences in response of changes in LT_{50} in the presence and absence of sulfide among taxonomic groups.

Results

We found a total of 68 published experiments involving 30 species of benthic macrofauna where the median lethal time (LT_{50}) was assessed in the presence and absence of sulfide. In all of these experiments the median lethal time in the presence of sulfide was lower than when the organisms were exposed to hypoxia alone (Fig. 1). The median lethal time under hypoxic conditions was reduced by, on average (± SE) 30 ± 2% in the presence of sulfide relative to that under hypoxia alone (Fig. 1).

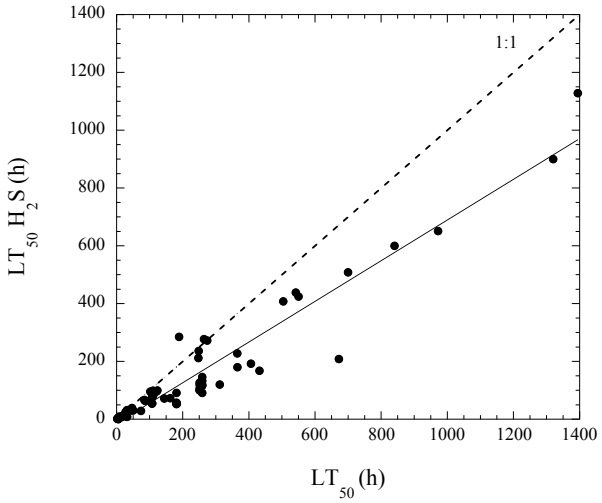

Figure 1. The relationship between the median lethal time in the presence of sulfide (LT_{50} H_2S) and the median lethal time without sulfide (LT_{50}). The solid line represents the fitted regression equation, LT_{50} H_2S (h) = -8.45 (± 13.74) + 0.70 (± 0.04)·LT_{50} (h) (R^2 = 0.84, p < 0.0001, n = 68). The dotted line represents the line 1:1. (*see* Annex 6 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/ for data sources)

A nested general linear model showed that the median lethal time in presence of sulfide (LT_{50} H_2S, h) decreased with increasing sulfide concentration (Log_{10} H_2S, μmol L^{-1}) and the interaction between the two variables, as shown by the fitted regression equation:

$LT_{50}\,H_2S = 135.6 - 39.7 \cdot Log_{10}\,H_2S + 0.5 \cdot LT_{50} - 0.3 \cdot [(LT_{50}-212.9) \cdot (Log_{10}\,H_2S - 2.9)]$ (Eq. 1)

($R^2 = 0.92$, $p < 0.0001$).

The model indicates no significant negative effect of sulfide concentration on marine benthic organisms at sulfide concentrations below 14 μmol L^{-1} (Fig. 2). The model shows that millimolar sulfide concentrations reduce the median lethal time to half of that in the absence of sulfide (Fig. 2). Even greater reductions in survival time, to 15% of those in the absence of sulfide, result from exposure of benthic animals to exceptionally high concentrations in the order of 10 mmol L^{-1} (Fig. 2). Such exceptionally high sulfide concentrations have indeed been reported in nature, with a value of 15000 μmol L^{-1} H_2S reported from sediment pore waters of the Gulf of California (Goldhaber and Kaplan 1974, Table 1), which, according to our model, would reduce survival time of the associated fauna by 89% relative to that in the absence of sulfide. Indeed, the reduction in survival time can be quite high for various benthic environments (Table 1), showing that sulfide can indeed substantially shorten the life span of organisms under hypoxia in nature.

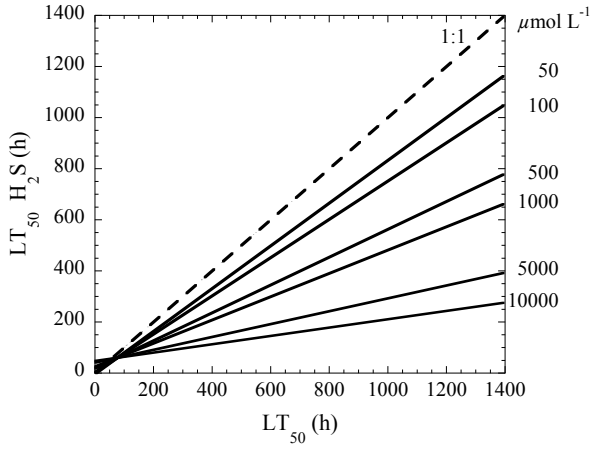

Figure 2. The decrease in median lethal time in presence of hydrogen sulfide (LT_{50} H_2S) at different sulfide concentrations (50, 100, 500, 1000, 5000, and 10000 μmol L^{-1}) predicted by the model described the effect of sulfide concentrations on survival fitted here (Eq. 1).

Analysis of covariance (ANCOVA) did not reveal any significant differences between taxonomic groups, either in the slope or intercept (*t*-test, $t = -1.08$, df = 64, $p = 0.29$), in the

regression equation relating LT_{50} in the presence or absence of sulfide under hypoxia. We found, however, ontogenic differences in the ratio of LT_{50} in the absence of sulfide and that in the presence of sulfide (ANOVA, $F_2 = 8.20$, $p = 0.0006$), with eggs having the shortest survival in the presence of sulfide relative to hypoxia alone, followed by adults and then by juveniles (Fig. 3). Juveniles did not show significant differences in this ratio from adults or eggs, whereas eggs and adults showed significant differences from each other in the extent of response to sulfide presence (Tukey post-hoc test, $p < 0.05$).

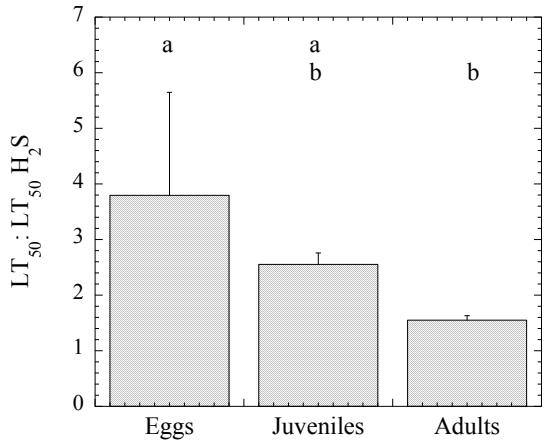

Figure 3. Ratio of LT_{50} in the absence of sulfide to LT_{50} in the presence of sulfide for different life stages. The letters indicate the results of the Tukey-Krammer honestly significant differences (HSD) test, whereby the ratio did not differ significantly for life stages with the same letter.

Discussion

The results presented here confirm the existence of a synergistic effect of hypoxia and the presence of sulfide in accelerating mortality of benthic macrofauna. The survival of benthic organisms under hypoxia is reduced by 30% in the presence of sulfide and are reduced even more for egg stages. Sulfide is quite toxic to aerobic aquatic animals (Theede et al. 1969) and plants (Terrados et al. 1999) at micromolar concentrations; 10 μmol L^{-1} sulfide causes *Posidonia oceanica* meadows to decline (Calleja et al. 2007). The reason why the survival of benthic macrofauna under hypoxia is reduced in the presence of sulfide is that the toxicity of sulfide, once its oxidation to thiosulfate is not possible due to the high sulfide content or the lack of oxygen necessary to oxidize it, operates through interferences with cytochrome *c* oxidase activity (Nicholls 1975*b*; Petersen 1977; Nicholls and Kim 1982). These interferences affect respiratory processes directly and indirectly through the depletion

of organic substrates and oxygen in sulfide oxidation by mitochondria, when animals are already compromised by low oxygen concentration. Nanomolar to low micromolar sulfide concentrations inhibit the cytochrome *c* oxidase of various organisms (Bagarinao 1992), with such toxic effects evident at sulfide concentrations ranging from 0.002 μmol L^{-1} H_2S for the bivalve *Mercenaria mercenaria* (Hand and Somero 1983) to 14 μmol L^{-1} for the annelid *Tubifex* sp. (Degn and Kristensen 1981). Mitocondrial respiration has been reported to be inhibited at 2 μmol L^{-1} H_2S for the annelid *Tubifex* sp. (Degn and Kristensen 1981) and at 38 μmol L^{-1} H_2S for the soil amoeba *Acanthamoeba castellannii* (Lloyd et al. 1981).

Under normoxic conditions, sulfide is present at some depth in the sediments. The depth of the sulfide front is set dynamically by the balance between the sulfide production through sulfate reduction in the anaerobic zone of the sediment and the diffusive and advective (though bioturbation) penetration of oxygen into the sediments. In particular, the depth of the sulfide horizon is set by the depth to which sufficient oxygen reaches to satisfy the respiratory requirements of the aerobic community and oxidize the sulfide diffusing upwards from the anoxic layers of the sediment. Depletion of water-column oxygen during hypoxic events leads to upward displacement of sulfide within the sediments, which may be accelerated by the loss of bioturbating fauna. Sulfide may even invade the water column, resulting in sulfide events that may be more common in hypoxic waters than reported because sulfides may be oxidized before they reach the air-sea interface. The extent of sulfide oxidation will depend on the balance between sulfide production and oxygen penetration, but sulfides can also be removed by chemolithotrophs. Indeed, Lavik et al. (2009) reported, for the fist time, an event of large-scale detoxification of sulfidic shelf waters by a bloom of chemolithotrophs, and postulate, on the basis of their results, that many sulfidic events in coastal waters may remain unnoticed because bacteria consume sulfide before it reaches the air-sea interface. Consequently, sulfide episodes in bottom waters on continental shelves may be more common than hitherto believed and could have an important but as yet neglected effect on benthic communities. Sulfide typically appears in the water column with a time lag, hours to days following the onset of hypoxia (Riedel et al. 2008). Hence, as hypoxia develops, the likelihood of mass mortality increases faster than anticipated, as the appearance of sulfide will shorten survival times.

There are multiple strategies that benthic organisms can adopt to survive in presence of hydrogen sulfide. Escape may help mobile organisms avoid areas with high sulfide content. Whenever escape is not possible, organisms may try to isolate themselves from

sulfide. For instance, bivalves can close their valves, but such responses are effective only over limited time spans (Hagerman 1998). Some organisms detoxify after sulfide intrusion by oxidizing the sulfide to thiosulfate, a capacity reported for the ostracod *Cyprideis torosa* (Jahn et al. 1996), the Baltic clam *Macoma balthica* (Jahn and Theede 1997), the priapulid worm *Halicryptus spinulosus* (Oeschger and Vetter 1992), the polychaetes *Heteromastus filiformis* (Oeschger and Vismann 1994) and *Arenicola marina* (Volkel and Grieshaber 1994) and the crustacean *Saduria entomon* (Vismann 1991), among others.

Sulfide oxidation can also be used as an energy source in some species, such as the ribbed mussel *Geukensia demissa* (Kraus and Doeller 2004), the polychaeta *Heteromastus filiformis* (Oeschger and Vismann 1994), and the lugworm *Arenicola marina* (Volkel and Grieshaber 1997). The mitochondria of this three species use sulfide as a respiratory substrate for ATP production.

Two main strategies appear to be involved in sulfide detoxification through sulfide oxidation: mitochondrial and blood-based sulfide oxidation (Grieshaber and Volkel 1998). Some organisms can oxidize sulfide in the hepatopancreas, such as the crabs *Bythograea thermydron* (Vetter et al. 1987) and *Sanduria entomon* (Vismann 1991). In natural environments, where the presence of sulfide is normally associated with oxygen deficiency, mitochondrial sulfide oxidation is only effective at micromolar ambient sulfide concentrations for most metazoans and requires the presence of oxygen to oxidize the sulfide to thiosulfate, sulfite or sulfate. Although the inhibition of mitochondrial respiration occurs at concentrations ranging from 2 to 38 μmol L^{-1} H_2S, sulfide acts as a mitochondrial substrate and stimulates oxygen consumption at slightly lower concentrations, reported at 5 to 15 μmol L^{-1} H_2S (Bagarinao 1992). Sulfide detoxification by mitochondrial oxidation involves a delicate dynamic balance wherein sulfide must be promptly oxidized before it reaches internal concentrations inhibitory to cytochrome *c* oxidase (Bagarinao and Vetter 1990). Mitochondrial sulfide oxidation is a detoxification that competes with organic molecules for oxygen, reducing oxygen available for aerobic respiration. The detoxification of sulfide by oxidation to thiosulfate is the most favorable energetically, as 1 mol of sulfide is removed for every 1.5 moles of oxygen, whereas the oxidation to sulfite and sulfate requires 3 and 4 mol of oxygen per mol of sulfide, respectively (Johns et al. 1997).

A last strategy is to switch to anaerobic metabolism. During hypoxia, sulfide will compete with the electron transport chain for oxygen and force animals to change to anaerobic metabolism at a higher oxygen content than this process would be triggered in the

absence of sulfide (Grieshaber and Volkel 1998). A complete inhibition of the respiratory chain occurs at hydrogen sulfide concentrations exceeding 2 to 38 μmol L^{-1} sulfide (Bagarinao 1992), and anaerobic metabolism is triggered even if the oxygen concentration is high enough to allow aerobic metabolism. Anaerobic pathways must proceed with the lowest possible consumption of energy reserves, both as a protection and to extend survival time (Hagerman 1998). That benthic animals may remain alive after exposure to anoxia or sulfide does not necessarily mean long-term survival, as the animals may be compromised and die subsequently. Processes occurring during recovery following exposure to sulfide are, therefore, important in determining the fates of the animals. Animals that turn to anaerobic metabolism and accumulate metabolites must restore their energy resources and re-oxidize at least part of the metabolites when aerobic metabolism is resumed (Hagerman 1998). Some organisms can eliminate the thiosulfate produced for sulfide oxidation though passive diffusion though the hindgut, as the echiuran worm *Urechis caupo* does (Julian et al. 1999), or through the body wall, as the lugworm *Arenicola marina* does (Hauschild et al. 1999).

After sulfide exposure some organelles can be damaged by sulfide. Wohlgemuth et al. (2007) proposed that electron-dense organelles (EDOs) represent transient organelles that sequester and degrade mitochondria or other cellular constituents damaged by sulfide. These EDOs are intracellular, membrane-bounded structures that appear electron-dense by transmission electron microscopy. EDOs are characteristic features of epithelial cells from marine annelids evolutionarily adapted to sulfide exposure and have been reported in all sulfide-adapted annelids that have been investigated using transmission electron microscopy (Wohlgemuth et al. 2007). An alternative pathway of sulfide detoxification is the precipitation of free sulfide by metals (commonly copper), as demonstrated in the Baltic clam *Macoma balthica* by Windoffer et al. (1999). This mechanism is temporary and only works at low sulfide conditions (10 μmol L^{-1}).

Although many marine benthic invertebrates have developed strategies to cope with sulfide exposure, we found no differences in the negative effects of sulfide exposure among broad taxonomic groups. Egg stages, however, showed more negative response to the presence of sulfide than adults and juvenile stages, indicating that a sulfide event can result in catastrophic consequences for hatching success and therefore population dynamics.

Table 1. Sulfide concentrations reported in different habitats, the species inhabiting these habitats and the reduction in survival time expected for the reported sulfide concentration as described by the lineal model reported here (Eq. 1). (*see* Web Annexes for data sources: Annex 6 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/)

Organism	Habitat	Reference	Sulfide concentration (μmol L^{-1})	Reduction in LT50
	burrows, Northern			
Urechis caupo	California	Arp et al. 1992	66	20%
	burrows in summer,	Völkel and		
Arenicola marina	intertidal flat, France	Grieshaber 1992	3.2-336	0-41%
	burrows in spring,	Völkel and		
Arenicola marina	intertidal flat, France	Grieshaber 1992	1.7-138	0-30%
Tubificoides	top 5 cm sediment,			
benedii	sand flat, North Sea	Dubilier et al. 1994	20-50	5-16%
Mud shrimps				
(Thalassinidea)	burrows, Scotland	Johns et al. 1997	260	37%
Burrowing	burrows, mudflat,			
animals	France	Völkel et al. 1995	36	12%
	top 1cm sediment,			
Streblospio	subtidal mud habitat,			
benedicti	Virginia	Llansó 1991	100	25%
	5 cm sediment			
	depth, North Sea and	Jahn and Theede		
Macoma balthica	Baltic Sea	1997	1-432	0-44%
Branchioasychis	intertidal mudflat,	Wohlgemuth et al.		
americana	Cedar Key, Florida	2007	10-170	0-32%
	sediment in Western	Oeschger and Vetter		
	Baltic	1992	665	49%
	water column, Port			
	Angeles harbor,			
	Washington	Ziebell et al. 1970	3	n.e.
	sediment pore water,	Goldhaber and		
	Gulf of California	Kaplan 1974	15000	89%
	Soft bottoms of			
	North Sea mud flats	Thamdrup 1935	180	33%

The meta-analysis conducted here shows that the presence of hydrogen sulfide will decrease survival times under hypoxia by an average of 30% in marine benthic communities. This reduction is concentration-dependent and varies with the sulfide levels that animals experience in their natural environments, and can reach a reduction in survival time of up to 90% at > 10 mmol L^{-1} sulfide concentrations, the highest levels reported in nature (Table 1). In contrast, other organisms, such as *Tubificoides benedii* that inhabits the upper 5 cm of the

sediment can encounter moderate sulfide concentrations, ranging between 20 and 50 μmol L^{-1} (Dubilier et al. 1994), which, using our fitted model, would lead to modest reductions in survival time under hypoxia ranging from 5% to 16% (Table 1). Current frameworks defining hypoxia consider oxygen concentrations alone and do not include the possible synergistic effect of hypoxia and other stresses, such as sulfide toxicity. Results presented here suggest that the survival of benthic invertebrates may be shorter than anticipated as sulfide appears, possibly accelerating mortality events. Other stresses, including pollutants and ammonia, may further reduce their survival. Aggravation of the negative effects of spreading hypoxia by the presence of sulfide suggests that the threats derived from hypoxia to marine biodiversity are greater than anticipated.

Acknowledgments

This research is a contribution to the 'Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery (WISER)' project, funded by FP7 (contract number #226273).

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Temperature effects on thresholds of hypoxia for marine benthic organisms.

Abstract

Global warming will contribute to decrease the global average dissolved oxygen in the oceans worldwide, and may also affect the oxygen requirements of marine benthic macrofauna. The effect of warming on the oxygen requirements and the survival of benthic organisms under hypoxia was tested using a meta-analysis of published experimental results evaluating the effects of increasing temperature on the median lethal time and median lethal concentration of benthic macrofauna under hypoxia. The meta-analysis confirmed that survival times under hypoxia are reduced by an average of 74% and that median lethal concentration increases by a mean of 16% in marine benthic organisms exposed to higher temperatures. Warming reduced survival times of marine benthic macrofauna under hypoxia by a median of 3.95 ± 1.67 hours $ºC^{-1}$ and increased the oxygen thresholds for hypoxia-driven mortality by a median of 1.02 ± 0.15 % saturation (i.e. 0.07 ± 0.01 mg O_2 L^{-1} $ºC^{-1}$). Assessment of the impact of the 4 ºC warming expected during the 21st Century on the survival time and the threshold oxygen concentrations for mortality of benthic macrofauna using the average Q_{10} values for median survival time (3.01 ± 0.29) and median lethal oxygen concentration (2.09 ± 0.20) derived here predict that survival times will decrease by a mean of 35.6 % under hypoxia and that the threshold oxygen concentrations for high mortality to occur will increase by, on average, 25.5 % in a 4 ºC warmer ocean. Hence, ocean warming is expected to increase the vulnerability of benthic macrofauna to reduced oxygen concentrations, increasing the mortality of benthic fauna and greatly extending the area of coastal ecosystems affected by hypoxia-driven mortality.

Introduction

Global warming is forecasted to lead to increase the mean global temperature by 1.8 to 4 ºC by the end of the 21st Century (Meehl *et al.* 2007), with important consequences on climate, hydrology, biodiversity, and biogeochemical cycles. Impacts from global warming will combine with those derived from other human pressures, such as the impacts derived from excess nutrient inputs, which is a major driver of the proliferation of hypoxia in the coastal ocean (Cloern 2001; Kemp *et al.* 2009). Dissolved oxygen is the property that has

changed more drastically in a shorter period of time in the marine environment (Diaz & Rosenberg 1995; Diaz 2001). Oxygen deficiencies have increased in frequency, duration, and severity in the world´s coastal areas during the last decades (Diaz & Rosenberg 2008). As a consequence hypoxia is emerging as a major threat to marine coastal biodiversity (Vaquer-Sunyer & Duarte 2008). Environmental factors, such as the presence of sulphide (Vaquer-Sunyer & Duarte 2010); hypercapnia and low pH (Boleza et al. 2001; Rosa and Seibel 2008), which are increasing with the increased pCO_2 and acidification of ocean waters (Caldeira & Wickett 2003; Zeebe *et al.* 2008), have been shown to enhance the sensitivity of marine organisms to hypoxia. Warming may also contribute to exacerbate hypoxia and its consequences for marine life.

Temperature is a key factor controlling the extent of hypoxia (Conley *et al.* 2007), acting through a multitude of interacting processes, including temperature effects on increasing stratification and reducing ventilation of marine waters (Sarmiento *et al.* 1998). The possibility of strengthened stratification alone, from increased surface water temperature, is enough to worsen hypoxia where it presently exists and will trigger its occurrence in other coastal areas (Rabalais *et al.* 2009). Stramma et al. (2008) documented the vertical expansion of the intermediate-depth low oxygen zones in the eastern tropical Atlantic and the equatorial Pacific during the past 50 years. The hypoxic boundary has shoaled up to 90 m in the Californian Current System (Bograd et al. 2008). The oxygen content of the Oxygen Minimum Zones (OMZ) has been documented to decline in the tropical Pacific, Atlantic and Indian Oceans (Stramma *et al.* 2009; Stramma et al. 2010). The expansion and shoaling of OMZ have been attributed to ocean warming (Keeling and Garcia 2002; Whitney et al. 2007).

The effects of climate change on water temperature are complex and modulated by multiple factors such as changes in wind patterns with subsequent changes in surface currents, circulation and mixing processes. Severe inner-shelf hypoxia off Oregon coast was documented in 2002 (Service 2004). The formation of this new hypoxic area was attributed to deviations in the circulation of the California Current System (Grantham et al. 2004) that further reflect large-scale wind stress anomalies present over the northeast Pacific in 2002 (Murphree et al. 2003). Increasing temperature can produce intensification of coastal upwelling (Bakun 1990; Bakun and Weeks 2004) with a subsequent oxygen decline in bottom waters bellow the upwelling system when low grazing pressure allow the sinking and subsequent respiratory decomposition of primary production (Bakun and Weeks 2004). In

these areas the upwelling of cold waters can lead to cooling, instead of warming, of seawater. Despite this decrease of water temperature in regions with intensified upwelling, there is a general trend toward a global warming of the upper ocean. Lyman et al. (2010) reported significant warming of the upper ocean (from 0 to 700m depth) at global scales over the past 16 years. Recent observational surveys have shown significant warming of ocean bottom-waters (Fukasawa et al. 2004; Masuda et al. 2010). The water temperature in shallow bays from the Swedish West coast has also been reported to have increased (Cossellu and Nordberg 2010). The seawater temperature at the West Mediterranean Sea has increased between 0.12 and 0.5ºC from 1948 to 2005 in the upper layers (0 to 200 m), between 0.05 and 0.2 ºC from 1948 to 2000 in the mid layer (from 200 to 600 m depth) and between 0.03 and 0.1ºC from 1948 to 2005 in the deep water (from 1000 to 2000 m) (Bladé et al. 2010). A coastal site (85 m depth) in the continental shelf of the Catalonian Sea (West Mediterranean) sampled weekly exhibited intense warming trends at all depths ranging from 0.03 to 0.04ºC/year along the period from 1974 to 2001(Vargas-Yanez et al. 2005). Hence, despite variable effects at specific locations, there is a well documented tendency for seawater temperature to increase over the top 700 m, where most hypoxic events have been documented, at the global scale.

Increasing temperature diminishes oxygen solubility (Carpenter 1966; Garcia & Gordon 1992), and increases the respiration rates of organisms (Jones 1977; Enquist et al. 2003), as temperature plays a fundamental role in regulating metabolic processes (Iriberri et al. 1985; White et al. 1991).

Increased temperature will likely affect the responses of marine benthic organisms to hypoxia because metabolic rates increase exponentially with temperature (Brown *et al.* 2004). Whereas both photosynthesis and respiration are enhanced with warming, within the limits imposed by resources (light, CO_2 and nutrients, and oxygen concentration, respectively), Metabolic Theory of Ecology (MTE, Brown *et al*., 2004) predicts that respiration rates should increase faster with warming than photosynthetic rates as activation energies for autotrophic processes are half of those for heterotrophic processes (Harris *et al.* 2006). On the basis of this differential response, Harris et al. (2006) predicted that an hypothetically four degree increase in the summer water temperature of a north-eastern Atlantic estuary will result in a 20% increase in net primary production and a 43% increase in heterotrophic metabolism, resulting in a 16% decrease of the P:R ratios and an increasing likelihood of system heterotrophy. These predictions, however, may be conservative, as they

refer to specific metabolic rates but do not consider possible effects of warming on autotrophic and heterotrophic biomass. Müren *et al.* (2005) showed that the heterotrophic to autotrophic biomass ratio increased 5 times and the production to respiration ratio decreased six times when temperature was raised from 5 to 10ºC in experimental mesocosms. A decrease in ecosystem P:R ratios with increasing temperature could result in a net decrease in oxygen concentration, increasing the frequency and severity of hypoxic events. However, increased temperature may also affect the vulnerability of organisms to low oxygen concentration, as the increased organismal respiration rates increases their oxygen demand, affecting the oxygen thresholds for hypoxia. Here we evaluate, on the basis of a meta-analysis of available experimental results, the effects of temperature on the oxygen thresholds for marine benthic macrofauna. Because the range of species where experimental assessment of temperature effects on thresholds of hypoxia is limited, the generality of the conclusions reached here must be tested further when data for species not included here become available.

Methods

We searched the Web of Science and Scholar Google for reports of hypoxia using the keywords 'hypoxia', 'marine', 'benthic' and 'sea', and their combinations to guide the search. This search delivered more than 6000 published reports of responses of benthic marine organisms to hypoxia, which were then examined further for the availability of experimental assessments of responses to reduced oxygen concentration that included temperature and/or evaluated them at different experimental temperatures. We also searched the list of papers cited in those retrieved by the search. This more restricted search delivered a total of 363 experimental assessments examining the median lethal time (LT_{50}), representing the statistically derived time interval at which 50% of a given population dies after exposure to low O_2 levels, involving 108 different species of marine benthos; and a total of 213 experimental assessments examining the median lethal concentration (LC_{50}), representing the statistically derived O_2 concentration at which 50% of the organisms in a given population die, involving 39 different species of marine benthos. Data on the experimental water temperature were derived from the paper, and where they were only reported in graphics were extracted digitalizing data using Graph Click 2.9.2 software.

Quantile regression was used to assess changes in the probability distribution of thresholds of hypoxia for marine benthic organisms with increasing temperature. The response of the thresholds of hypoxia, as described by LC_{50} (% sat. and mg O_2 L^{-1}) and LT_{50}

(h), to temperature was described by fitting the relationship between the 95%, 50% (median) and 5% quantiles for the distribution of these thresholds and water temperature. Quantile regression estimates multiple rates of change (slopes), from the minimum to maximum response, providing a more complete description of the relationships between variables missed by other regression methods focused on prediction of the mean value (Cade & Noon 2003). Quantile regression can be considered as an extension of classical least squares estimation of conditional mean models to the estimation of a compilation of models for several conditional quantile functions, considering the median as the central parameter (Koenker 2005). Statistical analyses were performed using JMP 7.02 for simple regression analyses, ANOVA and ANCOVA, and R for quantile regression.

Results

We found a total of 363 published experiments involving 108 species pertaining to 10 different taxonomic groups of benthic macrofauna reporting the water temperature at which the median lethal time (LT_{50}, h) was assessed (Fig. 1a) and 213 experimental assessments involving 39 species from 3 different taxonomic groups (mollusca, fishes and crustaceans) of benthic marine fauna reporting the incubation temperature at which the median lethal concentration (LC_{50}, % saturation and mg O_2 L^{-1}) was assessed (Fig 1b,c). The aim of this ample comparison is to test for evidence of a temperature-dependence in the thresholds of hypoxia for coastal benthic organisms. The experiments compiled include a diversity of procedures and species, which may add variability to the analysis, contributing to the residual variability.

Examination of the relationship between LT_{50} and experimental temperature showed that the range of LT_{50} values observed declined with increasing temperature, with most experiments conducted showing relatively low LT_{50} values at high temperature (Fig. 1a). This was confirmed using quantile regression fitted to the 95% and the 5% quantiles as well as the 50% quartile (median) of the change in LT_{50} with increasing temperature (Fig 1a). The 95% quantile regression, estimating the temperature dependence of the maximum LT_{50} expected for a given water temperature, indicated a decrease in the maximum LT_{50} by 63.62 ± 19.10 hours per each degree of temperature increase, whereas the 5% quantile regression, estimating the temperature dependence of the minimum LT_{50}, showed only a decrease by 0.45 ± 0.31 hours (27 minutes) for each degree Celsius increase. The median LT_{50} declined by 3.95 ± 1.67 hours for each degree Celsius of temperature increase (Fig 1a). The variability

in LT_{50}, as described by the 5% to 95% interquantile range, declined with increasing temperature from values of 12.95 to 1956.25 hours at the lower end of marine temperature (0 ºC) to 0 to 47.5 hours at the high end (30 ºC, Fig. 1a).

The intercepts and slopes of the regressions between LT_{50} and experimental temperature showed significant differences among taxonomic groups. No significant relationship between LT_{50} and temperature was found for fish, crustaceans, annelids, cnidarians, bryozoans, echinoderms and platyhelminthes, but Priapulidans showed the steepest decline in median lethal time (205.2 ± 2.08 hours º C^{-1}, $R^2 = 0.99$, $p < 0.007$, $N = 3$), whereas molluscs showed a much smaller decrease in survival time (41.90 ± 5.46 hours º C^{-1}, $R^2 = 0.25$, $p < 0.0001$, $N = 189$) with warming.

The relationship between LC_{50} (% sat.) and experimental temperature showed increasing variability in LC_{50} and increasing median lethal oxygen concentrations with increasing temperature. Quantile regression describing the relationship between the 95% quantile of LC_{50} and water temperature showed an increase in LC_{50} (% sat.) by 2.75 (± 0.47) % saturation (i.e. 0.15 (± 0.04) mg O_2 L^{-1}) per each degree Celsius increase, whereas the 5% quantile regression increased by only 0.50 (± 0.15) % saturation (i.e. 0.03 (± 0.01) mg O_2 L^{-1}) for each degree Celsius of temperature increase. The median LC_{50} (% sat.) increased by 1.02 (± 0.15) % saturation (i.e. 0.03 (± 0.01) mg O_2 L^{-1}) for each Celsius degree of temperature increase (Fig 1b,c). The variability in LC_{50} (% sat.), as described by the 5% to 95% interquantile range, increased with increasing temperature from 0.00 to 15.50 % saturation (i.e. 0.00 to 2.24 mg O_2 L^{-1}) at the lower end of marine temperature (0 ºC) to 11.63 to 98.00 % oxygen saturation (0.80 to 6.74 mg O_2 L^{-1}) at the high end (30 ºC, Fig.1b,c).

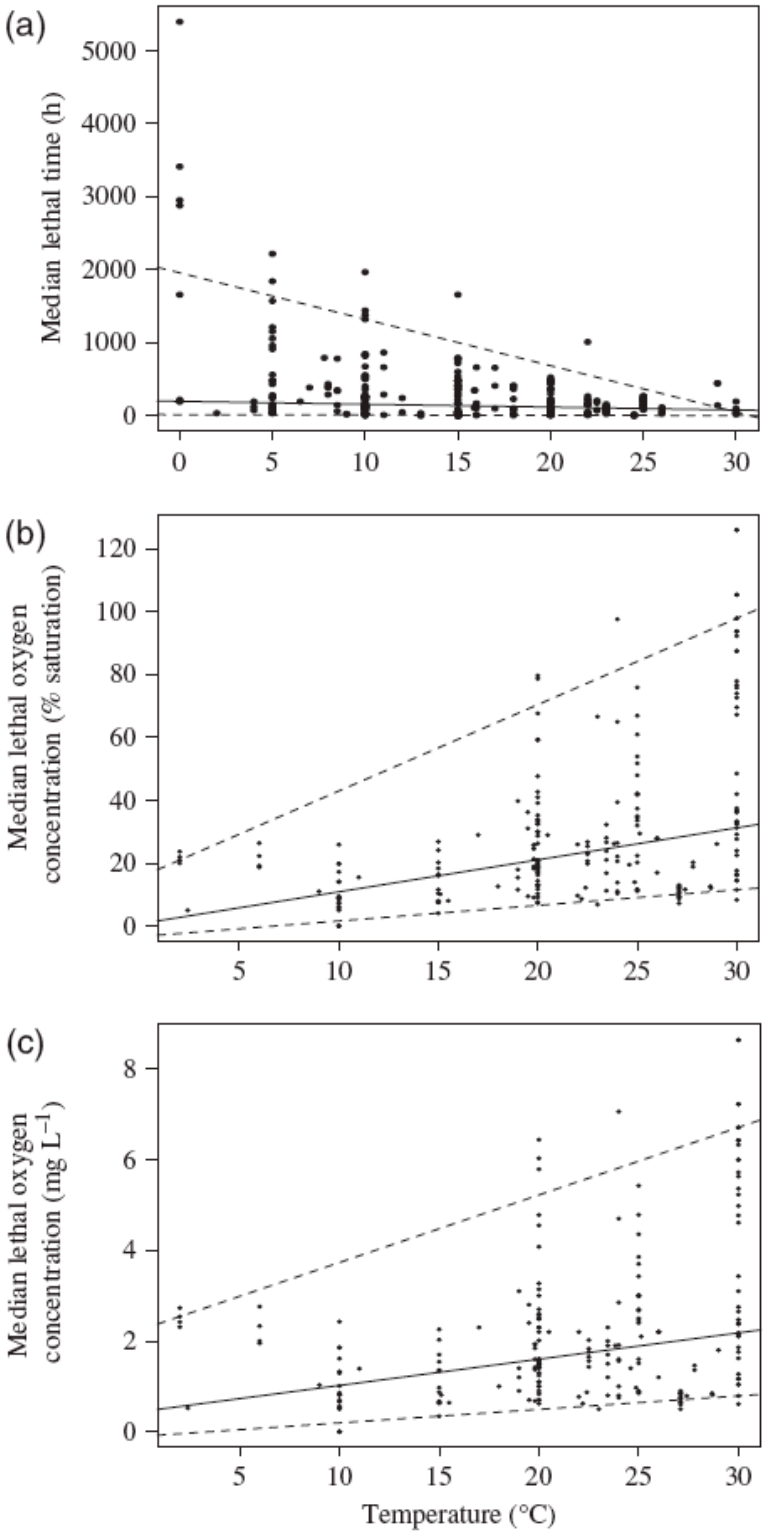

Figure 1. (a) The relationship between the median lethal time (LT50, h) and water temperature for the different experiments. The solid line represents the fitted regression for the median or the 50% quartile [LT50 (h) = 194.70 (±34.04)-3.95 (±1.67)* temperature; N=363, P<0.03]. The dashed lines represent the fitted regression for the 95% quantile [LT50 (h)=1956.25 (±430.54) - 63.62 (±19.10)* temperature; N =363, P<0.001] and the 5% quantile [LT50 (h)=12.95 (± 6.63)-0.45 (± 0.31)*temperature; N=363, P=0.15]. (b) The relationship between the median lethal concentration (LC50, %sat.) and the water temperature for the different experiments. The solid line represents the fitted regression for the median or the 50% quartile [LC50 (%sat.)=0.71 (±2.91)+1.02 (±0.15)* temperature; N=213, P<0.0001]. The dashed lines represent the fitted regression for the 95% quantile (LC50 (% sat.)=15.50 (±8.99)+2.75 (±0.47)*temperature; N=213, P<0.0001) and the 5% quantile (LC50 (% sat.)=-3.37 (±3.62)+0.50 (±0.15)* temperature; N=213, P<0.002). (c) The relationship between the median lethal concentration (LC50, $mgO_2\ L^{-1}$) and the water temperature for the different experiments. The solid line represents the fitted regression for the median or the 50% quartile [LC50 ($mgO_2\ L^{-1}$)50.44 (±0.27)+0.06 (±0.01)* temperature; N=212, P<0.0001]. The dashed lines represent the fitted regression for the upper 95% quartile [LC50 (mgO2L_1)52.24 (±0.68)+0.15 (±0.04)*temperature; n=212, P<0.0001] and the lower 5% quartile [LC50 (mgO_2L^{-1})=-0.10 (±0.32) +0.03 (±0.01)*temperature; N=212, P<0.02] (see Annex 7 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/ for data sources).

There were significant differences in the intercept and the slope of the relationships describing the temperature-dependence of LC_{50} (% sat.) for different taxonomic groups, as no significant relationship between LC_{50} (% sat.) and temperature was found for fish, which may be a consequences of the range of physiological capacities in the fish species included in the data set. Crustaceans showed the highest increase in median lethal O_2 concentration with warming (2.40 ± 0.36 % oxygen saturation $ºC^{-1}$, $R^2 = 0.26$, $p < 0.0001$, $N = 125$; i.e. 0.15 ± 0.03 mg O_2 L^{-1}, $R^2 = 0.21$, $p < 0.0001$, $N = 124$) and molluscs showed the lowest increase in LC_{50} with warming (1.42 ± 0.36 % oxygen saturation $ºC^{-1}$, $R^2 = 0.40$, $p < 0.0007$, $N = 26$; i.e. 0.09 ± 0.02 mg O_2 L^{-1}, $R^2 = 0.37$, $p < 0.001$, $N = 26$).

We found a total of 189 published experiments involving 21 species from 5 different taxonomic groups of benthic macrofauna where the median lethal time (LT_{50}, h) of the subject organism was assessed at different temperatures and a total of 165 published experiments involving 10 species belonging to 3 different taxonomic groups (fishes, crustaceans and molluscs) of benthic macrofauna where the median lethal oxygen concentration (LC_{50}) of the subject organism was assessed at different temperatures.

There was a strongly significant trend for the median lethal time under hypoxia to decrease as temperature increases (95.1% of the experiments), and only 2.9 % of the experiments reported LT_{50} to be unaffected by increasing water temperature (Fig. 2a, Wilcoxon ranked sign test, $p < 0.0001$). The median lethal time under hypoxic conditions was reduced by, on average (± SE), 74 ± 2 % when temperature was increased (Fig. 2a). There was a significant relationship between the ratio of LT_{50} (h) values at the minimum and maximum temperature tested ($\frac{LT_{50\,T\,\min}}{LT_{50\,T\,\max}}$) and the temperature increase (ΔT, º C), as described by the fitted regression equation:

$$\frac{LT_{50\,T\,\min}}{LT_{50\,T\,\max}} = 0.81 + 0.21\ (\pm 0.03)\ \Delta T\ (ºC)$$

($R^2 = 0.19$, $p < 0.0001$)

Calculated Q_{10} values for the change in LT_{50} (h) with increasing temperature showed a broad range of values with an average (± SE) Q_{10} describing the temperature-dependence of LT_{50} of 3.01 ± 0.29 (Fig. 3a). Analysis of covariance (ANCOVA) did not yield evidence of significant differences among taxonomic groups or life stages in slope or intercept (*t*-test, $p > 0.05$) in Q_{10} values for the change in LT_{50} with increasing temperature. Calculated Q_{10} values

for the change in LT_{50} (h) with increasing temperature showed statistically significant differences among organisms with different motility capacities, with sessile organisms having higher Q_{10} values than organism with mobility ($F = 5.35$, $p < 0.02$).

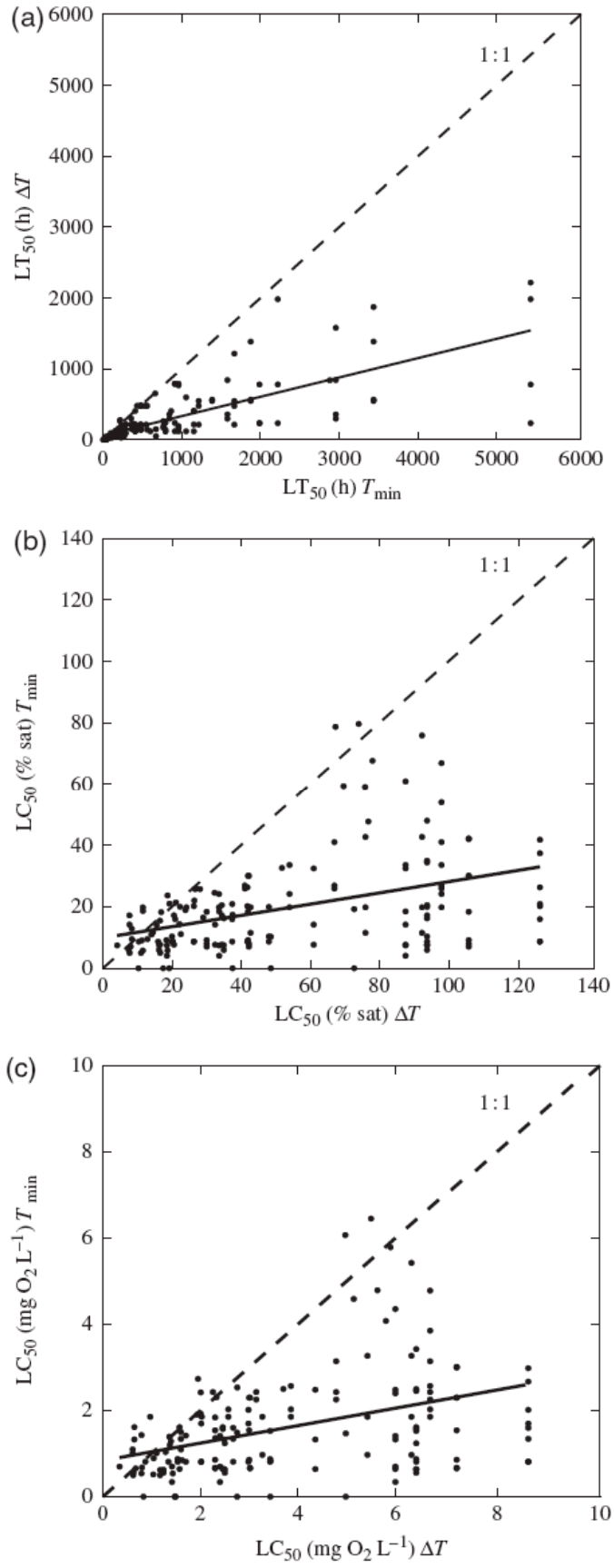

Figure 2. (a) The relationship between the median lethal time at increased temperature (LT50ΔT) and median lethal time at lower temperature (LT50) (units: h). LT50ΔT (h)=80.04 (±22.68)+0.26 (±0.02)*LT50 (h). (R^2=0.54, P<0.0001) n5189. The solid line represents regression line and dashed line represents the line 1:1. (b) The relationship between the median lethal concentration at one temperature (LC50 Tmin) and median lethal concentration at increased temperature (LC50ΔT) (units: % oxygen saturation). LC50 Tmin (% oxygen saturation)=9.85 (±2.01)10.18 (±0.03)* LC50ΔT (% sat.). (R^2=0.17, P<0.0001) N=165. The solid line represents regression line and dashed line represents the line 1:1. (c) The relationship between the median lethal concentration at one temperature (LC50 Tmin) and median lethal concentration at increased temperature (LC50ΔT) (units: mgO_2 L^{-1}). LC50 Tmin (mgO_2 L^{-1})=0.84 (±0.16)+0.20 (±0.04)*LC50ΔT (mgO_2 L^{-1}). (R^2=0.16, Po0.0001) N=165. The solid line represents regression line and dashed line represents the line 1:1 (see Annex 8 at http://imedea.uib-csic.es/users/raquel/phD_Annexes/for data sources)

There was a strongly significant trend for the median lethal oxygen concentration to increase with increasing temperature (93.3 % of the experiments, Wilcoxon ranked sign pair test, $p < 0.0001$, Fig. 2b). The median lethal oxygen concentration under hypoxic conditions increased by, on average (± SE), 81.7 ± 3.1 % and 79.9 ± 4 % when temperature was elevated in terms of % saturation (Fig. 2b) and concentration (mg O_2 L^{-1}, Fig. 2c), respectively. Calculated Q_{10} values for the change in LC_{50} with increasing temperature showed an average (± SE) Q_{10} describing the temperature-dependence of LC_{50} of 2.08 ± 0.20 (in % sat., Fig. 3b) and of LC_{50} 1.80 ± 0.17 (in mg O_2 L^{-1}, Fig. 3c). Analysis of variance (ANOVA) did not yield evidence of significant differences for taxonomic groups ($p > 0.05$). There were statistically significant differences for life stages, with larvae tending to have higher Q10 values for the change in LC_{50} (% sat) with increasing temperature than juveniles, but not than adults ($F = 3.43$, $p < 0.05$). Sessile organisms tended to have higher Q_{10} values for the change in LC_{50} (% sat) with increasing temperature than high mobile organisms or active swimmers, but not than organisms with reduced mobility ($F = 5.16$, $p < 0.006$).

Discussion

The results presented support the hypothesis that the thresholds of hypoxia for benthic marine macrofauna are significantly affected by temperature and provide estimates of the extent of change in thresholds of hypoxia with increasing temperature. However, is derived for a data set including a limited set of species, so that the generality of the conclusions reached here must be tested further when data for species not included here become available. Moreover, these results derive from experiments where temperature changes were imposed over short time scales, whereas microevolutionary changes may increase the resistance of organisms to hypoxia as the oceans warm along the 21st Century, so that the predictions resulting from this analysis may provide worst-case scenarios that can be refined with data derived from documented responses of benthic communities to hypoxia in the future.

The results from this meta-analysis indicates that the survival time of benthic organisms under hypoxia is reduced and the oxygen concentration at which high mortality (LC_{50}, % sat.) takes place increases with increasing temperature, indicating that the oxygen requirements of benthic macrofauna increase with increasing temperature. Moreover, the range of survival time and the median lethal oxygen concentration for benthic macrofauna are also significantly affected by increasing temperature.

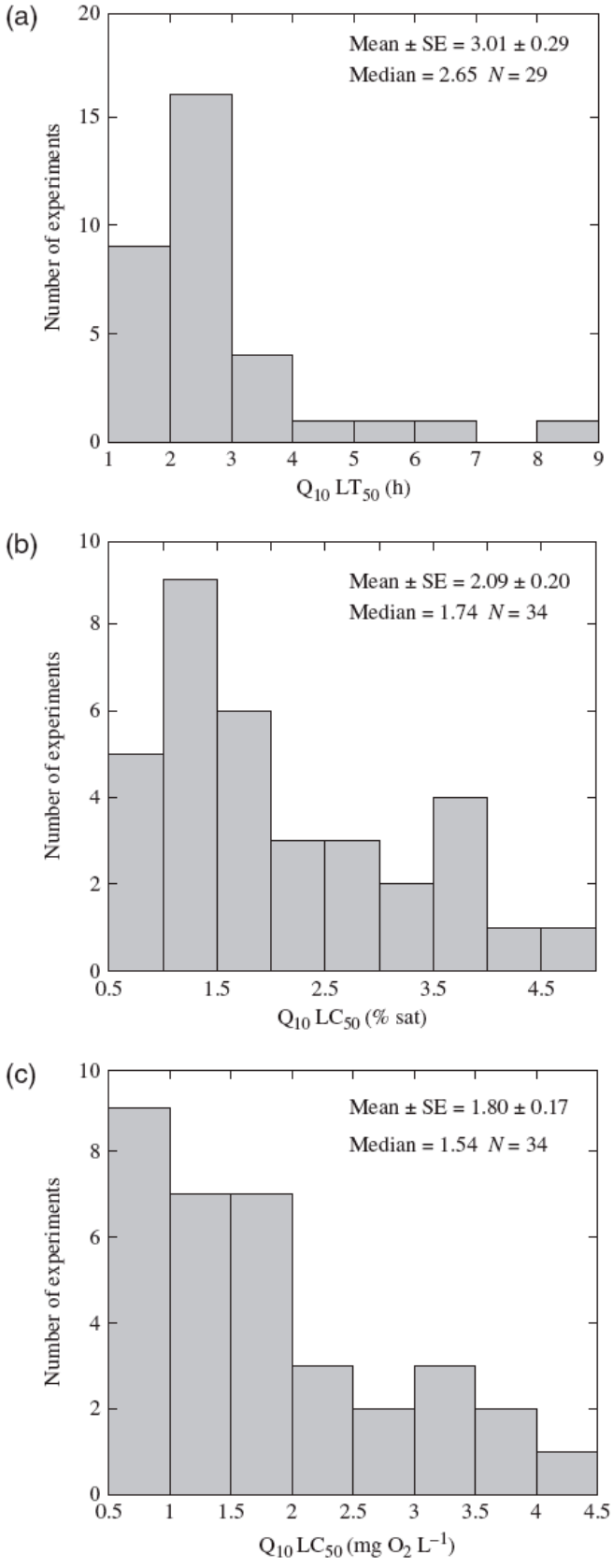

Figure 3. Frequency distribution for Q_{10} values for median lethal time (a) and for median lethal oxygen concentration in % sat. (b) and in mg O_2 L^{-1} (c). Data on Q_{10} for median lethal time calculated from (Gamble, 1970; Dries & Theede, 1974; Shumway et al. 1983; Oeschger & Theede, 1986; Stickle et al. 1989; Brooks et al. 1991; Johnson & McMahon, 1998; de Zwaan et al. 2001) and on Q_{10} for median lethal oxygen concentration calculated from (Shimps & Rice personal comment; Vargo & Sastry, 1977; Stickle et al. 1989; Schurmann & Steffensen, 1992; Hoback & Barnhart, 1996; Plante et al. 1998; Miller et al. 2002; Cerezo & Garcia, 2004; Ishibashi et al. 2005; Shimps et al. 2005; Goodman & Campbell, 2007).

Examination of the relationship between LC_{50} (% sat.) and water temperature showed that the range of median lethal O_2 concentration increased significantly with increasing water temperature, suggesting that the relationship between LC_{50} (% sat.) and water temperature is complex, probably driven by differences on the effect of temperature on metabolism, possible differences between the experimental temperature and the temperature within the ecosystem where the organisms were living, taxonomic differences in the sensitivity to hypoxia and warming, different life stages, and different species-specific physiological limitations, among others. Conversely, some tolerant species may benefit from reduced tolerance of predators, as the case of the quahog clam (*Mercenaria mercenaria*) that gained refuge from its less tolerant predators that are either excluded or less effective under hypoxia (Altieri 2008). Crustaceans, the group most vulnerable to hypoxia, were the organisms with the highest oxygen requirements for survival at any one temperature, showing an increase in the median lethal O_2 concentration of 0.24 mg O_2 L^{-1} per each degree Celsius of warming, and an even steeper increase for the 95% quantile (0.31 ± 0.06 mg O_2 L^{-1} $ºC^{-1}$). This confirms earlier indications that crustaceans are the most sensitive group to hypoxia (Vaquer-Sunyer and Duarte 2008), and shows that they are also the organisms with thresholds of hypoxia most sensitive to temperature.

Whereas the LT_{50} (h) and LC_{50} (% sat.) for individual species varies considerably for any water temperature, experimental temperature manipulations show an overwhelming tendency for individual species to the survival times under hypoxia and the oxygen requirements for survival to decrease and increase, respectively, with increasing temperature. The mean Q_{10} values describing the temperature-dependence of the thresholds of hypoxia for benthic macrofauna are comparable to those describing the temperature dependence of respiration rates (Q_{10} values around 2 - 3, cf. Neori & Holm-Hansen 1982; Raven & Geider 1988), providing evidence that the temperature-dependence of thresholds of hypoxia for benthic macrofauna is associated with increased metabolic oxygen demands at increasing temperature.

Q_{10} values either for median lethal time or median lethal oxygen content showed higher values for sessile species than for species with a higher degree of mobility. The implication of a high Q_{10} value for LC_{50} (%sat.) or LT_{50} (h) is that the oxygen requirements of sessile organisms will increase more with warming than for mobile organisms with the handicap that sessile organisms cannot escape the hypoxic area. The mean Q_{10} values for LT_{50} (h) for sessile species is 5.06, resulting in a decrease of their survival time by 25.3% and

47.7% with 1.8 ºC and 4 ºC warming, respectively. For a oyster (*Crassostrea virginica*) living in waters with 30 psu and 20ºC, this reduction means a decrease in its survival time from 20 to 10 days with a 4ºC warming and to 14 days with a 1.8ºC warming. Reductions in survival time to half can lead to a significant reduction of the oyster population in areas such as Chesapeake Bay, where persistent seasonal hypoxia occurs and temperature is one of the key controls of hypoxia development (Kemp et al. 2009). The average Q_{10} values for LC_{50} (% sat.) for sessile species is of 3.72, resulting in an increase of median lethal oxygen content by 21.1 % and 40.9 % with warming of 1.8 ºC, and 4 ºC, respectively. The consequences for an oyster living at 20ºC and 30 psu would be an increase of the oxygen requirements to survive, enhancing its LC_{50} (% sat.) from 19 % saturation (i.e. 1.46 mg O_2 l^{-1}) to 27 % or 23 % saturation (i.e. 1.95 or 1.71 mg O_2 l^{-1}) with warming of 4 or 1.8ºC respectively.

Three species showed an increase in survival time with warming, the bivalve *Corbicula fluminea* (Johnson and McMahon 1998), the polychaeta *Nephthys ciliata* (Dries & Theede 1974) and the crustacean *Corophium arenarium* (Gamble 1970). The cause for their decrease in survival at low temperatures is probably due to thermal stress, rather than hypoxia, because the experimental temperatures tested were not within the thermal niche of the species, as *Corbicula fluminea*, for example, is a warm-water specie (Johnson & McMahon 1998). When organisms are maintained outside their thermal optimal oxygen levels in body fluids can decrease, as a consequence of excessive oxygen demand at high temperatures or insufficient aerobic capacity of mitochondria at low temperatures (Portner 2001). In the case of the crustacean *Corophium arenarium* the difference in survival time was very small (0.83 h or 6% lower) for experiments made at 15ºC and 10ºC, compared with the differences in survival time measured at 20ºC and 15ºC (5.52 h, 39%) or between 10ºC and 5ºC (19.27 h, 60%). Only 11 experiments from a total of 165 belonging to 4 different species showed a higher LC_{50} at low temperatures than at warmer ones. These species were the Atlantic cod *Gadus morhua*, the crabs *Callinectes sapidus* and larval stages of *Cancer irroratus* and the gastropod *Thais haemastoma.* Two of these experiments were made to assess changes in the oxygen requirements of the Atlantic cod *Gadus morhua* with warming (Plante *et al.* 1998), two in the crab *Callinectes sapidus* (Stickle *et al.* 1989), 3 in the gastropod *Thais haemastoma* (Stickle et al. 1989), and four in larval stages of the crab *Cancer irroratus* (Vargo and Sastry 1977). In the case of the third and fifth zoea larval stage of the crab *Cancer irroratus*, the hypoxia tolerance decreased at 10ºC, indicating a failure in metabolic adaptation to this lower temperature, as their culture temperature was 15ºC (Vargo

and Sastry 1977). The published studies revealed either no effect of temperature on hypoxia tolerance of Atlantic cod (*Gadus morhua*) or decreasing tolerance with increasing temperature. The Atlantic cod (*Gadus morhua*) did not show measurable differences in survival under different temperatures ranging form 2 to 6ºC (Plante *et al.* 1998), but it showed decreasing hypoxia tolerance with warming in a later study where the temperature ranged from 2.4 to 17ºC (Schurmann and Steffensen 1992).

The results reported here imply the existence of synergistic effects of hypoxia and warming greatly increasing the vulnerability of marine biota to hypoxia in a warmer ocean. Assessment of the impact of warming across the range from 1 to 6 º C expected across regions along the 21st Century on the survival time and the threshold oxygen concentrations for mortality of benthic macrofauna using the average Q_{10} values reported here predict that survival times will decrease by a mean of 10.4 to 48.4 % and the threshold oxygen concentrations for high mortality to occur will increase by, on average, between 7.1 to 35.7 % with increasing warming (Fig. 4). Hence, ocean warming is expected to increase the thresholds of hypoxia-driven mortality of benthic macrofauna. Provided that ocean warming is already documented to affect, in most areas, the top 700 m of the ocean (Lyman et al. 2010), further warming is expected to extend the area of coastal ecosystems affected by hypoxia-driven mortality, except in areas where ocean dynamics may buffer warming, such as areas where upwelling maybe intensified. As for present hypoxia, the extent of impacts will vary among taxa, depending on physiological strategies, life stages and motility, but will also depend on the capacity of microevolutionary process to increase the resistance of benthic organisms to hypoxia along the 21st Century.

Oxygen concentrations are also expected to be reduced in a warmer ocean rendering the effects of ocean warming on hypoxia-driven mortality steeper than expected from the effect of temperature on the oxygen requirements of organisms shown here alone. In a recent paper, Conley *et al.* (2009) calculated that the hypoxic area in Danish coastal waters will double with a 4ºC increase as a consequence of changes in oxygen solubility alone, maintaining all other factors unchanged. At the global scale, ocean models predict declines in global average dissolved oxygen, due to ocean warming, over the next century ranging between 1% and 7% (Keeling *et al.* 2010). Shaffer *et al.* (2009) predicted long-term ocean oxygen depletion and a great expansion of ocean oxygen-minimum zones for scenarios involving high emissions or high climate sensitivity to green house emissions. Whereas Keeling et al. (2010) provide evidence for a global oxygen decline in ocean waters, rates of

oxygen decline tend to be greater in coastal waters compared to open ocean ones (Gilbert *et al.* 2009), consistent with observations around the world (Gilbert *et al.* 2005; Bograd *et al.*

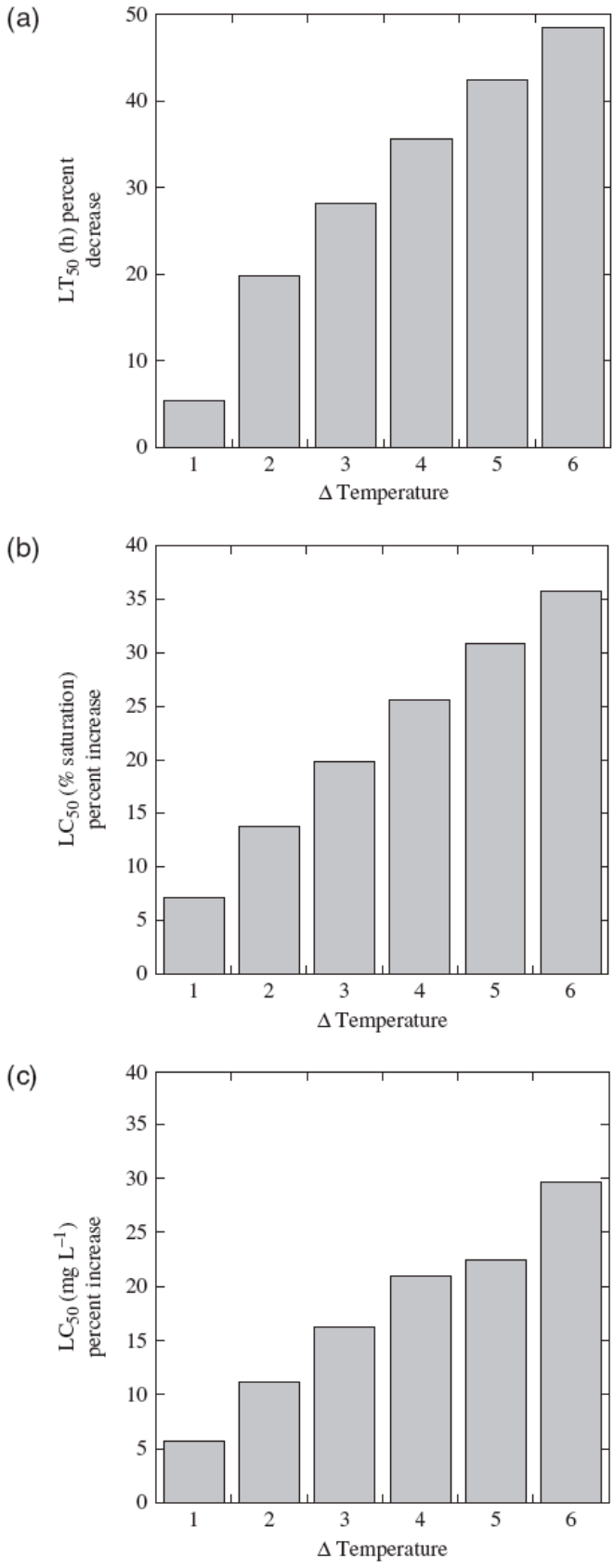

Figure 4. Changes in hypoxia thresholds calculated from Q_{10} values derived here. (a) Percentage of decrease of median lethal time expected with different water warming; (b) increase of the median lethal oxygen content expected for different water temperature increases in % sat.; (c) in mg O_2 L^{-1}.

2008; Diaz & Rosenberg 2008). Yet, reduced oxygen concentration with increasing temperature and increased oxygen requirements by benthic macrofauna are only two of a variety of processes affecting hypoxia in a warmer ocean, as a warmer ocean is also likely to show increased stratification, reducing ventilation, and an increased oxygen drawdown by respiratory processes, which are enhanced with increasing temperature (Brown *et al.* 2004; Harris *et al.* 2006).

Hence, ocean warming will produce an increase in the extent and severity of marine macrofauna mortality under hypoxia by the combined effect of reducing dissolved oxygen concentration in the ocean and increasing the oxygen requirements of organisms (Najjar *et al.* 2010) and their sensitivity to reduced oxygen concentrations. The combined effect will produce further reduction in the quality and spatial extent of suitable habitat for a wide range of aerobic organisms. For example, Niklitschek & Secor (2005) demonstrated, in a simulation on the combined effects of warming and hypoxia in the Chesapeake Bay system, that a small warming of 1 ºC during summer months could practically eliminate suitable habitats for juvenile Atlantic sturgeon (*Acipenser oxyrhynchus*). Indeed, the reduction of suitable habitat for fishery species could result in important losses for the fishery industry (Breitburg 2002). Suitable habitat for most metazoans is restricted by water temperature, among other multiple factors, as all organisms live within a limited range of water temperatures, allowing optimized structural and kinetic coordination of cellular, molecular, and systemic processes (Portner & Farrell 2008). Warming above the thermal window of the organisms can also trigger anaerobic metabolism (Portner & Farrell 2008).

The meta-analysis conducted here suggests that warming will negatively impact the survival of benthic organisms under low oxygen conditions by reducing survival times under hypoxia by a median of 3.95 ± 1.67 hours $ºC^{-1}$ and by increasing the oxygen thresholds for hypoxia-driven mortality by a median of 1.02 ± 0.15 % saturation $ºC^{-1}$ (0.07 ± 0.01 mg O_2 L^{-1} $ºC^{-1}$) across the species for which evidence is available. Hypoxia is already expanding globally across coastal waters (Diaz & Rosenberg 2008; Rabalais *et al.* 2009; Rabalais *et al.* 2010), parallel to increased flux of nutrients to the coastal zone and concurrent with a tendency for warming of coastal waters (Rabalais *et al.* 2009; Rabalais *et al.* 2010). The synergies between two global changes, oxygen depletion and warming the world's coastal waters, threaten benthic macrofauna in coastal ecosystems. Aggravation of the negative effects of spreading hypoxia by warming and the fact that warming will contribute to oxygen depletion in ocean waters suggest that the threats to marine biodiversity derived from hypoxia

will be greater than anticipated.

Acknowledgments

This research is a contribution to the 'Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery (WISER)' project, funded by FP7 (contract number #226273). We thank Alexandra Steckbauer for help with translations of German papers. C.M.D. was supported by a sabbatical fellowship from the Spanish Ministry of Education.

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General discussion

The results presented in the preceding sections confirm the temperature-dependence of metabolic processes in the two marine ecosystems experiencing the fastest warming rates, the Arctic Ocean and the Mediterranean Sea, and demonstrate that the thresholds of hypoxia are higher than hitherto believed, and dynamic, varying across taxa and responding to warming and the presence of sulphides in the environment.

We used two approaches to explore metabolic responses to warming: Experimental assessments of metabolic responses to warming and the assessment of the metabolic responses to *in situ* variability in temperature in natural systems. Experimental approaches were used to assess the responses of communities in the two areas of the world that are experiencing the steepest warming rates: the Arctic Ocean and the Mediterranean Sea. The results showed that the respiration rates of Arctic planktonic communities increase steeply with increasing warming. The experimental assessment of the responses of Arctic planktonic community respiration rates to warming revealed a Q_{10} (the relative rate of increase in respiration rate expected for a 10ºC temperature increase) value of 5 (Chapter 1). Our results suggest that a future 6 ºC warming of the Arctic Ocean surface water as expected by year 2100 may yield a mean increment in respiration rates of 62%, doubling the 30% increment expected for primary production (Wassmann et al. 2008), and 3.8-fold higher than the 16.1% increase in photosynthetic rates derived from Q_{10} values reported in the literature. The increase in respiration rates will be further aggravated during spring, when respiration rates may increase by, on average, 76% with a 6ºC warming (Vaquer-Sunyer *et al.* 2010).

The experimental assessment of the responses of benthic and planktonic communities metabolism in the Mediterranean Sea revealed no significant differences in the metabolic changes to warming between these two types of communities (Chapter 2). Our results suggest that a 6 ºC warming of the Mediterranean waters as expected by year 2100 may yield a mean increment in respiration rates of coastal Mediterranean communities of 73%, higher than the mean increase of 52% expected for gross primary production.

Results for both these ecosystems confirm earlier predictions of a higher increase in respiration rates than in primary production with warming (Harris *et al.* 2006; Lopez-Urrutia *et al.* 2006), with the subsequent consequences for Carbon and Oxygen cycling.

The examination of metabolic responses to temperature under natural conditions (i. e. examining the response to temperature variability *in situ*) yielded contrasting results. In Chapter 3, the calculated Q_{10} values for a Mediterranean semi-enclosed bay were very similar for GPP and CR, with the Q_{10} value for GPP being only marginally higher than that for respiration rates, 2.06 ± 0.39 and 1.98 ± 0.21, respectively. Moreover the Q_{10} values for respiration rate were well below those derived experimentally.

In general, activation energies, describing the temperature-dependence of metabolic processes, derived from natural communities at their in situ temperature are much lower than when derived from warming experiments. Indeed, most warming experiments derive similar activation energies across systems. The mean activation energy for planktonic respiration found for Mediterranean communities (114.7 ± 30.47 kJ mol^{-1} K^{-1}, Chapter 2) is similar to that found for temperate planktonic communities from the Menai Strait (113.0 ± 6.1 kJ mol^{-1} K^{-1}, Lefevre et al. 1994) and Arctic planktonic communities (115.5 ± 20.5 kJ mol^{-1} K^{-1}, Chapter 1). Although the activation energy for respiration is consistent with other activation energies for respiration derived from experimental studies, it is higher than the activation energy derived from the relationship between respiration rates and in situ water temperature for a Mediterranean costal bay (46.71 ± 7.39 kJ mol^{-1} K^{-1}, Chapter 3). The activation energies derived from warming experiments tend to be higher than those calculated from semi-empirical models, such as that derived by Lopez-Urrutia et al. (2006), where the predicted activation energy for community respiration was 27.02 kJ mol^{-1} K^{-1}, as well as values derived from the relationship between respiration rates, standardized to chlorophyll a concentration, and *in situ* water temperature for oceanic communities, which derived an activation energy for community respiration of 68.51 ± 5.79 kJ mol^{-1} K^{-1} (Regaudie-de-Gioux and Duarte *In press*).

The difference in activation energies derived from natural communities at in situ temperature and those calculated from experiments where the communities were exposed to sudden warming can be artefacts derived from the rapid increase in temperature applied in

the experiments compared to the more gradual warming experienced in their habitat. In natural communities the changes in temperature are gradual, allowing acclimation of the organisms. In contrast, in experimental assessments, organisms are suddenly exposed to higher temperatures, not allowing acclimation of the organisms. However, experiments also explore metabolic responses beyond the current thermal window of the organisms, which will be exceeded by predicted warming, whereas empirical assessments of relationships with in situ temperature explore the responses within current thermal windows, which may also explain the differences in the responses derived.

Although results derived in Chapter 3 do not confirm earlier findings, most previous studies coincide in predicting faster increases in respiration rates than in production (Chapters 1 and 2, Lefevre *et al.* 1994; Müren *et al.* 2005; Lopez-Urrutia *et al.* 2006; Yvon-Durocher *et al.* 2010) as predicted by Metabolic Theory of Ecology (Brown *et al.* 2004; Harris *et al.* 2006). Q_{10} derived in Chapter 3 seems to contradict MTE. However, *C. prolifera* photosynthesis is inhibited at temperatures above 30 ºC (Lloret *et al.* 2008). Hence, warming in Portocolom bay will probably reduce gross primary production in the bay when a 30 º C is exceeded. Indeed, warming will increase the likelihood of hypoxia in the bay, as the probability of finding hypoxia increases at higher temperatures (Chapter 3). The interactive effects of warming in proliferation of phytoplankton, inhibition of *C. prolifera* photosynthesis (Lloret *et al.* 2008), and decreasing nutrient uptake by the macroalgae (Grall and Chauvaud 2002) will probably led to increased eutrophication in the bay and decreased oxygen content, increasing the frequency and duration of hypoxic events. Global warming triggers a cascade of responses increasing the likelihood of hypoxia, with their associated negative consequences for marine life, and may, thus, have catastrophic consequences in Portocolom bay, as well as in other similar semi-enclosed Mediterranean Bays.

Hypoxia is an increasing problem worldwide (Diaz and Rosenberg 1995). Hypoxic events are increasing in number, frequency and severity around the world (Diaz 2001; Diaz and Rosenberg 2008). As a consequence, hypoxia is emerging as one of the major threats to marine biodiversity (Vaquer-Sunyer and Duarte 2008). Although eutrophication-driven hypoxia is a major concern, the widespread use of a threshold of 2 mg O_2 L^{-1} to designate waters as hypoxic lacks a robust empirical basis. Indeed, there is ample experimental evidence that a threshold of 2 mg O_2 L^{-1} may be inadequate to describe the onset of hypoxia,

as impacts of hypoxia occur at higher oxygen concentrations for many vulnerable organisms (Gray *et al.* 2002).

We examined the thresholds of hypoxia for marine benthic communities on the basis of a broad comparative analysis across a range of contrasting marine benthic organisms (Chapter 4). The results showed that hypoxia thresholds vary greatly across marine benthic organisms and that the conventional definition of 2 mg O_2 L^{-1} to designate waters as hypoxic is below the empirical sublethal and lethal oxygen thresholds for half of the species tested. The results of the meta-analysis provided evidence of the broad, order of magnitude, variability in the thresholds of oxygen concentrations for hypoxia among benthic marine organisms, which cannot be adequately captured by a single, universal threshold. This variability partially derived from significant differences in oxygen thresholds across taxa and also across organisms differing in mobility. A precautionary limit to avoid catastrophic mortality events and effectively conserve marine biodiversity could be set at 4.6 mg O_2/liter, the 90th percentile of the distribution of mean lethal concentrations, which would be expected to maintain the populations of most, except the 10% most sensitive, species. These results imply that the number and area of coastal ecosystems affected by hypoxia and the future extent of hypoxia impacts on marine life have been generally underestimated (Vaquer-Sunyer and Duarte 2008).

Thresholds of hypoxia for marine benthic communities are modulated by diverse factors, including environmental factors and intrinsic organismal features. Environmental stressors can have additive or synergetic effects in shortening survival time of marine organisms under hypoxia, such as increasing temperature, increased pCO_2 levels in the ambient waters (Portner and Farrell 2008), and the presence of hydrogen sulfide and contaminants, among others. Here, we explored the environmental modulation of hypoxia thresholds for marine benthic communities, specifically addressing the effects of the presence of hydrogen sulfide and warming (Chapter 5).

Low oxygen concentration does not only affects marine organisms but also fundamental biogeochemical processes (Conley *et al.* 2009). During the onset of hypoxia, electron acceptors are consumed, and prokaryotes shift to use alternative electron acceptors such as nitrite, nitrate, sulphate or metal oxides. Benthic metabolism shifts from aerobic to

anaerobic pathways when sediments and overlaying water becomes anoxic, leading to the accumulation of reduced metabolites such as sulphide. Sulphide is very toxic for most organisms, because it inhibits cytochrome *c* oxidase activity (Nicholls 1975), and reduces oxygen delivery to mitochondria in some species (Evans 1967), impeding respiration. The results presented in Capter 5.1 confirm the existence of a synergistic effect of hypoxia and the presence of sulphide in accelerating mortality of benthic macrofauna. The meta-analysis conducted on the effect of the presence of hydrogen sulphide on the survival of benthic organisms under hypoxia revealed that the presence of hydrogen sulphide decreases survival times under hypoxia by an average of 30% in marine benthic communities (Chapter 5.1). This reduction is concentration-dependent and varies with the sulphide levels that animals experience in their natural environments. The effect of sulphide on survival is higher for egg forms than for juvenile or adult stages (Chapter 5.1), with the consequent impacts on population dynamics.

Warming strongly affects biological processes because it plays a fundamental role in regulating metabolic processes (Iriberri *et al.* 1985; White *et al.* 1991). Temperature also affects the responses to hypoxia of marine benthic organisms because it accelerates metabolic processes as metabolic rates increase exponentially with temperature (Brown *et al.* 2004). In Chapter 5.2 the effect of warming on the oxygen requirements and the survival of benthic organisms under hypoxia was tested using a meta-analysis of published results of experiments evaluating the effects of temperature on the median lethal time and median lethal concentration of benthic macrofauna under hypoxia. Results confirmed that survival times under hypoxia were reduced by on average 74% and that median lethal concentration increased by on average 16% when marine benthic organisms were exposed to warmer temperatures. Warming reduced survival times of marine benthic macrofauna under hypoxia by a median of 3.95 ± 1.67 h $^{\circ}C^{-1}$ and increased the oxygen thresholds for hypoxia-driven mortality by a median of 1.02 ± 0.15% saturation $^{\circ}C^{-1}$ or 0.07 ± 0.01mgO_2 L^{-1} $^{\circ}C^{-1}$. The corresponding Q_{10} values averaged 3.01 ± 0.29 for the median survival time and 2.09 ± 0.20 for the median lethal oxygen concentration, similar to Q_{10} values for metabolic processes. Use of these Q_{10} values predicts that the 4 ºC warming expected during the 21st century will lead to survival times 35.6% lower under hypoxia and that the threshold oxygen concentrations for high mortality to occur will increase by, on average, 25.5% if bottom water temperature increased by 4 ºC. Hence, ocean warming is expected to increase the

vulnerability of benthic macrofauna to reduced oxygen concentrations and expand the area of coastal ecosystems affected by hypoxia (Vaquer-Sunyer and Duarte 2010b).

All studied processes and all the results obtained within this work reveal, in summary, that anthropogenic disturbances are significantly affecting coastal metabolism, and therefore, oxygen dynamics, leading to oxygen declines due to the combined effects of eutrophication and warming, threatening coastal biodiversity and ecosystems functioning. Continuing to explore consequences of anthropogenic disturbances on oxygen dynamics and in marine biodiversity, ecosystems functioning and food-web alterations and their consequences on fisheries is essential to better protect marine ecosystems, preserve biodiversity and set successful management targets for coastal waters.

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Conclusions

1. The increase in Arctic plankton respiration rates with a 6 ºC warming (62%) will far exceed the increase in photosynthetic rates expected from the temperature-dependence of photosynthetic rates reported in the literature (16-30%).

2.The role of Arctic communities as significant CO_2 sinks may weaken substantially, and even be reverted to become CO_2 sources to the atmosphere, with global warming.

3. The increase in Mediterranean coastal ecosystems respiration rates with a 6 ºC warming may yield a mean increment in respiration rates of coastal Mediterranean communities of 73%, higher than the mean increase expected for gross primary production of the 52%.

4. The probability of find lower oxygen content increases at higher temperatures. Warming will increase the likelihood of hypoxia in closed coastal bays in the Mediterranean Sea.

5. Hypoxia impacts occur at a broad range of oxygen concentrations, including oxygen concentrations well above the oxygen thresholds generally used to diagnose hypoxia at present. The vulnerability of coastal ecosystems to hypoxia is, thus, greater than currently recognized, with fish and crustaceans being the most vulnerable faunal components.

6. A precautionary limit can be set at 4.6 mg O_2 L^{-1}, the oxygen level above the lethal concentration for 90% of the available experimental tests, for effective conservation of biodiversity.

7. The number and extent of the coastal zones affected by hypoxia is likely to be greater than hitherto realized, and the prospects for future expansion of these areas more disturbing than currently forecasted.

8. Coastal hypoxia is emerging as a major threat to coastal ecosystems globally.

9. Survival times under hypoxia are reduced by an average of 30% in marine benthic communities exposed to hydrogen sulfide. Threats derived from hypoxia to marine

biodiversity are greater than anticipated on the basis of the direct effects of low oxygen concentration alone.

10.Warming impacts negatively on the survival of benthic organisms under low oxygen conditions:

-Reducing survival times under hypoxia by a median of 3.95 ± 1.67 hours $ºC^{-1}$

-Increasing the oxygen thresholds for hypoxia-driven mortality by a median of 1.02 ± 0.15 % saturation $ºC^{-1}$ or 0.07 ± 0.01 mg O_2 L^{-1} $ºC^{-1}$

11. Impacts of warming across the range from 1 to 6 º C of temperature increase (expected across regions by the end of the 21st Century) on the survival time and the threshold oxygen concentrations for macrofauna predict that survival times will decrease by 10.4 to 48.4 % under hypoxia and the threshold oxygen concentrations for mortality will increase between 7.1 and 35.7 % with warming.

12. The combined effects of eutrophication and global warming are negatively affecting coastal marine life and will probably continue in the future. Appropriate management strategies should be set to reduce nutrient loadings to coastal areas and to reduce CO_2 emissions at a global scale and mitigate consequences of global warming, to better protect marine biodiversity.

Acknowledgements

Since I was a child I was dreaming on be a scientist, doing research in the sea, on board, sailing the oceans, going to Antartica... When I grow up I thought that my dreams will never come true, since I met Carlos and he helped me to live my dreams. I'm highly grateful to Carlos, who introduced me to the research's world. He guided me all along my thesis, and shared with me his scientific knowledge and enthusiasm. Without his encouragement all this work would not been possible. Thanks for your patience and help all this years!

I also want to thank many people who I have collaborated with within different projects (Susana, Paul, Marit, Lena, Kriss, Christian, Camilla, Dan, Jacob and others), all the crew from the KB Svalbard, R/V Jan Mayen, Bio-Hespérides, García del Cid and Kilo Moana, and also to the UTM personnel who always helped whenever I had a problem on board.

I acknowledge the Spanish Research council (CSIC) for grants. This research is also a contribution to THRESHOLDS, WISER and ATP projects funded by the by the European Union 6th and 7th Framework Program. I also acknowledge the iAOOS-Norway project, the Floating Universities program from EUR-OCEANS and the International Polar Year (IPY), and the ESASSI project funded by the Spanish 'Plan Nacional' to allow me to conduct research in the Arctic Ocean, and in the Southern Ocean.

I also want to thank all the support I have recieved from friends and collegues who became friends working together (Maria, Laura, Moira, Aurore, Emma, Sergio, Pedrito, Sebas, Mica, Maite, Neus, María, Alexia, Alex, Regino, Rocío, Juan Carlos, Núria, Txetxu, Iris, Raquel, Paloma,Mar, Inés, Lucía, Ainhoa, Fiona, Berta, Clara, Zeeba, Amaya, Anita, Itzi, Fede, Silvia, Arantxa, Ana, Iñigo, Lorena, Natalia... and all the Precarious community). Moltes gràcies a tots!

També vull agrair a la meva familia per tot el seu suport i paciència. Gràcies mami, papà, Cristina, padrina, padrins, Yolanda. Gràcies a tots!

Vull agrair especialment a en Jaume el seu suport, paciència, ànims... moltes gràcies! Sense tú no hauria arribat mai fins aquí.

També vull agrair a totes les amigues i amics que m´han aguantat i donat suport tots aquest anys. Magda, Sigrid, Marian, Bea, Marta: Gràcies Akelarre! Johanny i Tià, Aineta, Laura, Rosa, Alber, Roi, Cris, Samu, Julen, Seve, Marta, Marcos, Maria, Manuel, Emma, Miquel Àngel, Gerard, Pere. Moltes gràcies!!! Bicos!

Thanks to all! Moltes gràcies a tots!!! Muchas gracias a todos!!! Gracinhas!!!