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Effect of Multiple Stressors on marine organism predicted and quantified through bioenergetic mechanistic models

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INDEX

Chapter 2 (Giacoletti et al., 2018 ECSS): Functional and energetic consequence of climate change on a predatory whelk

Chapter 3 (Giacoletti et al., 2018 JEMA): Predicting the effectiveness of oil recovery strategies in the marine polluted environment

Chapter 4 (Cheng et al., 2018 Aquaculture): Predicting effective aquaculture in subtropical waters: A dynamic energy budget model for the green lipped mussel, *Perna viridis*

Chapter 5 (Mangano et al., 2018 JSR): Dynamic Energy Budget provides mechanistic derived quantities to implement the ecosystem based management

Chapter 6 (Giacoletti et al., 2018 MERE *under review*): Predicting the multiple effects of acidification and hypoxia on *Mytilus galloprovincialis* (Bivalvia, Mollusca) life history traits

Chapter 7 (Mangano et al., 2018 Ecology Applications *under review*): Moving towards a strategy for addressing climate displacement of marine resources

ABSTRACT

Anthropogenic pressure on coastal ecosystems is vast and diverse, simultaneous impacts such as pollution, eutrophication and fishing pressure nowadays add up and interact with the effects of climate change (e.g., global warming, acidification and sea level rise). The magnitude of these effects on marine species and their replies can vary and the possible changes can depend on: i) species life-histories (LH) traits, ii) local environmental conditions and iii) contextual presence of more than one anthropogenic related stressor. The study of a single anthropogenic disturbance or Climate Change-derived alteration on multi-level ecological responses is misleading and generates unrealistic conclusions, and for this reason is actually recognized as the main limitation of the current ecosystem management approach. These climate change stressors exert negative effects on marine biota as single stressors, but at the same time they are also likely to have interactive effects on biodiversity and ecosystem functioning that are difficult to predict. Although the ecosystem based management (EBM) approach focuses on ecosystem *equilibria*, to provide realistic management measures for important activities at sea such as conservation, fisheries and aquaculture, there is a need of quantities. While ecological research has begun to document the individual effects of these various stressors on species and ecosystems, research into the cumulative and interactive impacts of multiple stressors is less frequent. This need is still cited as one of the most pressing questions in ecology and conservation. The effect of stressors on marine organisms has been frequently assessed using the Scope for Growth (SFG) approach, which lead to a static snapshot of the current physiological status of a target organism, used as an indicator of the ‘health’ of the ecosystem. In the last decade, eco-physiological studies have focused on linking the effect of climate change on species distributions based on organisms' physiological limits and, in some cases, with the overall relationship between environmental factors and physiological performance. In addition, past modelling efforts largely based on correlative Species Distribution Models (SDMs) also known as “bioclimatic envelope models”, “ecological niche models” or “habitat suitability models” used known occurrences of species across landscapes of interest to define sets of conditions under which species are likely to maintain populations. However, effective conservation management required models able to make projections beyond the range of available data. One way to deal with such an extrapolation is to use a mechanistic approach based on physiological processes underlying climate change effects on organisms. One such bio-energetic model, which has been successfully applied for modelling species distributions, is the Dynamic Energy Budget (DEB) model, which is able to deal with multiple stressors and other environmental parameters that are expected to affect the individual performance such as growth and reproduction. While **Chapter 1** was dedicated to frame of general topic of the

present thesis, **Chapter 2** experimentally investigated the effects of a novel prey and a chronic increase in temperatures on functional traits and global fitness of the whelk *Stramonita haemastoma*. In **Chapter 3,** we applied a new approach using DEB models to investigate the effects of an anthropogenic pollutant on Life-History (LH) traits of marine organisms, providing stakeholders and policy makers an effective tool to evaluate the best environmental recovery strategy. In **Chapter 4** we used DEB models to determine the effect of changing environmental conditions and pollution on the Indo-Pacific *Perna viridis* aquaculture. In **Chapter 5** we proposed a DEB application to study the link between future COP21 predicted temperature scenarios and varying food availability on LH-traits of some Mediterranean fishery and aquaculture target species, exploring the efficiency of Integrated Multitrophic Aquaculture as a potential management solution. A spatial contextualization of model outcomes allowed translating those results into useful figurative representations. Through **Chapter 6** we investigated the site-specific effects of environmental changes represented by Ocean Acidification and hypoxia on the functional and behavioural traits of the mussel *Mytilus galloprovincialis*. Finally, in **Chapter 7** we presented a proof-of-concept study using the European anchovy as a model species to show how a trait-based, mechanistic species distribution model can be used to explore the vulnerability of marine species to environmental changes. Scenarios of temperature and food were crossed to generate quantitative maps of selected mechanistic model outcomes.

LIST OF PAPERS

Published papers:

1. **Giacoletti A.**, Rinaldi A., Mercurio M., Mirto S. and Sarà G. (2016). Local consumers are the first line to control biological invasions: a case of study with the whelk *Stramonita haemastoma* (Gastropoda: Muricidae). Hydrobiologia, 772:117-129.
2. **Giacoletti A.**, Maricchiolo G., Mirto S., Genovese L., Umani M. and Sarà G. (2017). Functional and energetic consequences of climate change on a predatory whelk. Estuarine, Coastal and Shelf Science, 189:66-73. **CHAPTER 2**
3. Arcangeli A., Campana I., Angeletti D., Atzori F., Azzolin M., Carosso L., Di Miccoli V., **Giacoletti A.**, Gregorietti M., Luperini C., Paraboschi M., Pellegrino G., Ramazio M., Sarà G., Crosti R. (2018). Amount, composition, and spatial distribution of floating macro litter along fixed transborder transects in the Mediterranean basin. Marine Pollution Bullettin, 129:545-554.
4. Cheng M.C.F., Tan A.L.S, Rinaldi A., **Giacoletti A.**, Sarà G., Williams G.A. (2018). Predicting effective aquaculture in subtropical waters: A dynamic energy budget model for the green lipped mussel, *Perna viridis*. Aquaculture, 495:749-756. **CHAPTER 4**
5. Mangano, M.C., **Giacoletti, A.** and Sarà, G. *in press* (2018). Dynamic energy budget provides mechanistic derived quantities to implement the ecosystem based management approach. Journal of Sea Research, Sp. Issue 2018.
6. **Giacoletti, A.**, Cappello, S., Mancini, G., Mangano, M.C. and Sarà, G. (2018). Predicting the effectiveness of oil recovery strategies in marine polluted environment. Journal of Environmental Management, 223:749-757. **CHAPTER 3**

Under review papers:

7. Mangano, M.C., Mieszkowska, N., Johnson M., Patti, B., Cuttitta, A., Baiamonte, G., Bazan, G., **Giacoletti, A.**, Pranovi, F., Fiorentino, F., Mirto, S., Domingos, T., Sousa, T., Helmouth, B., Williams, G., Sarà, G., *under review* (2018). Moving towards a strategy for addressing climate displacement of marine resources. Ecological Applications. **CHAPTER 7**
8. **Giacoletti, A.** and Sarà G., *under review* (2018). Predicting the multiple effects of acidification and hypoxia on *Mytilus galloprovincialis* (Bivalvia, Mollusca) life history traits. Marine Environmental Research. **CHAPTER 6**
9. **Giacoletti A.**, Dupont, S., D'Acquisto L., Sabatino M.A. and Sarà G., *under review* (2018). Direct effect of ocean acidification on prey translates into indirect consequences on its predator.

In preparation papers:

10. **Giacoletti, A.**, Capaci, R., Giommi, C., Mangano, M.C. and Sarà, G. (2018). Effect of combined extreme thermal and hypoxia events on metabolic response and mortality of *Brachidontes pharaonis*. (in preparation for Journal of Experimental Biology).

11. **Giacoletti, A.**, Parisi M. G., Mauro M., Cammarata M. and Sarà G. (2018). Multi-level responses of *Mytilus galloprovincialis* (Lamarck, 1819) to multiple stressors. (in preparation).

CHAPTER 1: GENERAL INTRODUCTION

Anthropogenic pressure on marine ecosystems is vast and diverse, simultaneous impacts such as pollution, eutrophication and fishing pressure (Jackson et al., 2001) nowadays add up and interact with the effects of climate change (e.g., global warming, acidification and sea level rise). Now, climate change is also contributing to the erosion of resilience (Wernberg et al., 2010; Graham et al., 2015), because increasing temperatures are modifying key physiological, demographic, and community-scale processes (Bennett et al., 2015), driving species redistribution at a global scale and rapidly breaking down long-standing biogeographic boundaries (Poloczanska et al., 2013; Wernberg et al., 2012). The study of a single anthropogenic disturbance or Climate Change-derived alteration on multi-level ecological responses is actually recognized as the main limitation of the current ecosystem management approach (Hughes et al., 2005). Only a more adaptive management that integrates approaches that involve the full array of interactions within an ecosystem, including humans, rather than considering single issues in isolation, starting from an appropriate knowledge of species biological traits, will allow the translation of the effects of environmental change into realistic management measures (Carpenter and Folke, 2006). Thus, strategies of dynamic adaptation should be designed taking into account new more on-going favourable environmental conditions affecting the optimisation of the species' biological traits (Sarà et al., 2018a, b, c) to avoid unrealistic or inapplicable management measure. What is still poorly recognized and understood is how the effect of multiple drivers of environmental change vary in space and time at local scale (Mangano et al., 2018). Changing environments have the potential to alter the fitness of organisms through effects on components such as energy acquisition, metabolic cost, growth rate, survivorship, and reproductive output (Sebens et al., 2018). The capacity of organisms to respond to environmental variability, including unidirectional change, depends on phylogenetic, genetic, physiological, and developmental constraints, and such responses can be short term (acclimation, phenotypic plasticity) or long term (adaptation, selection). The overall success of any set of adaptations, including morphological, physiological, or life-history traits, can best be assessed by examining their effects on fitness (Endler, 1986; Kozlowski, 1993) which can also be used as a measure of natural selection (Arnold & Wade, 1984a, b).

The possibility of predicting the fitness of a species, expressed as the total number of eggs produced in their life cycle (*Darwinian fitness*, Stearns, 1992; Bozinovic et al., 2011), is crucial to fully understand marine ecosystems' dynamics. The number of locally-produced gametes by each individual inside a population is the main factor that conditions larval dispersion which, as widely known, is not unlimited. Several factors influence the extent of larval dispersion and the connectivity between microenvironments, but local densities of breeding adult and their Life-History traits are probably the most relevant factors. The ultimate body size, the number of reproductive events and the time needed to achieve sexual maturity (Roff, 1992) are crucial factors for the persistence of a population over time (Kearney et al., 2010). Their extent is essential not only for the conditions of local populations, but is the best guarantee of a constant flow of gametes in time and space able to ensure both larval connectivity between distant populations and high levels of biodiversity within large areas (Simberloff, 2009). In marine ectotherms, characterized by undetermined growth (Charnov & Berrigan, 1990), fitness changes together with body size (Strathmann & Strathmann, 1982) and the size of sexual maturity appears to be a fixed species-specific trait (Kooijman, 2010). This means that the constant supply of energy from food leads those organisms to a greater body size, greater reserves and consequently more energy allocated to reproduction. One of the most used strategies to get through stressful conditions or food limitation is to subtract energy at first from those allocated to reproduction and then to growth, devolving them to somatic maintenance (Kooijman, 2010). The change in fitness within marine ectotherms can therefore be defined by i) the amount of energy deriving from the habitat available food and ii) the amount of energy deriving from the food that can be managed by each individual. In this context, the definition of the functional role of each species becomes of fundamental importance, allowing a deeper understanding of ecosystem functioning, and of the specific energy and matter flows through ecosystems, as well as providing a deeper knowledge of the role played by biodiversity within ecosystem processes. The link between energy-individual-biodiversity and ecosystem functioning is often overlooked, although the loss of biodiversity arises from punctual alteration of individual's physiological, morphological and behavioural traits (Schoener, 1986). This commonly implies an alteration of the available energy for the metabolism that has indirect repercussions on the whole population and influences the ecological role it plays within the community.

Although the ecosystem based management (EBM) focuses on ecosystem *equilibria*, in order to provide realistic management measures for important activities at sea such as conservation, fisheries and aquaculture, there is a need of quantities. The Dynamic Energy Budget Theory (DEB models, Kooijman, 2010) feeding the functional trait-based approach (Schoener, 1986; Kearney and Porter, 2009; Sarà et al., 2014a, 2018c) may represents an effective and powerful mechanistic tool in providing those kinds of quantities. DEB models represent the “quantitative from scratch” framework which mechanistically investigate the fluxes of energy that one organism activate in order to optimize fitness during the life span (Marquet et al., 2014; Kearney et al., 2015). Up to date, DEB has not applied to assess the potential role of phenotypic plasticity in pushing adaptation of species under climate change; this will be a fruitful research ground for DEB scientists, evolutionary biologists and ecologists in the near future.

This study suggests the unification of ecological and mechanistic approaches as a way to study the response of marine organisms to combined impacts (*i.e.* multiples; e.g. the alteration of food availability, global warming, ocean acidification, coastal hypoxia phenomena, the dispersal of toxic compounds etc.) on model species specifically-chosen from those that inhabits the rocky intertidal or other characterizes by a commercial or ecological importance. A Dynamic Energetic Budget (DEB) application has been proposed to study the link between environmental change (due to several factor as temperature, pH, hypoxia, pollutants), food density and life-history traits of marine bivalves and fishes (*Perna viridis*, *Mytilus galloprovincialis*, *Crassostrea gigas*, *Engraulis encrasicolus*, *Dicentrarchus labrax*). Sensitivity analysis were often applied to simulate the effects of stressor or future environmental changes on LH-traits such the maximum length, the time needed to reach the commercial size, the length at first maturity, and the number of eggs produced. Moreover, when DEB outputs are translated at spatial level, by a spatially-contextualized and mapped analysis, it is possible to generate easy-to-read maps which are useful to engage with the stakeholders. They can easily identify and proactively implement adaptive site-specific management strategies tailored to target species. The use of mechanistic-derived quantities and high resolution spatial analysis in ecology and resource management science can help us to adopt a medical analogy: the "actual patient care should be highly individualized, and patient treatment should not be based on the results of broad-scale generalizations, without considering the patient's history, risk factors and other medications" (*literally* Helmuth et al., 2014). Thus predictions of environmental effects at local scale on biological responses should not be based on only mean conditions of environmental regimes (e.g. monthly, annually), but should rely on higher resolution data (at least daily). A broad-brush approach could be appropriate if high resolution data are lacking, but the present-day technology (e.g. satellite and remote sensing; Capodici et al., 2018) and recent scientific advancement (e.g. DEB theory) offer impressive improvement of the temporal and spatial resolution of many types of data needed to feed regional management strategies. Thanks to DEB, we are now able to incorporate such kind of "patient tailored" information needs together with multiple responses to environmental parameters, to develop appropriate tailored marine resource management.

1.1 The concept of disturbance

All natural assemblages are perturbed by both physical and biological forces. These agents of change occur with different intensities, frequencies, and spatial distributions (Paine et al., 1998). Traditionally, disturbances have been viewed as uncommon, irregular events that cause abrupt structural changes in natural communities and move them away from static, near equilibrium conditions (Karr et al., 1984; White, 1979). This definition has little utility in light of the following observations:

1. Evidence from long-term censuses suggests that few natural populations or communities persist at or near an equilibrium condition on a local scale (Connell and Sousa, 1983). There is no clear demarcation between assemblages in an equilibrium state and those that are not.
2. The change caused by any force can vary from negligible to extreme, depending on the intensity of the force and the vulnerability of the target organisms. How does one objectively decide what degree of change along this continuum constitutes a disturbance?

Given the complexities discussed above, it seems wisest to adopt the view (Karr et al., 1984) that disturbance lies near one extreme of the continuum of natural perturbations that affect organisms. There have been several others definition of disturbance across scientific literature. According to White and Pickett (1985) “A disturbance is any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment”. Mouillot et al. (2013) proposed a definition of disturbance in its widest sense as any event, natural or human-driven, that causes temporary and localized shifts in species demographic rates. He further classified disturbances in three categories as those caused by i) direct human impacts; ii) biotic pressure (mainly imposed by exotic species); and iii) environmental changes (abrupt shifts in abiotic conditions and habitat degradation).

Each disturbance event has a cause (e.g., lightning) and an effect (e.g., combustion), which triggers a species response (e.g., resprouting), directly affecting ecological processes. Each disturbance event has several aspects that can be measured. There are five key aspects of particular importance for ecological studies: intensity, timing, duration, extent, and disturbance interval (Miller et al., 2011). Disturbance is both a major source of temporal and spatial heterogeneity in the structure and dynamics of natural communities and an agent of natural selection in the evolution of life histories. These roles are clearly interdependent. The differential expression of life history attributes under different regimes of disturbance produces much of the spatial and temporal heterogeneity one observes in natural assemblages (Sousa, 1984). Anthropogenic disturbance has proven to be able to shape biodiversity through a driving in organismal Life Histories of organisms (Denny et al., 2009), in particular when associated with extreme events it may reduce a community’s biotic resistance to invasive species and increase rates of invasion (Gross et al., 2005, Altman and Whitlatch, 2007). Extreme events can cause sufficiently dramatic ecological change that recovery is greatly delayed or impossible. Such effects arise when populations are pushed below some minimum density threshold (e.g., the Allee effect), or when a community or ecosystem enters an alternate stable state (Allee, 1949, Folke et al., 2004).

Despite conservation efforts, biodiversity loss continues apace at regional or global scales across a wide range of ecosystems, due to increasing intensity of disturbances such as overexploitation of species, destruction of habitats, climate change, or invasion by alien species. As a feedback, biodiversity erosion is imperilling the sustainability of ecological processes and the provision of ecosystem services (Cardinale et al., 2012). Thus, there is an urgent need to quantify and predict the effects of disturbance on biodiversity patterns to guide conservation efforts and the management of ecological resources.

1.2 Ecological responses to Climate Change and anthropogenic impacts

Increases in the scale and extent of human activity in the last two centuries have brought about environmental changes that affect most of the globe. These global changes include directional shifts in climate, greenhouse gas concentrations, nitrogen fixation, and stratospheric ozone depletion (Vitousek et al., 1997). They also include biotic changes such as land cover change, biological invasions, and global loss of biodiversity (Lovejoy and Hannah, 2005; Mooney and Hobbs, 2000). A synthesis of human impact on marine ecosystems developed by Halpern et al. (2008) (Fig. 1) indicates that no area in the world is unaffected by human influence and that a large fraction (41%) is strongly affected by multiple drivers.

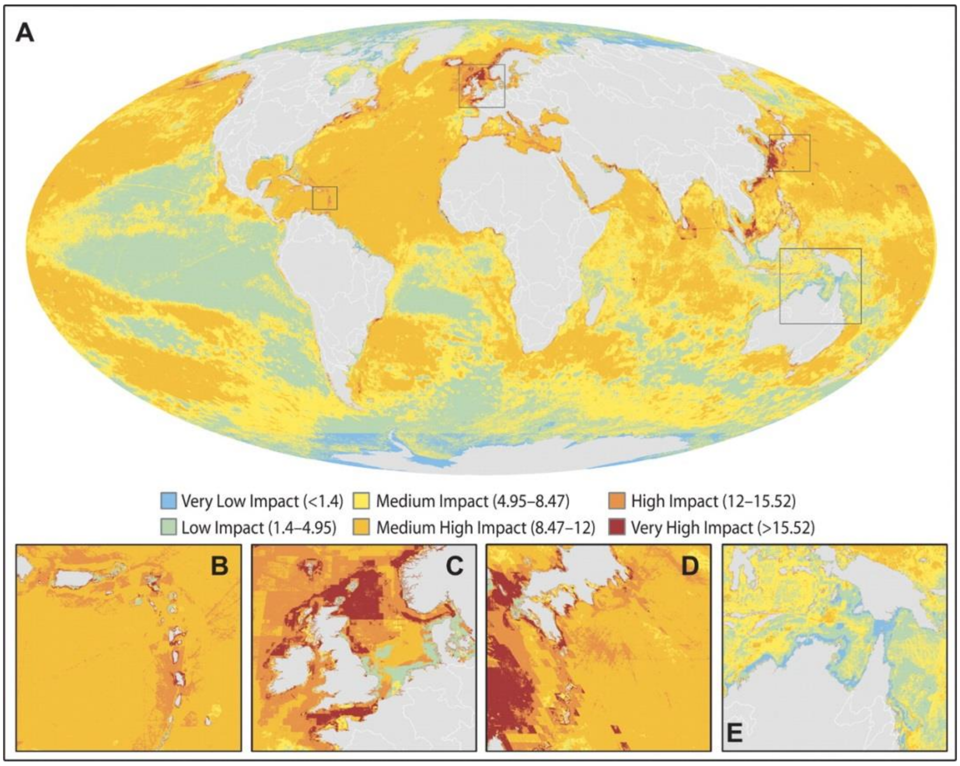

Fig. 1 Global map (A) of cumulative human impact across 20 ocean ecosystem types. (Insets) Highly impacted regions in the Eastern Caribbean (B), the North Sea (C), and the Japanese waters (D) and one of the least impacted regions, in northern Australia and the Torres Strait (E) (Halpern et al., 2008).

Climate change represents an on-going and rapidly growing threat at both global and local scale equally affecting the environment, societies and economies by forcing shifts in the distributional range and productivity of key commercial species (Lam et al., 2016). The magnitude of these effects on marine species (both in the wild and captive conditions) and their replies (e.g. increase or decline of abundance; Pecl et al., 2017) can vary and the possible changes can depend on: i) species life-histories traits, ii) local environmental conditions and iii) contextual presence of more than one anthropogenic related stressor (Gunderson et al., 2016; Pecl et al., 2017; Sarà et al., 2018a, Sarà et al., 2018b, Sarà et al., 2018c). Global change is further altering the distribution of aquatic and terrestrial species worldwide (Sunday et al., 2012).

In the marine environment, one of the main factors driving distribution changes is the climate-driven rise in sea water temperature (Harley et al., 2006), which is predicted to continue throughout the 21st century (IPCC, 2014). Marine ectotherms fully occupy the extent of latitudes tolerable within their thermal tolerance limits, and are consequently predicted to expand at their poleward range boundaries and contract at their equatorward boundaries with climate warming (Sunday et al., 2012). Seawater temperature, through the fundamental influence on metabolic machinery (Brown et al., 2004), plays a prominent role in driving functional and life history (LH) traits of most ectotherms, affecting their local persistence over time (*sensu* Sibly et al., 2012). Increasing temperature, as a consequence of future climate change (CC; IPCC, 2014), will probably determine cascading effects within natural communities, modifying current biodiversity (Gooding et al., 2009; Yamane and Gilman, 2009). CC may favour the spread of much more thermo-tolerant alien marine species, such as jellyfishes, bivalves and fishes by increasing the likelihood of reinforcing facilitation mechanisms (Southward et al., 1995; Stachowicz et al., 2002; Galil et al., 2015) or through the availability of empty niches in the invaded range (Hierro et al., 2005). According to theory (e.g. Arrhenius law; Kooijman, 2010; Sarà et al., 2014a), increasing temperature should be particularly effective in enhancing the consumption rates in ectotherms (Sibly et al., 2012; Seifert et al., 2014). As a consequence, it is possible that altered consumption dynamics may cause determine the destabilization of the entire community *equilibria* (Vasseur and McCann, 2005; Seifert et al., 2014). The modification of phenology (Duarte, 2007) and reproductive failure (Helmuth et al., 2014; Montalto et al., 2014, 2016) are moreover the two most important repercussions of temperature change in a context of CC claimed to have a strong effect on the persistence of local populations over time. A possible climate change scenario involving the warming of sea surface temperatures by ~2°C (IPCC, 2014), coupled with the presence of a richer diet represented by an alien species (Giacoletti et al., 2017), has proven as capable to alter the natural common temporal schemes of physiological processes and biotic interactions (Blois et al., 2013).

Although temperature is very often the main considered forcing variable inside bioenergetic studies, other aspects of the marine environment are also changing rapidly and can affect fitness of mussels and many other organisms. Ocean temperature is increasing globally, and acidification is occurring at a rate faster than has been experienced on the planet for at least the last 50 million years. A clear understanding of the ocean’s carbonate system is emerging and is essential to predictions of the organism-level feedbacks and impacts to be expected as a result of future increases of anthropogenic pCO_2 (Dickson, 2012; Doney et al., 2012; Feely et al., 2008, 2010; Hoegh-Guldberg and Bruno, 2010; Hoegh-Guldberg, 2012). In many coastal regions, effects of climate change are already evident (Crim, Sunday, & Harley, 2011; Gaylord et al., 2011; Gilman et al., 2010; Helmuth et al., 2010; Wootton & Pfister, 2012), and some locations have experienced low pH conditions long enough for local adaptation to have occurred already (Murray et al., 2015). These global drivers can interact with local change in environmental conditions (e.g., hypoxia events; Sarà et al., 2018a) complicating the situation, and highlighting the necessity of investigating both mechanical properties and life-history characteristics to forecast future effects on local biodiversity. Indeed, ecologically important species (e.g., keystone species, foundation species, ecosystem engineers) will be impacted by environmental change, causing unforeseen and often undesirable changes in community composition and species diversity (Maas et al., 2012; Menge, 2012; O’Donnell et al., 2013; Sarà et al., 2014b; Wethey et al., 2011).

Biodiversity is widely recognized as one of the fundamental provider of ecosystem services (Loreau et al., 2001; Tilman et al., 2014) affecting ecosystem productivity, nutrient cycling, stability and resistance to perturbations (Naeem et al., 1994; Smith et al., 2006; Mazancourt et al., 2013). On the other hand, anthropogenic disturbance is one of the most powerful driver capable to shape biodiversity (Miller et al., 2011) through a driving action on individual “life histories” (Denny et al.,2009; Sarà et al., 2018a). When Global Changes or a source of anthropogenic impact act, they do not affect only the individual level, but hierarchically, through the effect on hundreds of mussel-beds associated species (Suchanek, 1979), alter the ecosystem functioning in terms of provided good and services (Sarà et al., 2014a, b; Mangano and Sarà, 2017).

1.3 Multiple Stressors

We usually refer to a stressor as an abiotic or biotic (e.g. introduction of an alien species) variable that, as a result of human activity, exceeds its range of normal variation, and affects (whether negatively or positively) individual taxa, community composition or ecosystem functioning relative to a reference condition (Sanderson et al. 2002; Halpern et al. 2007a; Crain et al., 2008; Piggott et al., 2015). Abiotic and biotic stressors usually do not operate independently, but rather often interact to produce combined impacts on biodiversity and ecosystem functioning (Breitburg et al., 1998; Frost et al., 1999; Schindler, 2001). The worst threat for marine ecosystems derives from the interaction of stressors such including rising temperatures, changes to ocean circulation and mixing, eutrophication, ocean acidification, ocean deoxygenation (i.e. the global trend of decreasing oxygen as a result of ocean warming and increasing stratification), coastal hypoxia (i.e. low-dissolved oxygen environments due to increased organic enrichment and nutrient levels) and pollution (*sensu* Sarà et al., 2018a). These climate change stressors exert negative effects on marine biota as single stressors and are also likely to have interactive effects that are difficult to predict (Fabry et al., 2008; Pörtner and Langenbuch, 2005; Pörtner, 2008; Przeslawski et al., 2008; Widdicombe and Spicer, 2008). Environmental physiologists and physiological ecologists interested in understanding how the distribution of organisms will be impacted by biotic and abiotic changes in habitats have long used carefully controlled laboratory experiments that manipulate a single environmental variable (e.g., temperature) to determine some aspect of organismal performance, for example, temperature sensitivity (Q_{10}) or tolerance maxima (CT_{max}) (Todgham & Stillman 2013).

While ecological research has begun to document the individual effects of these various stressors on species and ecosystems, research into the cumulative and interactive impacts of multiple stressors is less frequent (Crain et al., 2008). The need to better understand the interactive and cumulative effects of multiple stressors was highlighted a decade ago (Breitburg et al., 1999) and is still cited as one of the most pressing questions in ecology and conservation (e.g. Sala et al., 2000; Zeidberg & Robison, 2007). The current approach, actually considering the effect of a single-stressor-per-time on biological and ecological responses, is misleading and generates unrealistic conclusions (Crain et al., 2008; Gunderson et al., 2016). This represents a significant limitation as it reduces the ability to cope with real challenges, linked to the use of marine resources and is now recognized as the main limitation of the ecosystem management approach (Hughes et al., 2005). Although it is recognized that multiple stressors can interact to generate complex detrimental effects on individual functional performances, up to population level, there has still been a pressing question of scientific research in ecology and conservation for almost the last two decades (Breitburg et al., 1999; Sala et al., 2000; Zeidberg & Robison, 2007). In terms of their impacts on organisms, multiple stressors can be viewed as having additive, antagonistic or synergistic effects (Fig. 2; Crain et al., 2008; Ghedini et al., 2013; Gunderson et al., 2016).

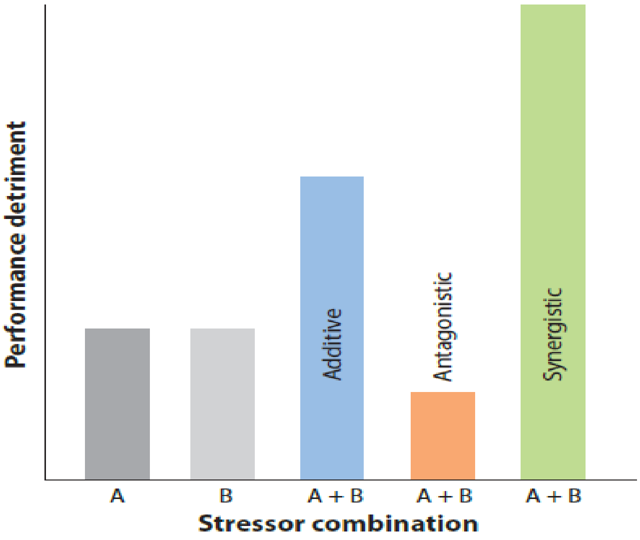

Fig. 2 Conceptual diagram of possible effects of multiple stressors on physiological performance (Gunderson et al., 2016).

The combined effect of multiple stressors is commonly assumed to be additive, *i.e.* equal to the sum of individual effects acting in isolation. However, this model is not always prevalent in ecological systems where antagonistic and synergistic interactions may dominate (Crain et al., 2008). Stressors act in synergy when their combined effect is greater than the sum of the impacts of individual stressors, whereas antagonistic interactions occur when the combined effect is less than expected based on their individual effects (Folt et al., 1999). In these conditions, the ecological benefit resulting from efforts to reduce any stressors acting additively can be predicted on the basis of the knowledge of its individual effect, whereas interactive effects could produce some "ecological surprises" (Paine et al., 1998). Predicting cumulative stressor effects is challenging due to various potential higher order interactions (HOI, *sensu* Billick & Case, 1994). First the stressors themselves can interact so that impacts change in the presence of additional stressors; second, species' response to a stressor may be context dependent and thus modified by additional stressors; third, species may respond similarly or differently to sets of stressors due to evolutionarily or ecologically derived tolerances (Vinebrooke et al., 2004). Finally, community response to stressors can differ due to changing interactions between component species under different stressor scenarios (Crain et al., 2008). Predicting community or ecosystem response to multiple stressors is additionally complicated by HOI among the component species themselves and factors such as species diversity, openness of a system that can influence dynamics of disturbance recovery, and environmental stochasticity (Breitburg et al., 1998).

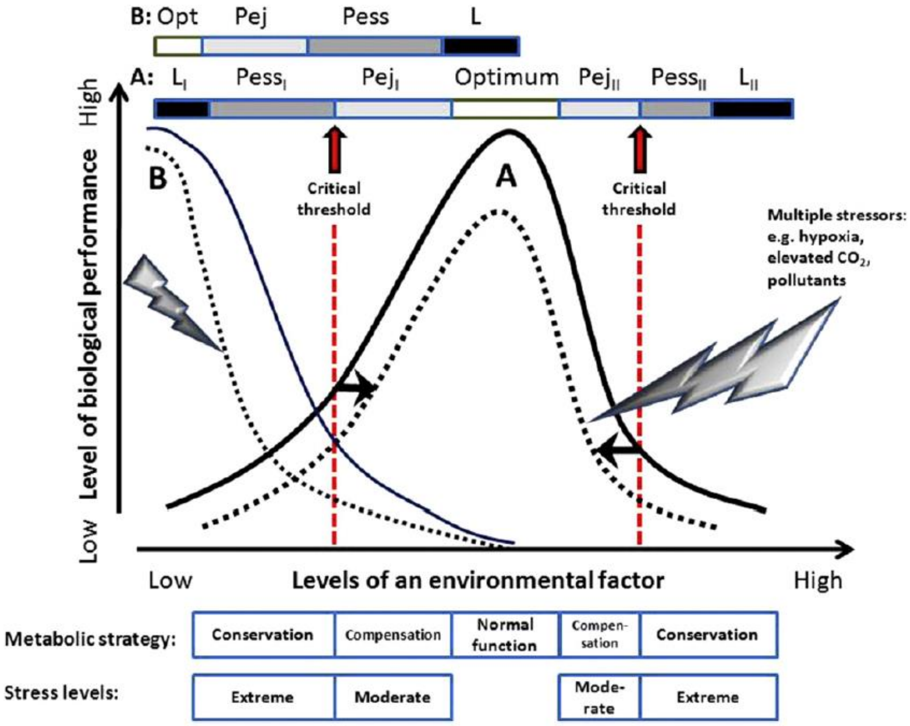

Fig. 3 The concept of energy-limiting stress tolerance limits and classification of environmental stressors based on their effects on energy balance. Solid lines refer to a single environmental factor/stressor situation, and dotted lines refer to a combined exposure to multiple stressors (e.g. hypoxia, hypercapnia or pollution) that can negatively affect the aerobic scope and thus narrow the tolerance window for another environmental factor/stressor (e.g. temperature) (Sokolova et al., 2012).

The explanation of why a single-stressor approach may generate unrealistic conclusions is simple and essentially connected to biological first principles driving the ecological functioning. Considering mechanisms, Sokolova et al. (2012; Fig. 3) provides a clear mechanistic physiological ground to increase our understanding of when, where and how multiple stressors alter functional performances of marine organisms. In an energy-based stress classification, the overall scope of the values of an environmental factor experienced by an organism is divided into several ecologically and physiologically relevant ranges (Pörtner and Farrell, 2008): 1) optimal range where the energy balance is positive and the maximum aerobic scope is available for activity, growth, development, reproduction and storage; 2) *pejus* range (from Latin "worse") where the aerobic scope is still positive but diminished compared to the optimal range due to the elevated basal metabolic costs and/or impaired aerobic capacity; 3) *pessimum* range (from Latin "the worst") where aerobic scope disappears and anaerobic metabolism is engaged to partially cover the energy costs of basal maintenance; 4) lethal range where the energy homeostasis is disrupted and a short-term survival depends on "emergency" stress protection such as molecular chaperones and antioxidants.

The level of the biological performance of an organism is proportional to the available aerobic scope and diminishes as an organism transits from the optimum to *pejus*, *pessimum* and then lethal range. The depicted curves (Fig. 3) show schematic representation of a generalized stressor, and the shape and the symmetry of the actual curves will depend on the nature of the stressor. Thus, for some stressors (such as temperature, salinity or levels of essential metals) the relationship between the level of the stressor and the organism's performance follows a bell-shaped curve (similar to curve A). As a result, there are lower and upper *pejus* (Pej_I and Pej_{II}), *pessimum* ($Pess_I$ and $Pess_{II}$) and lethal (L_I and L_{II}) ranges for these factors. For other stressors (such as pollutants, toxins or UV irradiation) the optimum lays near zero level of the stressor such as shown by curve B. For these stressors, there are only the upper *pejus*, *pessimum* and lethal ranges (Pej, Pess and L, respectively) (B: bar shown above the curves). The critical thresholds indicating the transition from moderate to extreme stress range correspond to the transition from the *pejus* to *pessimum* ranges. The shift in the critical thresholds of tolerance induced by multiple stressors is indicated by black horizontal arrows. Ranges depicting different metabolic strategies (conservation vs. compensation) and stress levels (moderate vs. extreme) refer to the single-stressor situation shown by the solid line (curve A). This conceptual framework is theoretically applicable to any stressor that negatively affects the aerobic scope of an organism and has been experimentally tested for temperature, oxygen levels, pollution and their combinations; additional studies on other stressors are needed to further test the generality of this conceptual framework. This energy-based classification of environmental stressors allows comparing the effects of various unrelated stressors and focuses on those physiological effects that are linked to fitness and thus can directly translate into the population-level consequences. The position and breadth of the stress tolerance windows (encompassing the *optimum* and *pejus* ranges on Fig. 3) are flexible within the limits and can be shifted by adaptation, acclimation or acclimatization. Each species is characterised by specific thresholds at which a particular environmental variable becomes stressful. If the stress threshold is passed, the energy required to overcome the stress event increases with increasing stress intensity (Sokolova et al., 2012).

The timing of environmental stressor events and fluctuations (*sensu* Miller et al., 2011) in their magnitude are also important for our understanding of how organisms respond to changing conditions. Gunderson et al. (2016) pointed out that the impact of multiple stressors depends critically on the intensity and timing of each stressor, that has an important role in addressing the type and strength of multiple stressor interactive effects, leading the approach to stress thresholds, from which the bio-ecological responses ultimately depend on.

Given the increasing multiplicity of environmental stressors associated with global change, there is an urgent need to develop a better understanding of the interactive effects of multiple stressors on ecosystems to better predict their responses to a changing environment (Vinebrooke et al., 2004).

1.4 The concept of Ecomechanics

Ecomechanics is a discipline that studies not only how individual organisms work, but also how these organisms interact with each other and with their environment, and how changing environmental conditions are likely to affect populations, communities, and the distribution of species (Carrington, 2002; Denny & Helmuth, 2009; Denny and Gaylord, 2010). The principles of ecomechanics are useful to analyse the link between the effects of anthropogenic disturbance and ecological responses and investigate the possible implications on biodiversity and ecosystem functioning (Knowlton & Jackson, 2008). At the same time is considered a practical solution to provide accurate predictions on species abundances in a changing world (Araùjo & Rahbek, 2006). This discipline is based on the analysis of functional behavioural (e.g. food preferences, searching and handling, swimming or mating behaviour etc.), physiological (e.g. thermal tolerance, oxygen consumption, ingestion rate etc.) and morphological (e.g. shape) traits that all contribute in maximizing the energy intake (Shoener, 1986; Kohel, 1989; Denny & Helmuth, 2009; Sarà et al., 2014a). In its simplest definition, a trait is a surrogate of organismal performance, and this meaning of the term has been used by evolutionists for a long time. Following Darwin’s (1859) proposal, traits were initially mainly used as predictors (proxies) of organismal performance. Over the last three decades, developments in community and ecosystem ecology have forced the concept of trait beyond these original boundaries, and trait-based approaches are now widely used in studies ranging from the level of organisms to that of ecosystems. Within each discipline, diverse types of traits are thus used to assess inherent components involved in the explanation of complex processes defined at higher organizational levels (Fig. 4).

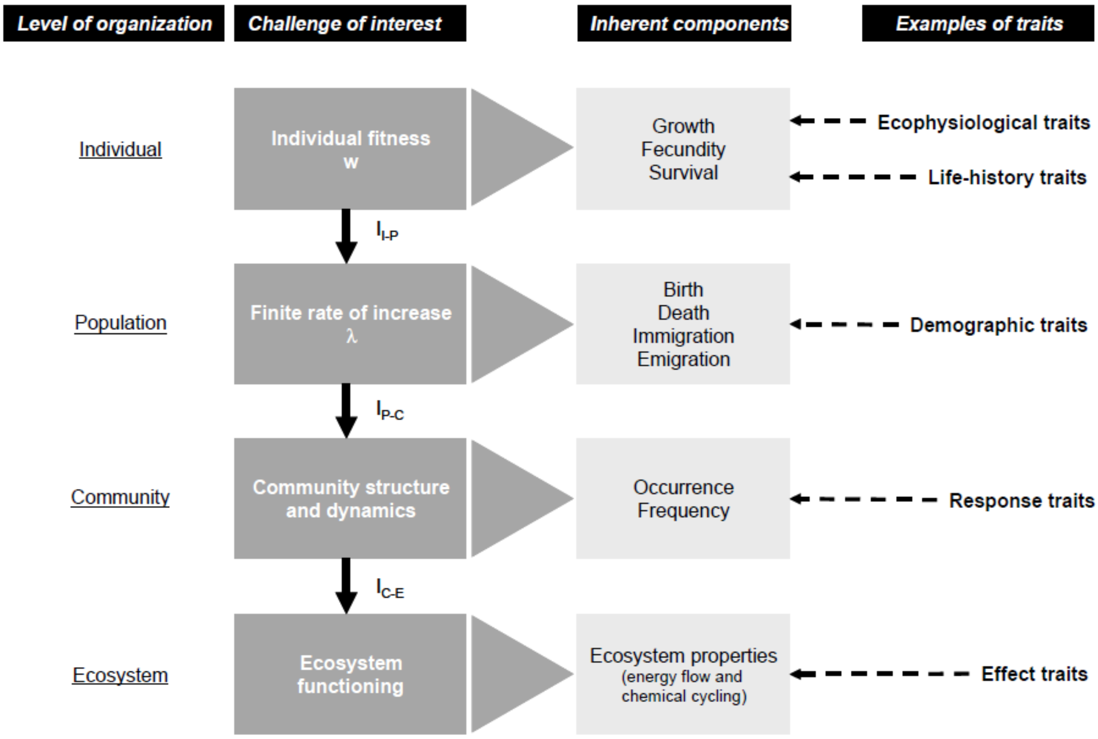

Fig. 4 Pathways linking the challenge of interest of different organizational levels, through their related inherent components, to some examples of traits found in the literature. Without trait-based information, scaling-up to higher organizational levels needs complex integration information (I). Thus fitness components of an individual determine the components of the finite rate of increase (λ) of the population (I_{I-P}). Occurrence and frequency of species at the community level encompass components of λ through complex integration (e.g. biotic interactions) (I_{P-C}). Finally, scaling-up to ecosystem properties can be done by combining functional property of each species of the community (I_{C-E}) (Violle et al., 2007).

A trait is generally a feature measurable at the level of the individual, which does not require additional information from the environment or at any other organizational level. Functional traits (FT) are in particular all those traits that lead to define a species in terms of its ecological role (Diaz & Cabido, 2001). FT are also all those morpho-physio-phenological traits which impact fitness indirectly *via* their effects on growth, reproduction and survival, the three components of individual performance (Violle et al., 2007). Functional traits include tolerance and sensitivity to environmental conditions such as physiological limits of thermal tolerance (Kearney & Porter, 2009) or mechanisms involved in the response to hypoxia (Portner, 2010) and hypercapnia (Hendriks et al., 2010). The possibility of obtaining energy from food is also a functional trait, and follows the so-called Functional response (Holling, 1959; Denny & Benedetti-Cecchi, 2012) or all those behavioural (swimming, habitat use, coupling strategies) and morphological (i.e. mega-parameters: Schoener,

1986) traits that contribute to the optimization of energy efficiency (Krebs & Davies, 1992). These functional traits are directly involved in the quantitative expression of the traits of Life-History traits (Stearns, 1992) which plays a crucial role in determining the density and dynamics of populations of each species (such as body size and the number of reproductive events during the whole life cycle: Roff, 1992; Stearns, 1992; Charnov, 1993).

1.5 Mechanistic-bioenergetic models

Models are useful tools for predicting the impact of global change on species distribution and abundance. Marine ectotherms are being challenged to adapt or track changes in their environment, either in time through a phenological shift or in space by a biogeographic shift. The effect of pollutants or other stressors on bivalves has been frequently assessed in the past by using the Scope for Growth (SFG) approach (Widdows and Staff, 2006; Mubiana and Blust, 2007), which allowed essentially to gain a static snapshot of the current physiological status of target organisms (Widdows et al., 1995). The success of SFG (Sobral and Widdows, 1997; Sarà et al., 2000, 2008; Widdows and Staff, 2006; Halldórsson et al., 2007; Sarà and Pusceddu, 2008; Ezgeta-Balic et al., 2011) was based on the provision of an instantaneous measure of the energy status of these key-species which was used as an indicator of the ‘health’ of the ecosystem (Thompson and Bayne, 1974; Widdows et al., 1995; Kearney, 2012). Nevertheless, SFG did not maximize the mechanistic power of a bioenergetic approach when assessing the bottle-necks in the energy flow from the environment to the organisms, neglecting a full translation of effects in terms of Life History (LH) traits (e.g. habitat body size, spawning events and Darwinian fitness; Kearney, 2012).

Past modelling efforts have also largely been based on correlative Species Distribution Models, which use known occurrences of species across landscapes of interest to define sets of conditions under which species are likely to maintain populations. Conceptually, these models aim to determine and map components of a species’ ecological niche through space and time, and they have become important tools in pure and applied ecology and evolutionary biology. Most approaches are correlative in that they statistically link spatial data to species distribution records (Kearney and Portner, 2009). The practical advantages of this correlative approach are its simplicity and the flexibility in terms of data requirements. However, effective conservation management requires models that make projections beyond the range of available data. One way to deal with such an extrapolation is to use a mechanistic approach based on physiological processes underlying climate change effects on organisms.

In contrast, most recently developed bioenergetics frameworks, such as the mechanistic functional trait-based (FT) models, which rely on the Dynamic Energy Budget Theory (DEB; Kooijman, 2010; Sarà et al., 2014b), allow an easier spatially-explicit contextualisation of effects (Sarà et al., 2011; Sarà et al., 2013a; Sarà et al., 2018a,b; Mangano et al., 2018) promising to trace new paths for future restoration strategies by predicting organismal functional traits and capturing variation across species (Pouvreau et al., 2006; Pecquerie et al., 2010; Lika et al., 2011; Sarà et al., 2011; Kearney, 2012; Sarà et al., 2012; Sarà et al., 2013a; b; Sarà et al., 2018a,b; Mangano et al., 2018).

1.5.1 Dynamic Energy Budget Theory

Metabolic theories aim to capture how organisms acquire energy and matter from their surroundings and allocate it to growth, maintenance, development and reproduction. These processes subsequently affect a wide range of fundamental biological phenomena such as ageing and body size. These individual-level processes and outcomes show enormous variation across species and are well known to be reflected in patterns at higher levels of biological organisation including communities and ecosystems. Mechanistic theories of metabolism that can capture this variation should therefore provide a powerful basis from which to answer a very wide range of problems in ecology and evolutionary biology (Lika et al., 2011). The Dynamic Energy Budget theory (DEB; Kooijman, 2010) is such a mechanistic theory that is unique in its generality and comprehensiveness (Kooijman, 2010; Sousa et al., 2008, 2010). It has been developed to provide an integrative approach to link basal physiological information with environmental conditions, in a mechanistic-bioenergetic framework. DEB theory comprises a complete theoretical asset, at the whole organismal level, to link habitat, functional traits and life history of any living organism. It is the core of the “functional trait-based approach” (Kearney & Porter, 2009) and it represents the ‘quantitative from scratch framework’ providing first principles to investigate mechanistically how every species manages the available energy from the habitat, and how the utilisation of this energy is prioritised (i.e. the important choices that one organism has to activate unconsciously to optimise fitness along the life span) (Sarà et al., 2014). In essence, the DEB model captures the processes of energy acquisition and utilization in an organism under varying environmental conditions based on First Principles (Nisbet et al., 2000; Van der Meer, 2006a; Filgueira et al., 2011) as applied to a suite of parameters that describe the physiology responses of a species to environmental variation. The DEB model, therefore, integrates the energy allocation strategies adopted by species, and allows subsequent prediction of LH traits (e.g., growth and reproductive potential) in response to changes in environmental variables such as temperature and food density (Van der Meer, 2006a; Filgueira et al., 2011; Sarà et al., 2013a).

A key feature of DEB theory (Kooijman, 2010; Fig. 5, Tab. 1) is the partitioning of mass into the abstract quantities of “structural volume”, (V) and “reserve” (E). The reserve, which may consist of fat, carbohydrates and amino acids scattered across the body, is used and replenished and hence does not require maintenance. The structure is the ‘permanent’ biomass such as proteins and membranes and requires energy and matter for its maintenance (protein turnover and the maintenance of concentration gradients and ionic potentials) in direct proportion to structural volume. The rate of energy assimilation $\dot{p}_A$ is explicitly related to food density through a functional response curve $\dot{p}_A = \mathrm{f}\{\dot{p}_{Am}\}V^{2/3}$, where f is the scaled functional response (ranging from 0 to 1) and $\{\dot{p}_{Am}\}$ is the maximum assimilation rate per unit surface area (note that, in DEB theory notation, square and curly brackets denote volume-specific and surface area-specific terms, respectively). DEB theory follows the flows of both energy and matter and does not necessarily assume that energy per se is limiting. Development, growth and reproduction are predicted dynamically according to the κ-rule whereby a fixed (throughout ontogeny) fraction κ of the energy matter in the reserves flows to growth and to somatic maintenance, the rest to increasing and maintaining the level of maturity E_H and to reproduction once maturity is reached. The rate of change in the structural volume at constant food density is equal to $\frac{dV}{dt} = \frac{\kappa \mathrm{f}\{\dot{p}_{Am}\}V^{2/3} - [\dot{p}_M]V}{\kappa \mathrm{f}[E_m] + [E_G]}$ where t is time, $[E_m]$ is maximum reserve density (which, at constant food, reaches steady state at $\mathrm{f}[E_m]$), $[\dot{p}_M]$ is the somatic maintenance costs per unit volume and $[E_G]$ is the total energetic cost of structure (tissue energy content plus overheads for synthesis) per unit structural volume (van der Meer, 2006 b; Kooijman, 2010). For a constant food density, this equation is equivalent to the von Bertalanffy growth curve, although based on very different principles (Kooijman, 2010). The rate of change of the reserve density (which must be multiplied by structural volume, converted to mass and added to the structure to obtain a wet weight) is equal to $\frac{d[E]}{dt} = \frac{\{\dot{p}_{Am}\}}{V^{1/3}}\left(\mathrm{f} - \frac{[E]}{[E_m]}\right)$. Once maturity is reached under the standard DEB model, a fixed fraction of assimilates is continually transferred from the reserve to the reproduction buffer (after accounting for maturity maintenance) and then ‘packaged’ as eggs and dispensed as soon as an appropriate threshold amount for a clutch is reached. The energy allocated to the reproduction buffer per unit time is $\dot{p}_r = (1-\kappa)\dot{p}_c - \dot{p}_j$, where $\dot{p}_c = \left(\dot{p}_{Am}[E]V^{2/3}\right)/[E_m] - [E](dV/dt)$is the reserve mobilization rate and $\dot{p}_j = \dot{k}_j E_H$ is the maturity maintenance rate, with $\dot{k}_j$ the maturity maintenance rate coefficient.

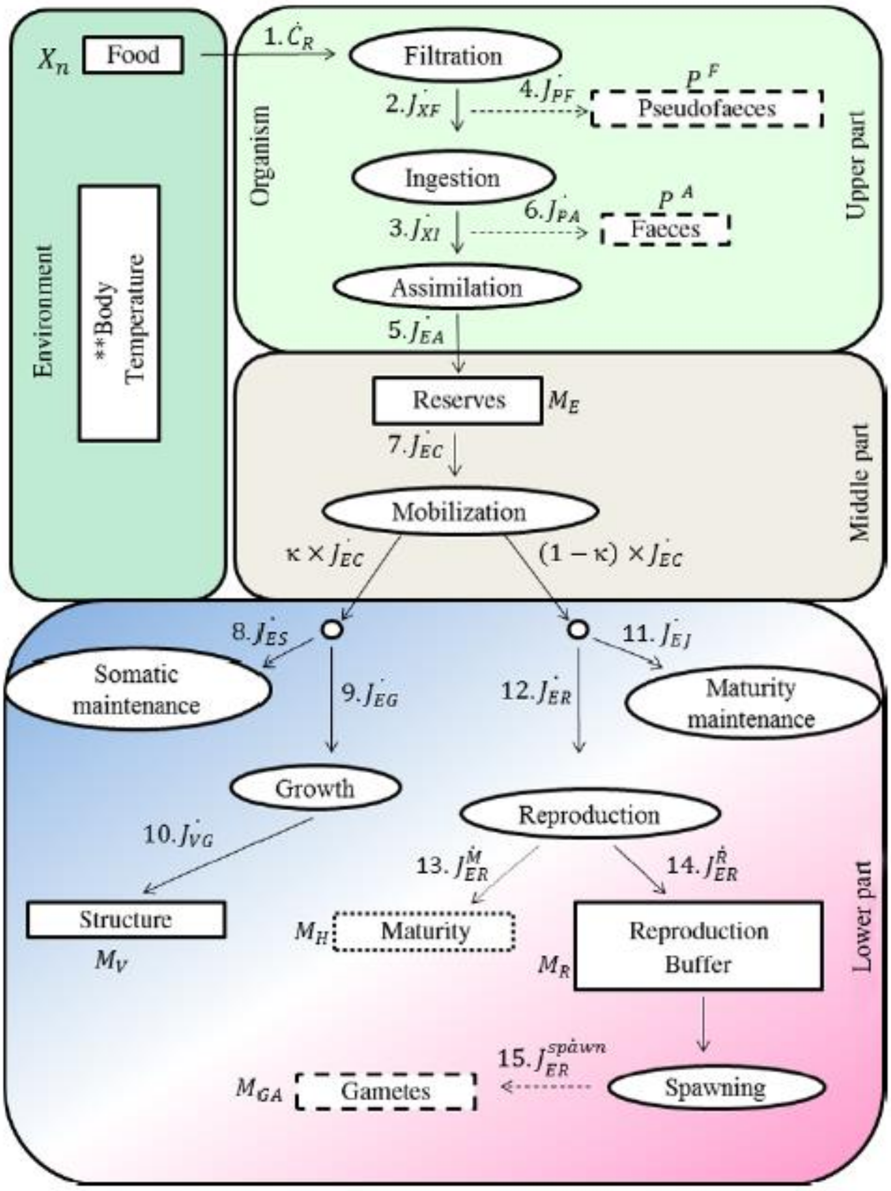

Fig. 5 Schematic representation of a standard DEB model (Kooijman, 2010; figure from Sarà et al., 2014, adapted for bivalves) describing the fluxes of energy (see Table 1) through an organism coming from the environment. The "Upper part" deals with feeding process that describes how energy coming from food is stored as metabolites (e.g. stored proteins, lipids, carbohydrates); Middle part: reserve in which the energy is first stored then made available for direct use following the κ-rule; Lower part: energy coming from reserve is allocated to maintenance and transformed into structure (i.e. growth) and offspring (i.e. reproduction). *indicates physiological parameters modified according to the different time-scale models; J_X = maximum ingestion rate, J h^{-1}, $[\dot{p}_M]$ = somatic maintenance costs, J h^{-1}.

Table 1 – Description of the main energy fluxes in the DEB model and their formulation. This table is adapted for bivalves, for more details see Kooijman (2010).

Process	**No.**	**Symbol**	**Description**	**Units**	**Formulation**
Filtration	1	$\dot{C}_R$	*Clearance - filtration rate*	$m^3\ h^{-1}$	$\dot{C}_R = \frac{\{\dot{C}_{Rm}\}}{1+\sum_{i=0}^{1}\frac{X_i\{\dot{C}_{Rm}\}}{\{\dot{J}_{XFm}\}}} V^{2/3}$
	2	$\dot{J}_{XF}$	Filtration rate	$molC\ d^{-1}g\ d^{-1}$	$\dot{J}_{XF} = CRX_n$
Ingestion	3	$\dot{J}_{XI}$	Ingestion rate	$molC\ d^{-1}g\ d^{-1}$	$\dot{J}_{XI} = \frac{\rho\ X_i\ I\dot{J}_{XF}}{1+\sum_i^n\frac{\rho\ X_i\ I\dot{J}_{XF}}{\{\dot{J}_{XI}\ I_m\}}}$
	4	$\dot{J}_{PF}$	Pseudofaeces production rate	$molC\ d^{-1}g\ d^{-1}$	$\dot{J}_{PF} = \dot{J}_{XF} - \dot{J}_{XI}$
Assimilation	5	$\dot{J}_{EA}$	Assimilation rate	$molC^E\ d^{-1}$	$\dot{J}_{EA} = \dot{J}_{EA^E} + \dot{J}_{EA^V}$
	6	$\dot{J}_{PA}$	Faeces production rate	$molC\ d^{-1}$	$\dot{J}_{PA} = \dot{J}_{XI} - \dot{J}_{EA}$
Mobilisation	7	$\dot{J}_{EC}$	Mobilization flux	$molC^E\ d^{-1}$	$\dot{J}_{EC} = \frac{[E]}{\frac{[E_G]}{\mu_E}+\kappa[E]}\left(\frac{[E_G]}{\mu_E}\dot{\upsilon}V^{2/3} + \dot{J}_{ES}\right)$
Somatic maintenance	8	$\dot{J}_{ES}$	Somatic maintenance	$molC^E\ d^{-1}$	$\dot{J}_{ES} = \frac{[\dot{p}_M]}{\mu_E}V$
Growth	9	$\dot{J}_{EG}$	Flux allocated to growth	$molC^E\ d^{-1}$	$\dot{J}_{EG} = \left(\kappa\dot{J}_{EC}\right) - \dot{J}_{ES}$
	10	$\dot{J}_{VG}$	Growth	$molC^V\ d^{-1}$	$\dot{J}_{VG} = y_{VE} - \dot{J}_{EG}$
Maturity reproduction	11	$\dot{J}_{EJ}$	Maturity maintenance	$molC^E\ d^{-1}$	$\dot{J}_{EJ} = \dot{\kappa}_J\ M_H$
	12	$\dot{J}_{ER}$	Flux allocated to reproduction/maturity	$molC^E\ d^{-1}$	$\dot{J}_{ER} = (1-\kappa)\,\dot{J}_{EC} - \dot{J}_{ES}$
	13	$J_{ER}^{\dot{M}}$	Flux to maturity	$molC^E\ d^{-1}$	$J_{ER}^{\dot{M}} = \begin{cases}\dot{J}_{ER}, if\ M_H < M_H^p \\ 0, otherwise\end{cases}$
	14	$J_{ER}^{\dot{R}}$	Flux to reproduction buffer	$molC^E\ d^{-1}$	$J_{ER}^{\dot{R}} = \begin{cases}0, if\ M_H < M_H^p \\ \dot{J}_{ER}, otherwise\end{cases}$
Spawning	15	$J_{ER}^{sp\dot{a}wn}$	Spawning	$molC^E\ d^{-1}$	$J_{ER}^{sp\dot{a}wn} = \begin{cases}k_R M_R / R^{spawn}, if \\ GSR \geq GSR^{spawn} \wedge T \geq T^{spawn} \\ 0, otherwise\end{cases}$

Based on strict physical, chemical and thermodynamic laws (the so-called first principles) governing the functioning of the world (Denny & Benedetti-Cecchi, 2012) this model aims to provide predictive scenarios of organismal functioning. DEB can provide a fundamental explanation of, for example, how, why and where organisms are present (or absent) throughout their distributional ranges (at the net of biotic interactions), how an organism is able to respond to environmental variability and multiple anthropogenic stressors (Sokolova et al., 2012), and the magnitude and spatio-temporal scale of ecological response. The mechanistic properties of this approach rely on energy and matter flows from habitat through organisms. But flows of energy and matter (and time) through habitats and organisms are subjected to conservation laws (Denny and Helmuth, 2009; Denny and Benedetti-Cecchi, 2012; Carrington et al., 2015) and, consequently, they are traceable (and budgetable) processes. DEB use these principles to mechanistically predict the functioning of each species and thereby the magnitude and variability of LH traits (Kearney et al. 2010, 2012; Sarà et al. 2013b). The components of an organism’s energy budget are functionally linked together, so that changes in any of the processes have consequences for one or more of the others so, according to the DEB theory, when general environmental conditions deviate from common natural patterns, reproduction and growth can be consequently affected. In order to survive and to maximize Darwinian fitness, the organism must be able to balance its energy gains from the environment against its metabolic losses and to ensure an optimal allocation of surplus energy to somatic growth and to reproduction (Koz1owski, 1992; Perrin and Sibly, 1993). The advantages of the DEB models are that they make use of the generalities found in terms of animal physiology and can therefore be applied to species for which little data or empirical observations are available. This mechanistic functional-trait (FT) based approach further allow an easy spatially-explicit contextualisation of model outputs by predicting organismal functional traits and capturing variation across species (Pouvreau et al., 2006; Pecquerie et al., 2010; Lika et al., 2011; Sarà et al., 2011a; Kearney, 2012; Sarà et al., 2012; Sarà et al., 2013a; b; Sarà et al., 2018a, b; Mangano et al., 2018).

1.5.2 Model outputs

The mechanistic nature of the standard DEB model allows to correlate the bioenergetic characteristics of an organism to environmental conditions, so that the ultimate fitness can be predicted. This is possible only if the body temperature of an organism and the amount of food present in the environment are known, and only that all the DEB parameters of the organism have been estimated. The present model allows to quantify:

1. **The Maximum Habitat Individual Size** ($MHIS$), calculated from $MHIS = \kappa \times \frac{\{\dot{p}_{am}\}}{[\dot{p}_M]}$, where κ is the fraction of energy allocated to somatic maintenance and growth (Kooijman, 2010), $\{\dot{p}_{am}\}$ is the area specific assimilation rate and $[\dot{p}_M]$ is the specific volume somatic maintenance rate. MHIS is used to explain the link between energy balance and body size on a local spatial scale. It is dependent on the energy that can be allocated to growth and to maintenance. Locally individuals will reach their asymptotic size if all the assimilated energy is constantly used for maintenance, when no other energy is available for growth (i.e. the growth ceases when these two terms are equal). Hence, MHIS will be a direct function of the amount of food available for consumption, through its relationship with the Holling's functional response (Kearney, 2012; Sarà et al., 2014). Implicitly this means that all the energy available from food (within the fundamental thermal niche of a species, as expressed by thermal tolerance limits, Saraiva et al., 2011b) is a determining factor for the fitness of ectotherms.
2. **Maturation Time** (MT) defined as the time (expressed in days) needed by the individual to reach the minimum necessary size for the development and maturation of the gametes. A primary requirement is therefore to collect information on the smallest size at maturity for the considered species. Therefore, once known the minimum size at maturity, the flow of energy 1- κ (coming from the existing reserves and/or the amount of net energy assimilated from food), and assuming that it is used for reproductive purposes (development and maturation of gametes, packaging of energy within the gametes), it is possible to estimate the time necessary to reach the maturity. MT is strictly habitat-specific (i.e. depends on the thermal conditions and the abundance of available food) as it depends on the time required to reach the minimum threshold for early sexual and reproductive maturity.
3. **The number of reproductive events during the whole life** (RE) is another important part of organismal life-histories, which occurs every time that the amount of energy in the reproduction buffer has reached a certain density, so that it overflows in the form of gametes. The RE is strictly related to environmental conditions, since the energy that fills the reproduction buffer depends on the availability of food. Furthermore, the standard DEB model

assumes that only when the body temperature is above a certain threshold (*optimum*), then the organism releases the gametes (Gabbott & Bayne, 1973), this implies that also the temperature represents a constraint for the occurrence of a RE.

4. **Total Reproductive Output** (TRO) is given by the total number of eggs produced during the whole life of the organism and it is possible to assimilate it to Bozinovic's et al. (2011) fitness. When the reproduction buffer energy reaches threshold limit, it is packaged in the form of gametes, which are produced in a discrete number of deposition events. Since DEB assumes that the energy needed to produce one single gamete is generally constant (e.g. 0.0019 J for an egg in bivalves, van der Veer et al., 2006) and this cost is species-specific, the TRO will depend on the amount of energy available for reproduction from the reserves and it is stored in the reproduction buffer.

1.5.3 Estimation of DEB parameters

The Dynamic Energy Budget (DEB) theory for metabolic organisation captures the processes of development, growth, maintenance, reproduction and ageing for any kind of organism throughout its life-cycle. However, the application of this theory is challenging because the state variables and parameters are abstract quantities that are not directly observable. In order for the model to generate outputs several parameters are needed. A wide collection of over 1230 parameterised species (1235 entries at 07/09/2018), is available online at https://www.bio.vu.nl/thb/deb/deblab/add_my_pet/ and represent an important source of energetical and ecological referenced data, and implied properties. Van Haren and Kooijman presented the first estimates of DEB parameters in 1993, using data from the literature. After that, van der Veer et al. (2006) developed a protocol to estimate a complete set of DEB parameters from factorially designed experiments. Kooijman et al. (2008) provided further guidance by structuring the estimation of parameters in 10 steps with a minimum set of data and the use of the regression routines included in the software package DEBtool (http://www.bio.vu.nl/thb/deb/deblab/debtool/) for MATLAB (The MathWorks, Natick, MA, USA). These routines used several generic algorithms to obtain the best fit, from slow algorithms with a large domain of attraction (genetic algorithms, Nelder–Mead method), to fast algorithms with a small domain of attraction (Newton–Raphson method). These previous estimation procedures have taken the strategy of estimating "compound parameters" that represent amalgams of the core DEB parameters that are more easily estimated from typical empirical data sets (Kooijman et al., 2008; van Der Meer, 2006a). However, for many applications, such as comparative analyses of evolutionary trends in physiological traits and ecological or geographical constraints on abundance, all parameters are needed, since only then it is possible to evaluate the full energy and mass budgets dynamically. Lika et al. (2011) provided a method based on the simultaneous minimization of the weighted sum of squared deviations between data sets and model predictions in one single-step procedure, including physiological constraints on the estimated parameter set (covariation method). The intended physiological consistency, apart from optimizing the goodness of fit for all available data, can be obtained using the concept of sloppy constraints, where ‘pseudo-observations’ are fitted for particular parameters, simultaneously with real observations (Kooijman et al., 2008). By choosing the weight coefficients in the regression procedure that minimize the weighted sum of squared deviations, the observations can be obtained (high weight coefficient for observations) without high deviation of the standard parameters (slightly lower weight coefficient). The covariation method aims to estimate the core DEB parameters from i) real data (zero- and uni-variate data) based on empirical observations and ii) pseudo-data i.e. a set of values of primary or compound parameters for a generalized animal obtained from a large collection of estimated parameters from various data sets for a wide variety of species (Kooijman et al., 2010).

Table 2 - Core parameters of the standard DEB model. Symbol, typical value (T_{ref}=293 K) and range limits.

Zoom factor $\boldsymbol{z = L_m/L_m^{ref}}$ with $\boldsymbol{L_m^{ref} = 1cm}$. Controls the maximum length $\boldsymbol{L_m} = \kappa\{\boldsymbol{p^{\cdot}\_Am}\}/[\boldsymbol{p^{\cdot}\_M}]$ via the specific assimilation $\{\boldsymbol{\dot{p}_{Am}}\}$. It also affects the life-stage parameters and the ageing acceleration as primary parameters. Most derived quantities (being functions of parameters) are affected by this factor in ways that follow from DEB theory. In combination with the zoom factor, the shape coefficient $\boldsymbol{\delta_M}$ controls the length–weight relationship, where weight has contributions from reserve and, possibly, the reproduction buffer. An increase in the zoom factor gives an increase in the specific assimilation rate and so an increase in the maximum reserve capacity and in the contribution of reserve to weight. Range: >0; likely range <150.

Maximum surface-area-specific searching rate $\{\boldsymbol{\dot{F}_m}\}$, 65 dm^3 d^{-1} cm^{-2}: controls food intake if food is not abundant and has no effect at abundant food; its value and even its dimension is species-specific; for some taxa the unit dm^2 d^{-1} cm^{-2} is more appropriate. A low value means a low food density at which food starts to limit ingestion. Range: >0.

Digestion efficiency $\kappa_{\boldsymbol{X}}$, 0.8: specifies the fraction of energy in food that is fixed in reserve. Since this factor is applied to the maximum specific assimilation to obtain the maximum specific food intake, a low value for $\kappa_{\boldsymbol{X}}$ gives a high food intake. Range: >0, >1.

Defecation efficiency $\kappa_{\boldsymbol{X}}^{\boldsymbol{p}}$, 0.1: specifies the fraction of energy in food that ends up as faeces. This parameter doesn't affect the state variables (structure, reserve, maturity), but controls faeces production and mineral fluxes (CO_2, H_2O, O_2, NH_3).Note that $\kappa_{\boldsymbol{X}}$+ $\kappa_{\boldsymbol{X}}^{\boldsymbol{p}}$, else energy (and CO_2) uptake will occur directly from the environment. Range: >0, <1.

Energy conductance $\boldsymbol{\dot{v}}$, 0.02 cm d^{-1}: controls the reserve mobilisation. A high value gives a high growth rate, short development time to reach birth or maturity, a low maximum reserve density, a rapid occurrence of problems during starvation. The maximum size is not affected by this parameter. The energy conductance is unlikely to deviate strongly from the typical value independent of the body size of the species. Range: >0.

Allocation fraction to soma κ, 0.8: controls the allocation of mobilised reserve to somatic maintenance and growth as opposed to maturity maintenance and maturation of reproduction. A high value gives rapid growth to a large size, long development times and low reproduction. A small value ($\kappa < 0.5$) can reduce growth and reproduction, since food uptake is linked to size. Range: >0, <1.

Reproduction efficiency κ_R, 0.95: is the fraction of reserve allocated to reproduction that is fixed in the offspring's reserve. A high value gives a high reproduction rate and a low CO_2 production linked to reproduction.Range: >0, <1.

Volume-specific somatic maintenance $\boldsymbol{\dot{p}_M}$, 18 J d^{-1} cm^{-3}: controls the sink of reserve linked to structural volume, mostly due to turnover of structure and behaviour, transport. A high value reduces growth as well as the maximum size, so indirectly also reproduction. This effect can be cancelled by increasing the zoom factor. Range: >0.

Surface-specific somatic maintenance $\{\boldsymbol{\dot{p}_T}\}$, 0 J d^{-1} cm^{-2}: controls the sink of reserve, but now linked to structural surface area (e.g. heating in endotherms and osmotic work in freshwater organisms). It thus depends in environmental temperature and salinity. As long as endotherms are in the thermal-neutral zone, $\{\boldsymbol{\dot{p}_T}\} = 0$. A high value reduces the ultimate length, but the von Bertalanffy growth rate does not depend on this rate. Range: ≥0.

Maturity maintenance rate coefficient $\{\boldsymbol{\dot{k}_J}\}$, 0.002 d^{-1}: is the third primary parameter that controls the sink of reserve, but now linked to maturity. Metabolic switches (birth, puberty) occur at threshold values for maturity. A high value delays development and reduces reproduction. Maturity density (maturity per structure) is constant if $\boldsymbol{\dot{k}_J} = [\boldsymbol{\dot{p}_M}]/[\boldsymbol{E_G}]$. Range: >0; likely range $<[\boldsymbol{\dot{p}_M}]/[\boldsymbol{E_G}]$.

Specific cost for structure $[\boldsymbol{E_G}]$, 2800 J cm^{-3}: is the reserve energy that is required to synthesise a unit volume of structure. It includes the energy content of the tissue plus the overhead costs of the anabolic machinery. A high value reduces the growth rate (but not the ultimate size), and decreases the size at birth (and puberty). If toxicants increase this value, hormesis occurs for reproduction (i.e. low levels of toxicants increase the reproduction rate). The value is proportional to the specific density d_v for dry mass (in g cm^{-3}). Range: > $\mu V[MV]=\mu_V d_V/w_V$.

Maturity at birth $\boldsymbol{E_H^b}$, $0.275z^3$ J: controls the timing of and the size at birth, i.e. the moment assimilation is switched on. Food abundance affects the timing, because of the maternal effect (reserve density at birth equals that of the mother); eggs with little reserve take longer to develop into a hungry neonate. Increasing the value causes an increase in the size at birth and a decrease of the reproduction rate. Range: >0, $<\{\boldsymbol{\dot{p}_{Am}}\}^{\boldsymbol{3}}[\boldsymbol{E_G}](\boldsymbol{1-k})\boldsymbol{k^2}/[\boldsymbol{\dot{p}_M}]^{\boldsymbol{3}} - \boldsymbol{L_m^3}[\boldsymbol{E_m g}(\boldsymbol{1-k})]$.

Maturity at puberty $\boldsymbol{E_H^p}$, $166{*}z^3$ J: controls the timing and the size at puberty, i.e. the moment at which investment into maturation is re-directed to reproduction. Food abundance affects the timing sensitively. Increasing the value causes an increase in the size at puberty. Range: >0, $<\{\boldsymbol{\dot{p}_{Am}}\}^{\boldsymbol{3}}[\boldsymbol{E_G}](\boldsymbol{1-k})\boldsymbol{k^2}/[\boldsymbol{\dot{p}_M}]^{\boldsymbol{3}} - \boldsymbol{L_m^3}[\boldsymbol{E_m g}(\boldsymbol{1-k})]$; likely range $>\boldsymbol{E_H^b}$.

Weibull ageing acceleration $\boldsymbol{\ddot{h}_a}$, 10^{-6} z d^{-2}: controls the mean life span in a way that hardly depends on food density (because the increased respiration is cancelled by dilution by growth). Increasing the value reduces the mean life span and the survival probabilities at birth and puberty. Range: >0.

Gompertz stress coefficient $\boldsymbol{s_G}$, 0.01: also controls the mean life span, but in ways that depend on food density. A positive value (around 0.5) can be expected in endotherms and elongates life at caloric restriction. Negative values can occur if damage inducing compounds can be degraded (found for *Daphnia magna*). Increasing the value decreases the mean life span, but increases survival at young age, relative to the mean life span. Range: ≥∞, <∞; likely range ≥0.5, <0.5.

1.6 Study aims and thesis outlines

While ecological research has begun to document the individual effects of these various stressors on species and ecosystems, research into the cumulative and interactive impacts of multiple stressors is less frequent. The need to better understand the interactive and cumulative effects of multiple stressors was highlighted a decade ago and is still cited as one of the most pressing questions in ecology and conservation. The current approach actually considering the effect of a single-stressor-per-time on biological and ecological responses is misleading and generates unrealistic conclusions, reducing the ability to cope with real challenges linked to the use of marine resources. As there is a pressing need to identify a way to quickly assess the effect of single and multiple impacts on individuals and populations, this thesis aims to test a simple tool based on the principles of eco-mechanics at the individual level, as a possible mechanism to disentangle the effects of single and combined impacts in determining an alteration of LH-traits, and consequently on potential biodiversity loss. The mechanistic functional trait-based (FT) approach, which rely on the Dynamic Energy Budget Theory, is based on flux of energy and mass through an organism (and not on a snapshot as in a context of the SFG approach), which is a traceable process being subject to conservation laws. Those models represent an effective and powerful tool in providing those kinds of quantities that allow an easy spatially-explicit contextualisation, leading to translate complex results into useful figurative representations for stakeholders.

The aim of this study was to introduce a comprehensive approach that integrates quantities generated by a mechanistic DEB based application, and in order to complete the framework, to include a spatially-explicit module that allow to spatially visualize potential management issues and predicted future scenarios of climate effects on target species. While **Chapter 1** was dedicated to frame of general topic of the present thesis, the **Chapter 2** experimentally investigated the effects of a novel prey and a chronic increase in temperatures on functional traits and fitness of the whelk *Stramonita haemastoma*. In **Chapter 3,** we apply a new approach using DEB models to investigate the effects of an anthropogenic pollutant on Life-History (LH) traits of marine organisms, providing stakeholders and policy makers an effective tool to evaluate the best environmental recovery strategy. In **Chapter 4** we used DEB models to determine the effect of changing environmental conditions and pollution on the Indo-Pacific *Perna viridis* aquaculture. In **Chapter 5** we proposed a DEB application to study the link between future COP21 predicted temperature scenarios and varying food availability on LH-traits of some Mediterranean fishery and aquaculture target species, exploring the efficiency of Integrated Multitrophic Aquaculture as a potential management solution. A spatial contextualization of model outcomes allowed translating those results into useful figurative representations. Through **Chapter 6** we investigated the site-specific effects of environmental changes represented by Ocean

Acidification and hypoxia on the functional and behavioural traits of the mussel *Mytilus galloprovincialis*. Finally, in **Chapter 7** we presented a proof-of-concept study using the European anchovy as a model species to show how a trait-based, mechanistic species distribution model can be used to explore the vulnerability of marine species to environmental changes. Scenarios of temperature and food were crossed to generate quantitative maps of selected mechanistic model outcomes.

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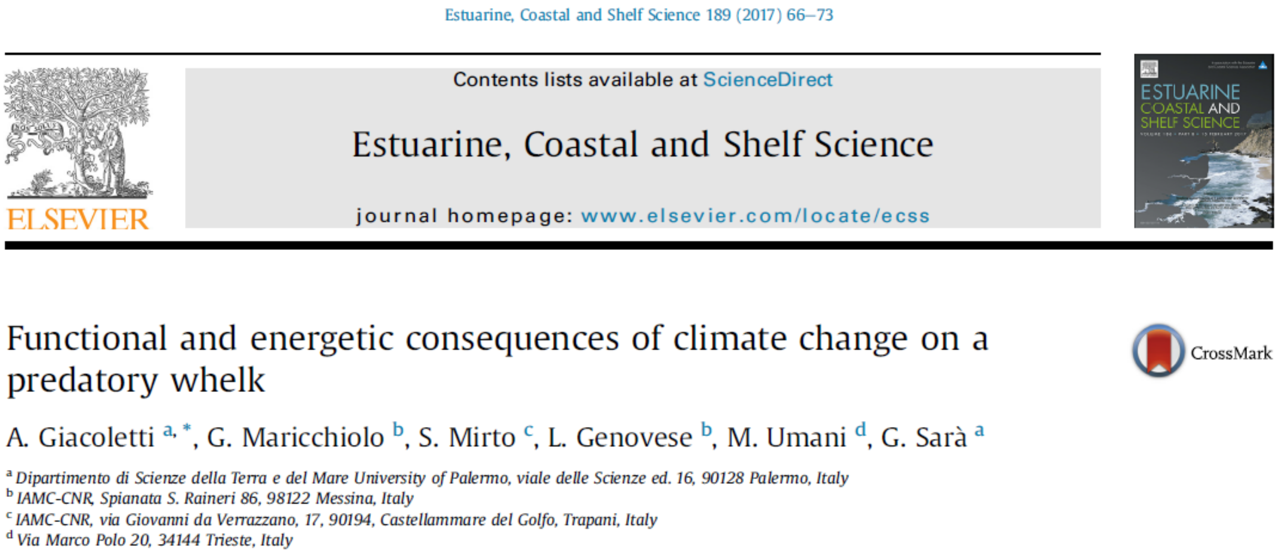
CHAPTER 2



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Functional and energetic consequences of climate change on a predatory whelk

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ABSTRACT

The increasing rise in sea surface temperature caused by human activities currently represents the major threat to biodiversity and natural food webs. In this study we used the Lessepsian mussel *Brachidontes pharaonis*, one of the most recent invaders of the Mediterranean Sea, as a model to investigate the effect of a novel prey and a chronic increase in temperatures on functional parameters of local consumers, compared to the native mytilid species *Mytilaster minimus*. In particular we focused on the whelk *Stramonita haemastoma*, a widespread Mediterranean intertidal predator that actively preys on bivalves, barnacles and limpets, by studying the direct effects of such multiple stressors on feeding and growth rate, projected into a future climate change scenario (RCP8.5) relative to 2046-2065 with higher hypothesized temperatures of 2°C. Gastropods showed a significantly higher feeding rate (ADFR) on *M. minimus* at high (6.45 ± 0.43) vs low temperatures (5.15 ± 0.33) compared to *B. pharaonis* (2.84 ± 0.37 vs 2.48 ± 0.27). Ingestion rate (ADIR), however, recorded higher values for *B. pharaonis* at high (1.71 ± 0.22) and low (1.49 ± 0.16) temperatures, compared to *M. minimus* (0.17 ± 0.01 vs 0.14 ± 0.01). Prey significantly influenced growth rate, condition index and the length-weight relationship (LWR) of whelks, while only ADFR seemed to be influenced by higher temperatures. In conclusion the extra amount of energy from the novel prey, together with temperature side effects, successfully influenced growth rates and reproductive events, positively affecting the global fitness of whelks.

Key words: Invasive species; Climate change; Multiple-stressor; *Stramonita haemastoma*; *Brachidontes pharaonis*; RCP8.5.

1 INTRODUCTION

Biotic interactions of local species are naturally shaped by environmental variability (Vasseur et al., 2007; Post, 2013). Seawater temperature, through the fundamental influence on metabolic machinery (Brown et al., 2004), plays a prominent role in driving functional and life history (LH) traits of most ectotherms, affecting their local persistence over time (*sensu* Sibly et al., 2012). Increasing temperature, as a consequence of future climate change (CC; IPCC, 2014), will probably determine cascading effects within natural communities, modifying current biodiversity (Gooding et al., 2009; Yamane and Gilman, 2009). Intertidal shores are harsh habitats with regard to temperature; the body temperatures of ectotherms can vary greatly according to daily tidal cycles and seasonal weather conditions (Helmuth, 1998; Helmuth and Denny, 1999). Intertidal organisms are, for this reason, considered to live “on the edge” and thus CC is expected to modify the structure and species composition of those communities. CC may favour the spread of much more thermo-tolerant alien marine species, such as jellyfishes, bivalves and fishes by increasing the likelihood of reinforcing facilitation mechanisms (Southward et al., 1995; Stachowicz et al., 2002; Galil et al., 2015) or through the availability of empty niches in the invaded range (Hierro et al., 2005). Invasive species originated from the Red Sea (also called Lessepsian) and introduced to the Mediterranean Sea through the Suez Canal, are considerably more thermo-tolerant and better able to cope with highly changing thermal conditions than most Mediterranean species (Zerebecki and Sorte, 2011). Thus, when these species interact with local ecological equivalents, they will be advantaged by their major innate ability to survive under harsher conditions (Sarà et al., 2008). Invaders may then be able to replace native species in the local food webs (Simberloff et al., 2013) and consequently, local native consumers may count on a larger selection of prey. Nonetheless, local consumers, for their part, will also have to cope with increasing temperatures due to CC. However, although the effect of temperature is essentially pervasive (Gillooly et al., 2001), there is still little research to investigate the crossed effect between an alien prey and the increasing temperature on feeding behaviour (e.g. prey preference and consumption rate) of a local consumer apart from a single study dealing with the planktonic food webs (Seifert et al., 2014). According to theory (e.g. Arrhenius law; Kooijman, 2010; Sarà et al., 2014), increasing temperature should be particularly effective in enhancing the consumption rates in ectotherms (Sibly et al., 2012; Seifert et al., 2014). As a consequence, it is possible that an altered scheme of consumption dynamics of an abundant local predator has important local implications for the destabilization of the entire community equilibria (Vasseur and McCann, 2005; Seifert et al., 2014). Consumers are widely believed as able to adapt their feeding behaviour, and in particular the quality and quantity of food consumed in order to adjust their energy intake as a response to a varying environment (Tylianakis et al., 2008; Kordas et al., 2011; Kaspari et al., 2012). Ecologists use the term “plasticity” to describe this ability of organisms to modify their feeding behaviour, with compensatory feeding patterns, e.g. increased predation or ingestion rate to compensate for low quality resources or stressful conditions (Duarte et al., 2015). The same kind of plasticity can also be observed when consumers maximize consumption of high quality resources (Jacobsen and Sandjensen, 1994; Falkenberg et al., 2013) at the same time satisfying their energetic requirements and enhancing the individual fitness as suggested by classical ecological theory (Optimal Foraging Theory; Pyke, 1984). Here, we designed an experimental set-up to test if a widespread intertidal carnivorous gastropod, *Stramonita haemastoma*, when fed with an invasive bivalve, *Brachidontes pharaonis* (Sarà et al., 2000, 2003) under constant increased temperature of a few degrees (IPCC, 2014; RCP8.5 scenarios relative to 2046-2065), will show any difference in the individual fitness with respect to when it fed with the native ecological equivalent prey, the bivalve *Mytilaster minimus*. Thus, we investigated whether (i) functional traits, such as those involved in feeding processes (i.e. predation and ingestion) were modified, and (ii) if any repercussions on life history (LH) traits, such as growth and fecundity, were evident. *Brachidontes* and *Mytilaster* (and *Stramonita*) represent a perfect model for this study: the former is one of the first time invaders (Pallary, 1912; Sara et al., 2000, 2003), forms dense clusters on lower mid-littoral and subtidal rocks where *Stramonita* lives and spreads to the Western Basin (Sarà et al., 2013; Sarà et al., 2018). If present, it out-competes *M. minimus* (Safriel et al., 1980) that usually, in the absence of the alien species, proliferates and represents one of the most frequent items in the *Stramonita* diet (Safriel et al., 1980). Results from the present experiment comprise an important tool to evaluate the species colonization process and predict future spread, but will also be useful when assessing the potential expansion of Lessepsian species under higher temperatures and salinity conditions in the Mediterranean Sea, as a result of current global warming, where Lessepsian species would have a distinct advantage over native species (Sarà et al., 2008).

2 MATERIALS AND METHODS

Specimens of *S. haemastoma* were collected alive at low tide during the month of June 2014 from the intertidal shores near San Vito Lo Capo and the natural reserve of Monte Cofano (Castelluzzo, TP) (LAT: 38°6'23.42"N; LONG: 12°42'17.84"E), where the mussels *B. pharaonis* and *M. minimus* were both present, although with different densities. As in surveys conducted along 100-m transects, whelks were relatively abundant in this site with a density of ~0.56 ind./m^2 (Giacoletti et al., 2016), while *M. minimus* reached densities of ~19,753.3 ± 9445 ind./m^2 (G. Sarà, unpublished data), and *B. pharaonis* showed an occasional distribution. Whelks were brought back to the Experimental Aquaculture Facility at IAMC-CNR in Messina, and acclimated at room temperature (20-22°C) and seawater salinity (37-38), and starved for one week to reduce stress generated by manipulation and transport (Garton and Stickle, 1980), before being transferred to experimental tanks (1350 L). The first tank (Tank A) was equipped with a 1500 W electric heater in order to maintain a higher temperature of 2°C than the second tank (Tank B), which received water at ambient temperature. Water from each tank (A, B) was distributed into 16 1 L plastic compartments, divided in two groups of 8, each containing a single whelk. Natural variation of temperatures due to the open water flow were continuously monitored through the use of temperature data loggers (model: iButton G1, prec. ± 1°C, res. ± 0.5°C, http://www.alphamach.com). Each group of whelks was fed for the first 7 days with *B. pharaonis* or *M. minimus,* respectively, in order to allow whelks to experience handling with their prey (Rovero et al., 1999). After this last period of adaptation, animals were starved for another week prior to the start of the experiment: this step allowed for hunger levels to be standardized (Garton and Stickle, 1980). No drill mortality was detected during the experiment.

2.1 Prey-size selection

Size-classes for each prey species (*M. minimus* and *B. pharaonis*) used during the experiment were determined through a preliminary prey-choice test, following the experimental design proposed by Underwood and Clarke (2005). In practice, we tested 36 specimens of *S. haemastoma* of the same size, and acclimated as above. In the first experiment two size-classes of *M. minimus* (5-10 mm and >10 mm) were offered simultaneously, without replacement, to each single whelk in their respective 1-L plastic compartment. The experiment was replicated twice. In the second experiment, four size-classes of *B. pharaonis* (10-15, 15-20, 20-25 and 25-30 mm) were offered simultaneously to each single whelk, with the same experimental setting. These prey class-sizes were chosen as they corresponded to the most common sizes observed in the field where the whelks were observed feeding (unpubl. obs.). Each experiment was replicated twice. Attacks were considered to start when the prey was put in the arena, and ended as soon as specimens of *S. haemastoma* had made their choice. To meet the independence criterion (*sensu* Underwood, 1997), each whelk was used only once, and later they were killed by gentle freezing.

2.2 Growth rates and fecundity

The experiment involved 48 medium size (30-40 mm) individuals of *S. haemastoma*, and lasted for 135 days, from July 2nd to November 19th, 2014, in order to evaluate the influence of prey and temperature on the growth rate of our model predator. As before detailed, water from each experimental fiberglass tanks was distributed in two groups of 8 whelks, and each group was fed with a different diet. The first diet (D1) consisted of a fixed daily density (n = 12) of the indigenous mussel *M. minimus,* while the second diet (D2) involved the same density of the Lessepsian mussel *B. pharaonis*. Each day consumed items were replaced, in order to maintain a constant density of the prey, allowing the whelks to feed ad libitum. Specimens of *S. haemastoma* were weighed and measured every two weeks, by taking five different variables (Fig. 1): total weight (TW), shell length (SL), shell width (SW), aperture height (AH), and aperture width (AW) (Chiu et al., 2002), in order to determine growth throughout the experimental period. Four whelks for each group (total = 16) were initially sacrificed in order to investigate the flesh organic content at the beginning of the experimental period.

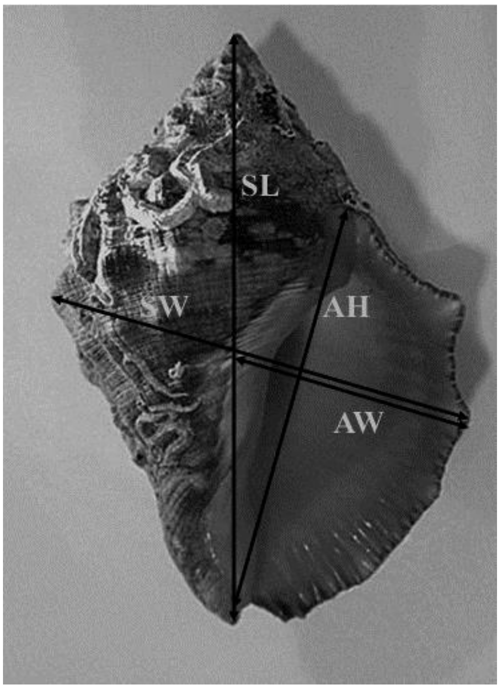


Fig. 1 Morphometric variables of *Stramonita haemastoma*: shell length (SL), shell width (SW), aperture height (AH), aperture width (AW).

The biomass of whelks exposed under two different temperatures and fed with two different diets was compared by estimating the individual dry weight (oven 95°C for 24 h) and the ash content (muffle furnace at 450°C for 4 h) of tissue to determine the Ash Free Dry Weight (AFDW) to the nearest 0.001 g. The Condition Index (CI) of whelks was estimated as: $\mathrm{C.I.} = \left(\frac{\mathrm{FDW}}{\mathrm{SDW}}\right) * 100$ (Davenport and Chen, 1987), where FDW is the flesh dry weight, and SDW is the shell dry weight. The relationship (LWR) between shell length (SL) and total weight (TW) was calculated, as reported by Merella et al. (1997), in order to better estimate the growth rate of whelks

through the comparison of the allometric regression slopes (b). Such a descriptor is powerful in capturing also the slightest differences in growth performance of marine invertebrates (*sensu* Gould, 1966). Fecundity and timing were estimated through simple daily visualization and here, unfortunately, no estimates of number of eggs or amount in grams are reported. Accordingly, whelks from both tanks were daily inspected, during the whole reproductive period, in order to annotate the presence or absence of released egg capsules. The proportion of individuals with eggs is used, in this study, as a proxy for fecundity.

2.3 Predation rates of whelks

Individual feeding rates of whelks was determined in single 1-L plastic compartment, as suggested by other authors (Palmer, 1983; Garton and Stickle, 1980). Prey of uniform length (*M. minimus* of

~10 mm; *B. pharaonis* of 15-20 mm) was selected to remove size as a variable within the experimental design. Feeding rates were determined by daily removing and counting the number of consumed prey from the fixed density (n = 12) for both *M. minimus* and *B. pharaonis*, constantly maintained through the experiment. The daily ingestion rate was determined by estimating the average prey tissue from n = 100 mussels of same size, and multiplying it for the predation rate. The predation rate was measured as the average daily feeding rate (ADFR, prey per drill/day; Garton and Stickle, 1980) and the ingestion rate as the average daily ingestion rate of tissue (ADIR, g of tissue per drill/day) during the 20-day experiment. The determination of ADFR and ADIR was the first part of the experiment, and took place after the acclimation and conditioning period, with daily measurement for 20 consecutive days on the same 48 experimental whelks, in order to compare potential differences between treatments (temperature and diet). After that whelks were left feeding ad libitum with their respective prey, in order to calculate the growth rate.

2.4. Statistical analysis

In order to test for significant differences in predation, ingestion, and growth rates, ANOVA were performed using feeding or morphometric variables as fixed factors, with two levels of temperatures (T Low: seawater ambient temperature and T High: seawater ambient temperature + 2°C). When significant differences were detected, the Student-Newman-Keuls (SNK) post-hoc pair wise comparisons of means were used (Underwood, 1997). Cochran's test was used prior to ANOVA to test the assumption of homogeneity of variances (Underwood, 1997). Pearson χ^2 test was used to test significant differences in temperatures, and data from prey size experiments (Darmaillacq et al., 2006). In order to test for significant differences in the condition index of whelks, a Permutational Multivariate Analysis of Variance (PERMANOVA) was used, considering time (initial - final), temperature (low - high), prey (*Brachidontes* - *Mytilaster*) as a fixed factor (2 levels). The Euclidean similarity measure was used, and all p-values were calculated using 9999 permutations of the residuals under a reduced model (Anderson, 2001).

3 RESULTS

Different temperatures were significantly ($\chi^2 = 29.5$, df = 140, $p < 0.05$) maintained through the 135-day experimental period (Fig. 2). Mean temperatures recorded through the whole experimental period were 24.65 ± 1.65 for Tank A and 22.64 ± 1.76 for Tank B.

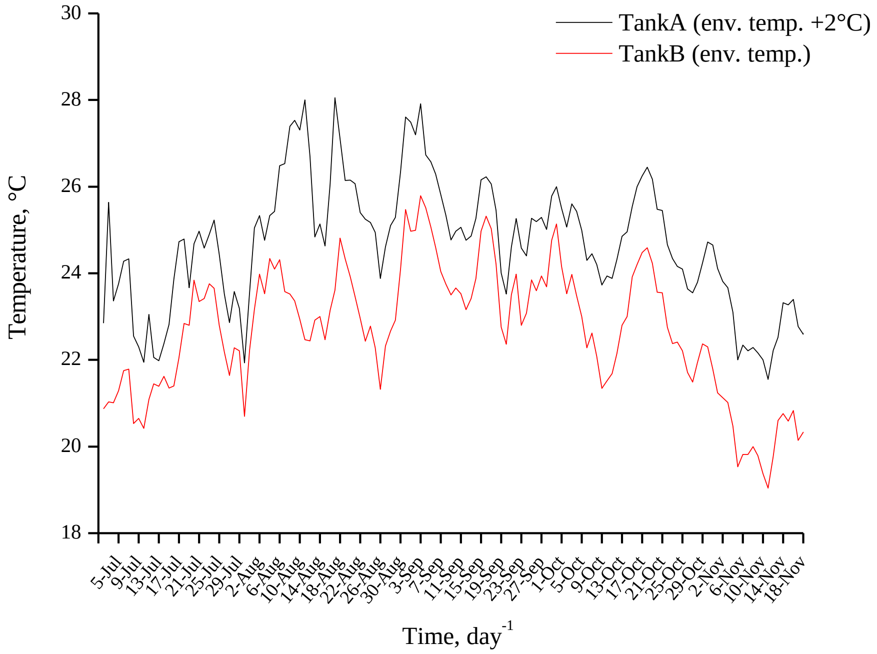


Fig. 2 Temperatures of tanks (A, B), continuously monitored through an electronic submersible data logger, for the whole length of the experiment (135 days).

3.1 Prey-size selection

S. haemastoma showed a greater and significant preference for the >10 mm class size of *M. minimus* ($\chi^2 = 12.1$, df = 68, $p < 0.05$), and for the 15-20 mm specimens of *B. pharaonis*, although not statistically significant ($\chi^2 = 1.81$, df = 68, $p > 0.05$), (Fig. 3). Around 11% of whelks fed with *B. pharaonis* and 22% of specimens fed with *M. minimus* manifested no feeding or other activity, with a closed *operculum*.

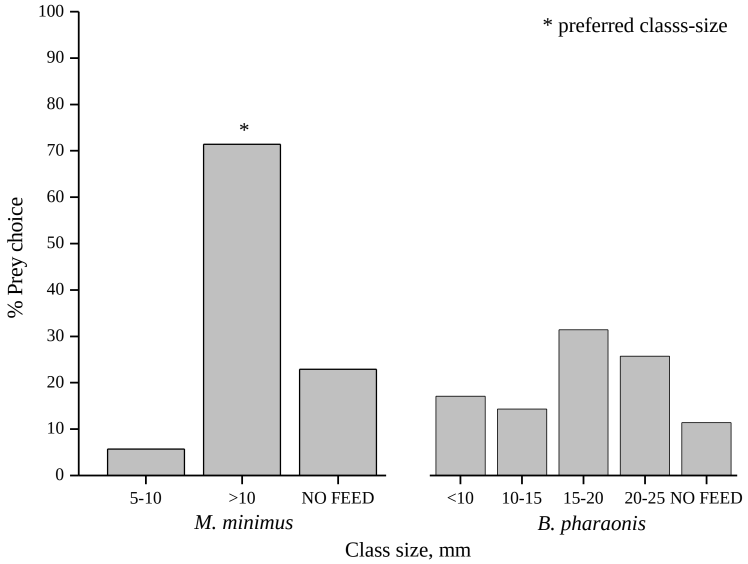


Fig. 3 Prey-size experiment, % prey choice on two different sizes of *M. minimus*, and on four different sizes of *B. pharaonis* (Preferred classes are indicated by *).

3.2 Growth rates and fecundity

Shell length (SL), aperture height (AH) and aperture width (AW) were not significantly influenced by *Brachidontes* at T High (SNKs; Fig. 4; Table 1). Condition index (CI) of whelks was derived from dry weight (DW) of flesh and shell, and resulted significantly influenced by time (PERMANOVA $p < 0.001$) and diet (PERMANOVA $p < 0.01$), while temperature revealed no significant effect (PERMANOVA $p > 0.05$; Table 2).

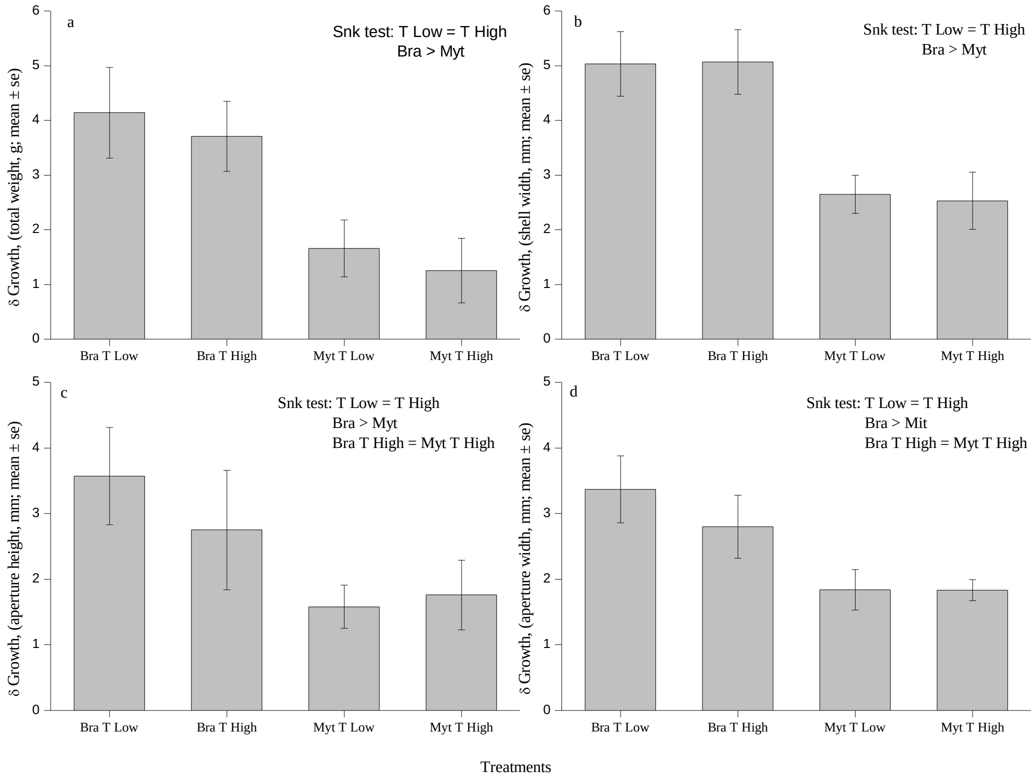


Fig. 4 (a-b-c-d) Growth rates of *S. haemastoma* analysed between treatments. Compared variables are from left to right, and from top to bottom: (a) total weight (TW), (b) shell width (SW), (c) aperture height (AH), (d) aperture width (AW).

Table 1. ANOVA on morphometric variables of *S. haemastoma* feeding on two different prey species at different temperatures (* = p <0.05; ** p <0.01; *** = p <0.001; ns = non-significant difference). TW = Total weight, SL = Shell length, SW = Shell width, AH = Aperture Height, AW = Aperture width.

	df	TW (g)			SL (mm)			SW (mm)		
		MS	F	p	MS	F	p	MS	F	p
Temp	1	1.41	0.41	ns	5.94	1.02	ns	0.014	0.01	ns
Prey	1	48.81	14.23	***	63.53	10.89	**	48.44	22.48	***
Temp*Prey	1	0.0024	0.00	ns	1.16	0.2	ns	0.047	0.02	ns
Residuals	28	3.43			5.83			2.15		

	df	AH (mm)			AW (mm)		
		MS	F	p	MS	F	p
Temp	1	0.81	0.23	ns	0.67	0.55	ns
Prey	1	17.85	5.02	*	12.5	10.38	**
Temp*Prey	1	1.99	0.56	ns	0.63	0.52	ns
Residuals	28	3.55			1.2		

Table 2. (PERM)ANOVA table of result and group analysis for the Condition Index (C.I.) of *S. haemastoma* of tank A (env. temp. +2°C) and tank B (env. temp). (* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; ns = not significant). T0 = Start of experimental period; Tf = End of experimental period; Bp= *B. pharaonis*; Mt = *M. minimus*.

Source	df	MS	Pseudo-F	P(perm)	Unique perms	Groups	t	P(perm)	Unique perms
Time	1	144.14	27.742	***	9821	T0, Tf	5.2671	***	9842
Temperature	1	0.2306	0.04438	ns	9847	Bp, Mt	3.1558	**	9846
Prey	1	51.745	9.9589	**	9822				
Time*Temp	1	1.4081	0.271	ns	9855				
Time*Prey	1	1.5876	0.30555	ns	9822				
Temp*Prey	1	0.0818	0.01574	ns	9831				
Time*Temp*Prey	1	0.0299	0.005771	ns	9812				
Residuals	38	5.1958							

The L-W relationship of whelks at the end of the experimental period revealed higher allometric b values for the whelks that fed on *B. pharaonis* at both temperatures (T High 2.62 ± 0.32; T Low 2.43 ± 0.38), while lower values were recorded when feeding on *M. minimus* at T Low (2.34 ± 0.41), and in particular at T High (1.76 ± 0.92). Comparing the different growth rates of SL (Fig. 5), whelks showed a tendency to slow down shell growth during the reproductive period up to the month of August, whereafter SL increased again. The delta (ƌ) shell length has been calculated as the relative average growth rate (in mm) for each period by subtracting two consecutive time intervals (e.g. T1-T0; T2-T1; T3-T2, etc.) of experimental whelks.

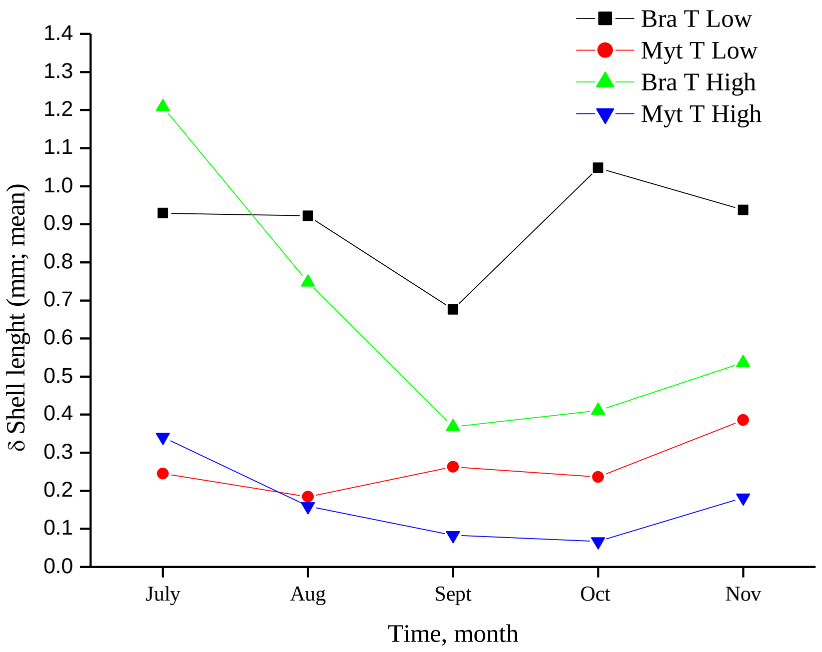


Fig. 5 Growth rates of *S. haemastoma* expressed in terms of ƌ shell length (SL) during the whole experimental period (135 days), compared between treatments.

Overall, every morphometric variable was significantly influenced by the prey consumed: TW ($p < 0.001$), SL ($p < 0.01$), SW ($p < 0.001$), AH ($p < 0.05$), AW ($p < 0.01$), while they were not affected by the different temperatures (ANOVA $p > 0.05$; Table 1; Figs. 4 and 5). Lastly, approx. 38% of the whelks feeding on *B. pharaonis* at normal temperature, and 50% at high temperatures, released egg capsules during the whole reproductive period, while no whelks that fed on the native prey completed the reproductive event (i.e. no eggs were produced). Under the higher temperature treatment, whelks produced eggs approx. 7 ± 3 days before those at ambient temperature.

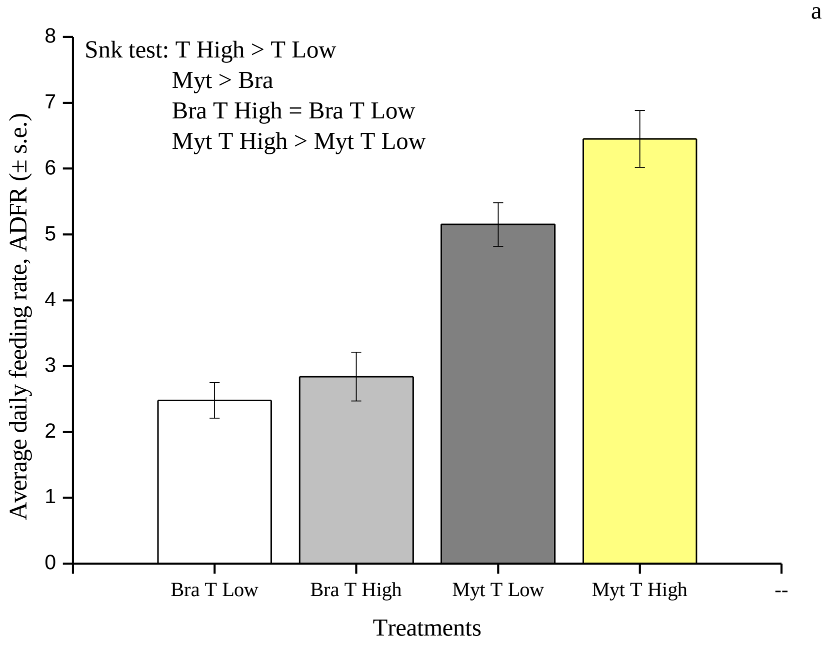
3.3 Predation rates of whelks

The feeding experiments showed that temperature significantly affected the average daily feeding rates (ADFR) (ANOVA $p < 0.05$; Table 3). Significantly different ADFR resulted also on the two different prey species (ANOVA $p < 0.001$; Table 3).

Table 3. Reported values and ANOVA table of results of: Average Daily Feeding Rate (ADFR) and Average Daily Ingestion Rate (ADIR) of *S. haemastoma* on two different prey species (*B. pharaonis* and *M. minimus*) at two different temperatures (environmental; env. + 2°C) (* = p<0.05; ** = p<0.01; *** = p<0.001; ns = not significant).

VALUES			ADFR		ADIR		ANOVA	ADFR				ADIR			
Tank	Temp	Prey	mean	s.e.	mean	s.e.		df	MS	F	p	df	MS	F	p
A	High	Bra	2.84	0.37	1.71	0.22	Temp.	1	5.57	5.58	*	1	0.25	2.72	ns
A	High	Myt	6.45	0.43	0.17	0.01	Prey	1	78.91	79.09	**	1	41.67	454.99	***
B	Low	Bra	2.48	0.27	1.49	0.16	tempXprey	1	1.73	1.74	ns	1	0.025	0.27	ns
B	Low	Myt	5.15	0.33	0.14	0.01	Residuals	28	0.997			28	0.091		

SNK test showed a significantly higher ADFR on *M. minimus* at T High compared to T Low, while the ADFR on *B. pharaonis* was not significantly different between the two temperatures (Fig. 6a). The ADFR always resulted higher on *M. minimus* compared to *B. pharaonis* (Table 3). A non-significant effect of temperature (ANOVA p > 0.05; Table 3) was detected on the average daily ingestion rate (ADIR), but prey elicited significant differences in ADIR (ANOVA p < 0.001; Fig. 6b; Table 3). A significantly higher ADIR was evidenced when *Stramonita* fed on *B. pharaonis* compared to *M. minimus* in both tanks (A-B) (Table 3).



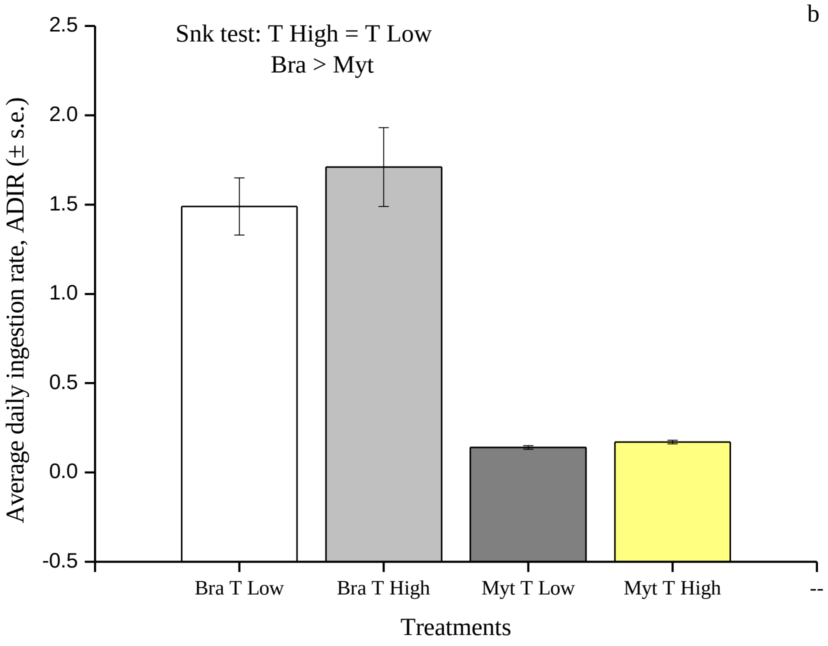


Fig. 6 (a-b) Predation rate experiment on two prey species with different temperatures: (a) Average daily feeding rate (ADFR); (b) average daily ingestion rate (ADIR).

4 DISCUSSION

The modification of phenology (Duarte, 2007) and reproductive failure (Helmuth et al., 2014; Montalto et al., 2014, 2016) are the two most important repercussions of temperature change in a context of CC claimed to have a strong effect on the persistence of local populations over time. Here, we showed that *S. haemastoma* consumption rates of two different bivalves were affected by increased temperature. This is consistent with what has already been shown by other authors when whelks fed on oyster spat (*C. virginica*; Garton and Stickle, 1980). On the contrary, temperature did not directly influence growth rate: the ~2°C higher temperature condition based on a projected future climate change scenario (IPCC, 2014; Paris COP21) was not sufficient (or not maintained for sufficient time) to significantly affect the growth rates of our whelks. However, this result may be consistent with the theory that assumes ectotherms follow the Von Bertalanffy growth function (VBGF), where the asymptotic length in VBGF does not strictly depend on body temperature (Kooijman, 2010). This supports the concepts that ectotherms such as gastropods and bivalves, living under different body temperature conditions (as our experimental animals did), should not record significant deviations from natural common patterns (as those at ambient temperature did) in reaching the maximum/asymptotic size. In contrast, theory would suggest that the maximum body size of an individual is dependent on energy allocated to growth and maintenance requirements, and that it is directly dependent on food characteristics (i.e. quality and density). Such a fact will have consequences on the approaching velocity to the VBGF asymptotic size. Thus, the time needed to reach maturity (if the amount of energy is sufficiently larger than that necessary for somatic and reproductive maintenance) will be influenced. In indeterminate growing ectotherms, maximum length is directly linked to the absolute reproductive output (i.e. Darwinian fitness), and then food quality and quantity may become main determinants of fitness of our investigated species. That growth rate can be directly linked with food value and density has been shown in many studies (Walne, 1963; Verity and Villareal, 1986; Kawamura et al., 1998), in particular when food is supplied *ad libitum* under experimental conditions. The diet relied on by the Lessepsian species promoted the most rapid growth and achievement of egg production, while growth of whelks relying on the indigenous *M. minimus* was slower and the energy ingested was not sufficient to reach reproduction; temperature did not influence these processes. Although a previous study showed a similar energetic value of flesh for g^{-1} for the two prey species (Giacoletti et al., 2016), the differences in the amount of edible tissue per individual were, on average, of such a magnitude to promote the Lessepsian mussel as the most profitable prey with respect the native bivalve. All whelk morphometric variables measured here followed the same increasing pattern as they were significantly influenced by the prey eaten rather than temperature. The overall beneficial effect of the alien species diet on whelk performance is also testified by the condition index (CI). CI estimates were higher in specimens that actively fed on the alien rather than the native species, although a significant influence of temperature was not always evident. The analysis of allometric coefficients (b) further corroborated this, even though it revealed a kind of masked effect of the higher temperature treatment on mass-length ratios, not previously captured by other growth descriptors. Further research is needed to investigate this phenomenon, which could be inconsistent with what has been previously observed and with theory. Most whelks fed with *B. pharaonis* (38% and 50% at normal temperature and high temperature, respectively) released egg capsules. In contrast, not surprisingly, whelks fed with the native species showed a massive reproductive failure (100% of individuals never produced eggs). At both temperatures, it appeared that whelks, particularly those who fed upon the Lessepsian mussels, started to divert energy from structure allocation (shell and flesh) towards reproduction as the temperature approximated to that of the reproduction threshold (~21-22°C). This agrees with recent bioenergetics (e.g. Kooijman, 2010; Sarà et al., 2014) as also seen in other gastropods *Littorina keenae* (Chow, 1987). Reproductive failure was evident in those whelks fed on the natives and this was likely due to the lower food value of that species, which might be assumed to be sub-optimal (*sensu* Krebs and Davies, 1997) in the diet of our whelks. Consequently, in sites with no Lessepsian bivalves, whelks in nature probably prey on more than one species, selecting each time the more profitable item, using a strategy of trophic integration to outstrip the intrinsic energetic limitations of a diet based exclusively on *M. minimus*. While bioenergetic theory reports that size at the maturity is fixed (Kooijman, 2010), current research shows that prey promoting more rapid growth may be able to induce an anticipation of the age at the first reproduction; the more optimal the prey, the quicker the first maturity size is reached. In addition, a better diet should influence the so-called maternal effect that in whelks may comprise more and larger egg capsules (not measured in this study, but see Palmer, 1983). Our results are consistent with this theory as the whelks we studied, under a higher temperature relying on the alien species, anticipated a number of days the beginning of egg production and with the observation on the planktonic larval duration time in fish and invertebrates. The planktonic larval duration time was shorter in the case of increasing temperature affecting the dispersal distance and optimal trophic conditions for the young stage (Duarte, 2007; O'Connor et al., 2007). Increased temperatures produced a similar effect in our whelks that may be subjected to similar effects influencing the ability of local populations to assure the best trophic conditions for new juveniles (O'Connor et al., 2007) with direct implications for population structure and then local diversity.

5 CONCLUDING REMARKS

Our experiments allowed us to investigate how a novel prey may result as optimal in the diet of a predator, and even if through a borderline descriptor (allometric slopes) increased temperature showed a side effect in only a few months of exposure. Such observations should further raise awareness on the possible role of multiple stressors (here alien vs. increasing temperature; Vye et al., 2015) in reinforcing the effect of increasing temperature on life history traits of marine invertebrates. This means that a possible climate change scenario involving the warming of sea surface temperatures by ~2°C (IPCC, 2014), and the presence of a richer diet represented by an alien species, may alter the natural common temporal schemes of physiological processes and biotic interactions (Blois et al., 2013). In this context, the food acquisition process may represent a key point allowing the organismal energetic balance (Kooijman, 2010). Overall, the alien species, *B. pharaonis*, provided an extra amount of energy compared to the native mussel, *M. minimus*, at the same time promoting the fastest growth and release of egg capsules during the whelk's reproductive period, while none of the individuals feeding on *M. minimus* did the same. Altogether, these results appear to demonstrate that *M. minimus* will not be able to satisfy energetic requirements of whelks in a global climate change scenario, while the alien species will be.

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CHAPTER 3

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Research article

Predicting the effectiveness of oil recovery strategies in the marine polluted environment

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ABSTRACT

Many recent studies have focused their attention on the physiological stress experienced by marine organisms in measuring ecotoxicological responses. Here we suggest a new approach for investigating the effects of an anthropogenic pollutant on Life-History (LH) traits of marine organisms, to provide stakeholders and policy makers an effective tool to evaluate the best environmental recovery strategies and plans. A Dynamic Energy Budget (DEB), coupled with a biophysical model was used to predict the effects of a six-month oil spill on *Mytilus galloprovincialis'* LH traits and to test two potential recovery strategies in the central Mediterranean Sea. Oxygen consumption rates were used to check for increasing energetic maintenance costs $[\dot{p}_M]$ respectively in oil-polluted system treatments (~76.2%) and polluted systems with physical (nano-bubbles ~32.6%) or chemical treatment (dispersant ~18.4%). Our model outputs highlighted a higher growth reduction of intertidal compared to subtidal populations and contextually an effect on the reproductive output and on the maturation time of this latter. The models also enabled an estimation of the timing of the disturbance affecting both the intertidal and subtidal populations' growth and reproduction. Interestingly, results led to the identification of the chemical dispersant as being the best remediation technique in contexts of oil spill contamination.

Key words: DEB model, *Mytilus galloprovincialis*, Oil pollution, Remediation, Mediterranean sea, Good environmental status.

1 INTRODUCTION

The need to investigate and predict the possible effects of anthropogenic pollutants on natural and managed ecological systems is one of the most pressing challenges facing science today. Oil spills represent one among the worst risks for marine biodiversity due to high oil container traffic (REMPEC, 2008; Portopia, 2016; Zodiatis et al., 2016) and the number of oil drilling platforms often crowding semi-enclosed seas, such as the Mediterranean basin (Mangano and Sarà, 2017). The list of unexpected oil spill accidents in the last decade is long (https://en.wikipedia.org/wiki/List_of_oil_spills) and there is a pressing urgency to deepen the potential effects on marine biota on both short (days) and mid-terms (months). Thus, studies of oil spill impact and the possible quick-intervention recovery techniques using chemical-physical compounds and/or mechanisms (e.g. oil skimmer, boom floating, sorbent and dispersant) on the coastal biodiversity should be encouraged in basins that harbour large biodiversity and are particularly vulnerable to unexpected extreme acute pollution events (Mangano and Sarà, 2017). Once spilled, oil often reaches and accumulates on coastal intertidal habitats (De la Huz et al., 2005), the zone between the high- and low water marks, which is recognised worldwide as crucial in providing ecosystems goods and services (Sarà et al., 2014a) but highly threatened by human activities (Barber et al., 1995; Ansari and Ingole, 2002; Orbea et al., 2006; Xia and Boufadel, 2010). Mangroves, lagoons, salt lakes, ponds, rocky shores and pools - where the worldwide marine biodiversity concentrates (Danovaro and Pusceddu, 2007) – become potential targets as already happened in the last decade (Mexico, 2010; Philippines, 2013; Bangladesh, 2014; India, 2017). As a main consequence, investigating the potential effect of oil spills on biodiversity and the degree of recovery needed could increase our understanding of how these detrimental and extreme events can be absorbed by biota. Recovery would need to include varying strategies that use chemical-physical compounds and/or mechanism such as oil skimmer, boom floating, sorbant and dispersant methods. To disentangle the effect of oil on biodiversity is likely to be difficult because of the complexity and heterogeneity of species' responses to environmental change and the choice to perform experimental studies on sentinel organisms is historically preferred by scientists (Rice et al., 1979). Since the dawn of ecological marine scientific research, marine bivalves - and more specifically mussels - have been widely used as 'sentinel' to monitor the wide spectrum of pollution effects on biological responses (e.g. 'Mussel Watch' monitoring programs). Being habitat-forming species – HFS, mussels can be easily adopted to infer on the likelihood of associated biodiversity loss (Widdows and Donkin, 1992; Widdows et al., 1995; Salazar and Salazar, 1996; Serafim et al., 2008; Sarà et al., 2013a, 2014a). Mussels can survive in the presence of both moderate trophic enriched conditions (i.e. suspended chlorophyll-a concentration around 1 μg l^{-1} and beyond; Sarà et al., 2011b) and high pollution levels (Halldórsson et al., 2005) buffering the human-driven biodiversity loss and recording changes in the environmental quality status of aquatic habitats at local scale (Cajaraville et al., 1996). For all these reasons, mussels have been widely used as indicators of environmental pollution (Phillips, 1976) and adopted as model organisms for physiological, genetic, toxicological and ecological studies (Smolders et al., 2003; Luedeking and Koehler, 2004; Halldórsson et al., 2007; Moore et al., 2006; Browne et al., 2008). This is also testified by the growing interest in the biological monitoring role of these sessile filter feeders, recently included in the European Marine Strategy Framework Directive (MSFD, Descriptor 9, EU 2008; Gorbi et al., 2008; Scarpato et al., 2010; Andral et al., 2011) thus recognised useful site-specific bio-indicators to meet the EU Good Environmental Status (GES). The effect of pollutants on bivalves has been frequently assessed by using the Scope for Growth (SFG) approach (Widdows and Staff, 2006; Mubiana and Blust, 2007) which allowed essentially to gain a static snapshot of the current physiological status of target organisms (Widdows et al., 1995). The success of SFG (Sobral and Widdows, 1997; Sarà et al., 2000, 2008; Widdows and Staff, 2006; Halldórsson et al., 2007; Sarà and Pusceddu, 2008; Ezgeta-Balic et al., 2011) was based on the provision of an instantaneous measure of the energy status of these key-species which was used as an indicator of the 'health' of the ecosystem (Thompson and Bayne, 1974; Widdows et al., 1995; Kearney, 2012). Nevertheless, SFG did not maximize the mechanistic power of a bioenergetic approach when assessing the bottle-necks in the energy flow from the environment to the organisms, neglecting a full translation of effects in terms of Life History (LH) traits (e.g. habitat body size, spawning events and Darwinian fitness; Kearney, 2012). In contrast, most recently developed bioenergetics frameworks, such as the mechanistic functional trait-based (FT) models, which rely on the Dynamic Energy Budget Theory (DEB; Kooijman, 2010; Sarà et al., 2014b), allow an easier spatially-explicit contextualisation of effects (Sarà et al., 2011a; Sarà et al., 2013a; Sarà et al., 2018a,b; Mangano et al., 2018) promising to trace new paths for future restoration strategies by predicting organismal functional traits and capturing variation across species (Pouvreau et al., 2006; Pecquerie et al., 2010; Lika et al., 2011; Sarà et al., 2011a; Kearney, 2012; Sarà et al., 2012; Sarà et al., 2013a, b; Sarà et al., 2018a, b; Mangano et al., 2018). The FT-DEB approach is based on flux of energy and mass through an organism (and not on a snapshot as in a context of the SFG approach), which is a traceable process being subject to conservation laws (Denny and Helmuth, 2009; Denny and Benedetti-Cecchi, 2012; Carrington et al., 2015). Here, an FT-DEB was spatially explicit-contextualised along the Sicilian coasts (Helmuth, 1998; Kearney et al., 2010; Sarà et al., 2011a; Sarà et al., 2012; Sarà et al., 2013a, b) in order to test the role of an acute contaminant exposure and of two recovery strategies: a commercial chemical dispersant and a nano-bubble generator (see Materials and Methods section for more details). The effects of the acute contaminant exposure and the two recovery strategies was tested on the LH traits of the blue Mediterranean mussel (*M. galloprovincialis* Lamarck, 1819), one among the most abundant filter feeders in both natural and human hard substrata (e.g. harbours, oil-drilling platform; Andaloro et al., 2011; Maggi et al., 2014; D'Alessandro et al., 2016; Mangano and Sarà, 2017; Mangano et al., 2017). The Sicilian waters were chosen as a target oceanographic area, which is a recognised biodiversity hotspot (Medail and Quezel, 1999) subject to high risk of accidental oil spill because it holds a central crossroad position in the Mediterranean which is the largest oil traffic route in the world (Galgani et al., 2011) and hosts the second largest oil container harbour in Europe (Augusta, Southern Sicily).

The outcomes presented and discussed are the resulting integration of an experimental and modelling study settled up to investigate the acute effect of an accidental oil spill exposure and of two possible bioremediation techniques on intertidal and subtidal mussels throughout their full life cycle. First, we compared the effects of an acute (48h) hypothetical oil spill along with that of an oil spill plus two potential recovery treatments on the mussels' energetic maintenance costs (as expressed in the DEB by $[\dot{p}_M]$ parameter and estimated as a metabolic extra-cost as measured by the oxygen consumption) and then measured the effect at individual level. Subsequently we introduced the measured effect by tweaking the $[\dot{p}_M]$ parameter in an explicit contextualised DEB model to investigate the potential implications in terms of i) maximum total shell length; ii) maximum wet weight; iii) reproductive outputs as expressed by the number of eggs produced; iv) time to reach sexual maturity; v) timing of disturbance.

Insights from the testing of the proposed remediation measures might inform policy makers and environmental technicians when assessing the best remediation techniques that would allow a quick recovery when a benthic population might be subjected to unexpected and acute pollution effects.

2 MATERIALS AND METHODS

2.1 Sampling and acclimation

Specimens of *Mytilus galloprovincialis* of commercial size (mean shell length=65.7 ± 3.8 mm) were collected in late September 2017 from an aquaculture plant located in Lake Faro (38° 15′ 59.95″ N; 15° 38′19.56″ E), on the north-eastern point of Sicily (Messina, Italy). As previously described elsewhere (Cappello et al., 2011), Gas Chromatography–Flame Ionization Detection (GC-FID) analysis was used to reveal the presence of chemicals in lake water (data not shown). Mussels were collected by hand and transported within 30 min to the Mesocosm Facility of IAMC-CNR of Messina (Italy; Cappello and Yakimov, 2010). The mussels were carefully cleaned and placed in a 200 l aquarium filled with natural seawater at room temperature (18–20 °C) with a field salinity (37–38), and fed ad libitum with cultured *Isochrysis galbana*. According to common experimental procedures successfully adopted in studying the bioenergetics of bivalves (Sarà et al., 2008; Ezgeta-Balic et al., 2011), the mussels were acclimated for two weeks to reduce stress generated by manipulation and transport; following that 48 organisms were tagged with a permanent marker and transferred to mesocosms.

2.2 Experimental set-up

The mussels were housed in eight mesocosms of 120 L capacity to allow double replication (rectangular glass tanks 100 cm long, 30 cm deep, 40 cm wide), each filled with 100 L of natural seawater (Cappello et al., 2011) collected directly from the station “Mare Sicilia” (38° 11′ 43.54″ N, 15° 34′ 24.729″ E; Messina, Italy) by a direct pipeline from the sea (mean seawater temperature 20 ± 1 °C). Six mesocosms (indicated as OIL, OIL+D and OIL+B) were supplemented with 70 ml of Arabian Light Crude Oil (ENI Technology S.p.A; 900 mg l^{-1}) prepared as previously indicated elsewhere (Cappello et al., 2006, 2007). A commercial dispersant (Bioversal 0.1% vol/vol$_{OIL}$, BIOECOTECH s.r.l.) was added to mesocosms OIL+D, while mesocosms OIL+B were equipped with a commercial system for continuous nano-bubble generation (OxyDoser™ PUREair, Oxydoser USA). Two mesocosms without any addition of crude oil, dispersant and/or nano-bubble generator were used as a pristine control (CTRL). All treatments lasted 48 h.

2.3 Respiration rate

Oxygen consumption rates were determined as a proxy for stress effect and in order to determine the consequent alteration of the energetic cost of maintenance [ṗ$_M$] (expressed as J cm^{-3} h^{-1}) after oil exposition. Oxygen consumption rates were measured within respirometric glass chambers (0.3 L) in a temperature-controlled water bath, filled with air-saturated filtered seawater, and stirred with a magnetic stirrer bar beneath a perforated glass plate (e.g. small Petri dish with holes) that supported each individual (Sarà and Pusceddu, 2008; Ezgeta-Balic et al., 2011). The decline in oxygen concentration was measured with a calibrated oxygen fibreglass sensor connected to a data logger (PiroScience Firesting O2) capable of four sensor connections. A total of n=48 mussels were used, acclimated as above and fed *ad libitum* until the day before the experiment. The decline of four animals for each session was continuously recorded for at least 1 h, excluding an initial period of ~10 min, characterised by a more rapid decline in oxygen caused by a disturbance of the sensor's temperature equilibration. Respiration rate (RR, μmol O2 h−1) was calculated as:

$$RR = (C_{t0} - C_{t1}) x\, Vol_r x 60 (t_1 - t_0)^{-1}$$

according to (Sarà et al., 2008, 2013a; Ezgeta-Balic et al., 2011), where C_{t0} is the oxygen concentration at the beginning of the measurement, C_{t1} is the oxygen concentration at the end of the measurement, and Vol_r is the volume of water in the respirometric chamber.

2.4 Model description

According to the κ-rule (DEB theory; Kooijman, 2010) a fixed fraction (κ) of energy inside each organism is allocated to growth and somatic maintenance, while the remaining fraction (1 - κ) is allocated to maturity maintenance plus maturation or reproduction. If the general environmental condition deviates from common natural patterns (i.e. changes in temperature, food availability etc.) reproduction and growth are consequently reduced. A reduction in growth can be caused either by reduced food assimilation [ṗ$_A$], enhanced maintenance costs [ṗ$_M$], or enhanced growth costs [ṗ$_G$]. Using this approach, and through DEB parameters derived from (Sarà et al., 2011a), we simulated growth and reproduction of our model species, except for the variation in the maintenance costs [ṗ$_M$] experimentally estimated through this study. The idea of quantitatively assess the effect of a stressor including it as a modification of a specific parameter was first introduced by Jager et al. (2016) with the so-called stress factor “s” applied to assimilation, maintenance and costs of growth. Here, oxygen consumption rates were used to derive the quantitative percentage effect of a stressor by comparing metabolism of control and stressed organisms, and then by summing/subtracting this value to the [ṗ$_M$] parameter of *M. galloprovincialis* (Sarà et al., 2011a). To run DEB simulations local thermal series of selected sites were used, obtaining a first model with environmental conditions. A second model was run with the [$\dot{p}_M$] calculated from the oxygen consumption rate measurements on *M. galloprovincialis* specimens.

2.5 Water temperature data and chlorophyll-a

The main forcing driver of shellfish LH inside DEB models is represented by mean seawater temperature (Pouvreau et al., 2006; Kearney et al., 2010; Kooijman, 2010; Sarà et al., 2011a). Thus, to run DEB simulations we used both intertidal and subtidal conditions (body temperature was expressed by the mean seawater temperature; Montalto et al., 2014) with four-years hourly data (January 2006–December 2009) of seawater temperature measured about 1m below the surface at the closest meteo-oceanographic station held in 4 sites: Catania (LAT 37° 29′ 53.09″ N; LONG 15° 05′ 37.77″ E), Lampedusa (Pelagie Islands, Agrigento: LAT 35° 29′ 59.38″ N; LONG 12° 36′ 15.98″ E), Palermo (LAT 38° 07′ 17.08″ N; LONG 13° 22′ 16.79″ E) and Porto Empedocle (Agrigento; LAT 37° 17′ 08.72″ N; LONG 13° 31′ 36.74″ E). Data are available online from the Italian Institute of Environmental Research (ISPRA) web page (http://www.mareografico.it/). These sites were chosen in order to predict the effects of an oil spill around Sicilian coasts. The period of 4 years was chosen because it is consistent with the normal life span of most shellfish (Sarà et al., 2012, 2013b). Chlorophyll-a (CHL-a; μg L^{-1}), usually derived from satellite imageries, is widely adopted inside DEB models as a reliable food quantifier for suspension feeders (Kearney et al., 2010; Sarà et al., 2011a, 2012; 2013b, 2014b; 2018a, b; Mangano et al., 2018). Although the availability of satellite CHL-a data (EMIS website, http://emis.jrc.ec.europa.eu/) for the considered 4-year thermal dataset, our simulations were run with an average amount of food, imposing a value of 0.5 μg L^{-1} CHL-a to all models. While this condition might appear unrealistic (Miller et al., 2011), for the purpose of this study it will allow the exclusion of the effect of environmental variability of food conditions (CHL-a).

2.6 Effects on Life-History traits of bivalves

We answered to the following questions: 1) What is the effect of oil or of the two tested remediation techniques (dispersant and nano-bubbles) on the LH-traits of bivalves? and 2) Will it affect both intertidal and subtidal populations in the same way? Thus we ran models using the experimentally-derived [$\dot{p}_M$]. Outputs of simulations were (Sarà et al., 2014b): the maximum theoretical total length of shellfish (TL), the maximum total weight (TW), the total number of eggs (TRO) produced during a life-span of 4 years, and the time needed to reach gonadic maturity (TM) for each treatment. The average of these outputs across four different sites intends to answer our first question.

2.7 Timing effect

One of the less investigated aspects across the current literature is the timing effect of disturbance, i.e. the effect of disturbance at specific points during the life span of the mussels. This has recently been emphasised as a crucial aspect in investigating the role of disturbance, as the timing effect combines with frequency and intensity giving unpredictable scenarios. Thus, to determine if a particular timing (Miller et al., 2011) of the disturbance affects the LH-traits of the bivalves, we introduced into the model an oil spill event lasting 6-months by tweaking the DEB $[\dot{p}_M]$ parameter with the experimentally-derived one. In order to ascertain whether it might have different effects during the life span of mussels, we introduced the “six-month disturbance effect” once at the beginning of each year, obtaining a total of 104 models, both for intertidal (52 models) and subtidal population (52 models).

2.8 Statistical analysis

In order to test for significant differences in experimentally-derived respiration rates, a one-factor ANOVA with four levels (CTRL, OIL, OIL+D and OIL+B) was performed. When significant differences were detected, the Student-Newman-Keuls (SNK) post-hoc pair wise comparison of means was used (Underwood, 1997). Cochran's test was used prior to ANOVA to test the assumption of homogeneity of variance (Underwood, 1997).

3 RESULTS

3.1 Respiration rate

Our results revealed significantly higher oxygen consumption rates from *M. galloprovincialis* specimens exposed to crude oil (OIL) (Table 1; ANOVA, $p < 0.01$). The SNK Test revealed significant differences between the OIL system and the control experiment (CTRL; no oil, no dispersant and no nano-bubbles) and in particular between OIL and OIL+D and between OIL and OIL+B. In contrast, no significant differences resulted between CTRL and OIL+D or CTRL and OIL+B. The rate of oxygen consumption measured was respectively ~82% higher (respect to CTRL) for the OIL, ~16% higher for the OIL+D and ~31% for OIL+B treatment (Fig. 1a). The measured rates caused an increase in the $[\dot{p}_M]$ of ~76% for the OIL, ~18% for the OIL+D and ~33% for OIL+B treatment (Fig. 1b).

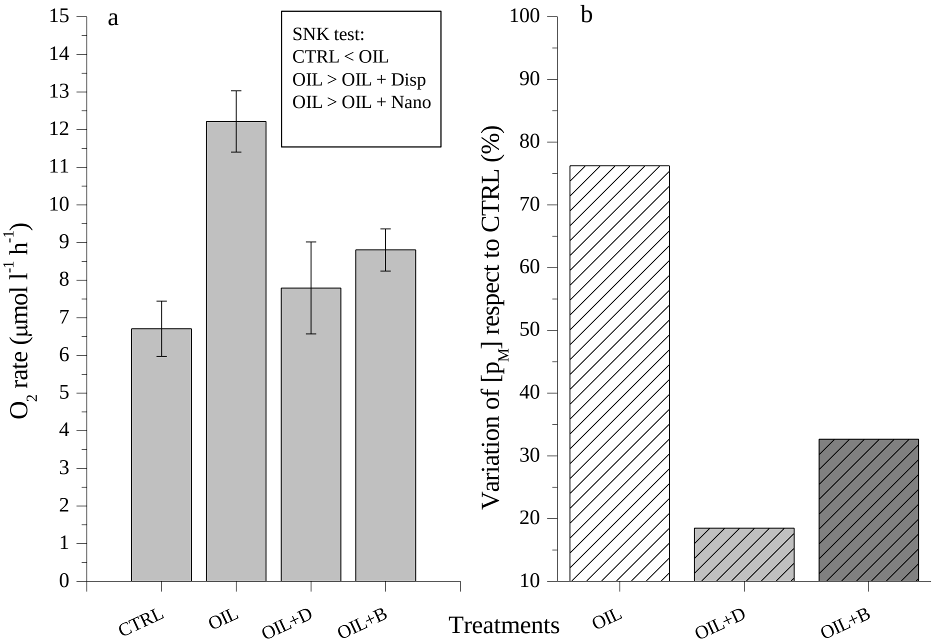

Figure 1 (a) Oxygen consumption rates (RR) of *Mytilus galloprovincialis* under different experimental conditions; **(b)** Effect of oil addition and recovery treatments on the energetic cost of maintenance $[\dot{p}_M]$, expressed in percentage in respect to control.

Table 1 ANOVA table of results: respiration rate (RR) of *Mytilus galloprovincialis*. (* = p<0.05; ** = p<0.01; *** = p<0.001; ns = not significant).

Source	df	RR		
		MS	F	P
Treatment (Tr)	3	45.43	7.58	***
Residuals	28	5.99		
Cochran's C				ns

3.2 Average effect on LH-traits

Outputs from model simulations with site-specific (Palermo, Catania, Porto Empedocle, Lampedusa) thermal series demonstrated a strong effect on the LH traits of intertidal and subtidal specimens of *M. galloprovincialis* (Table 2) compared to CTRL due to the hypothetical oil spill with a six-month duration.

Table 2. Average mean effect (%) of a contaminant (OIL) and of two different oil recovery strategies (dispersant and nanobubbles, OIL+D and OIL+B respectively) on LH-traits of intertidal and subtidal individuals of *M. galloprovincialis* following a six-month spillover in the central Mediterranean Sea. (TL = Total Length, WW = Wet Weight, TM = Time to Maturity, disp = dispersant, nano = nanobubbles).

Population	Treat	TL	WW	TRO	TM
Intertidal	OIL	-3.83	-10.47	-	3.76
Intertidal	OIL + D	-1.06	-2.99	-	0.81
Intertidal	OIL + B	-1.86	-5.16	-	1.48
Subtidal	OIL	-1.50	-4.39	-6.49	7.18
Subtidal	OIL + D	-1.11	-3.27	-4.20	1.46
Subtidal	OIL + B	-1.29	-3.80	-5.35	2.63

3.3 Effects on intertidal populations

Our simulations with the presence of crude oil (OIL) predicted an average reduction of ~3.8% in the total length (TL), and of ~10.5% in the total weight (TW) within the four sites. The treatment carried out with addition of dispersant (OIL+D) predicted a reduction of ~1% in the total length (TL), and of ~3% in the total weight (TW). The OIL+B treatment (presence of nano-bubble generator) predicted a ~2% reduction in the total length (TL) and a ~5.2% reduction in the total weight (TW). Outputs from simulation predicted a ~4% increase in the maturation time for the OIL treatment, followed by a ~1.5% for the OIL+B and 0.8 for the OIL+D treatment increase respectively (Table 2; Fig. 2). No reproductive events occurred in our intertidal simulation in the four sites (Catania, Lampedusa, Palermo, and Porto Empedocle). For full results see Supplementary Information Table S1 (Supplementary Data Chapter 3).

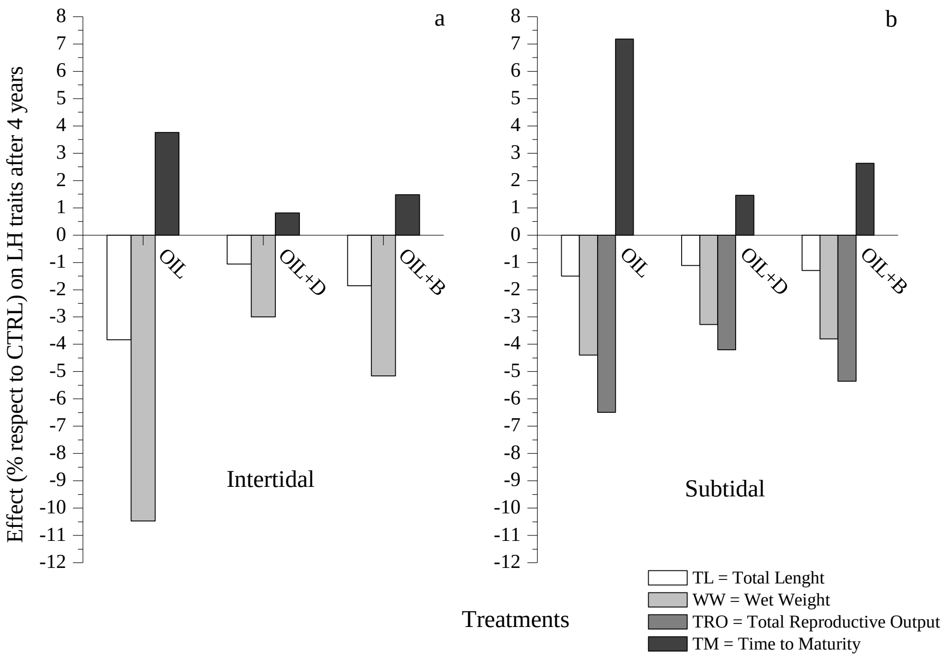

Figure 2 Average effects on intertidal and subtidal populations from DEB simulations expressed as percentage variation of LH-Traits such as Total Length (TL), Wet Weight (WW), Total Reproductive Output (TRO) and Time to Maturity (TM) in respect to CTRL.

3.4 Effects on subtidal populations

Simulations with presence of crude oil (OIL) predicted an average reduction of 1.5% in the total length (TL), and of ~4.4% in the total weight (TW) within the four sites. The OIL+D treatment predicted a 1.1% reduction in the total length (TL), and a ~3.3% reduction in the total weight (TW). The OIL+B treatment predicted a ~1.3% reduction in the total length (TL), and a ~4% reduction in the total weight (TW). Outputs from simulations predicted a ∼7% increase in the maturation time for the OIL treatment, followed by a ∼2.6% for the OIL+B and 1.5% for the OIL+D treatment increase respectively. The OIL treatment also predicted a 6.5% reduction in the number of eggs produced (total reproductive output, TRO), while the OIL+D and the OIL+B treatments predicted respectively a 4.2% and a ∼5.4% reduction (Table 2; Fig. 2). For full results see Supplementary Information Table S2 (Supplementary Data Chapter 3).

3.5 Timing effect of disturbance

Outputs from model simulations with a precise timing of the oil spill disturbance demonstrated a differing pattern of effect between intertidal and subtidal populations. Intertidal simulations showed that the oil addition (OIL mesocosms) determined an increasing negative effect on the total length (TL) from ∼0.9% in the first year to ∼6.4% in the fourth year, coupled with an increasing negative effect on the wet weight (WW) from ∼2.8% in the first year to ∼17% in the fourth year. The OIL+D treatment predicted an increasing reduction of the total length (TL) from 0.2% in the first year to 2% in the last year, coupled with an increasing reduction of the WW from 0.7% in the first year to 5.4% in the fourth year. The OIL+B treatment predicted an increasing reduction of the total length (TL) from 0.4% in the first year to ∼3.5% in the last year, coupled with an increasing reduction of the WW from ∼1.2% in the first year to ∼9.3% in the fourth year. No reproductive events occurred in our intertidal simulations but predictions reported the highest effect on the maturation time in correspondence with the second year, with an increase of 10%, 2.2% and 4.1% respectively with addition of crude oil (OIL) and two treatments (OIL+D, OIL+B) (Table 3; Fig. 3).

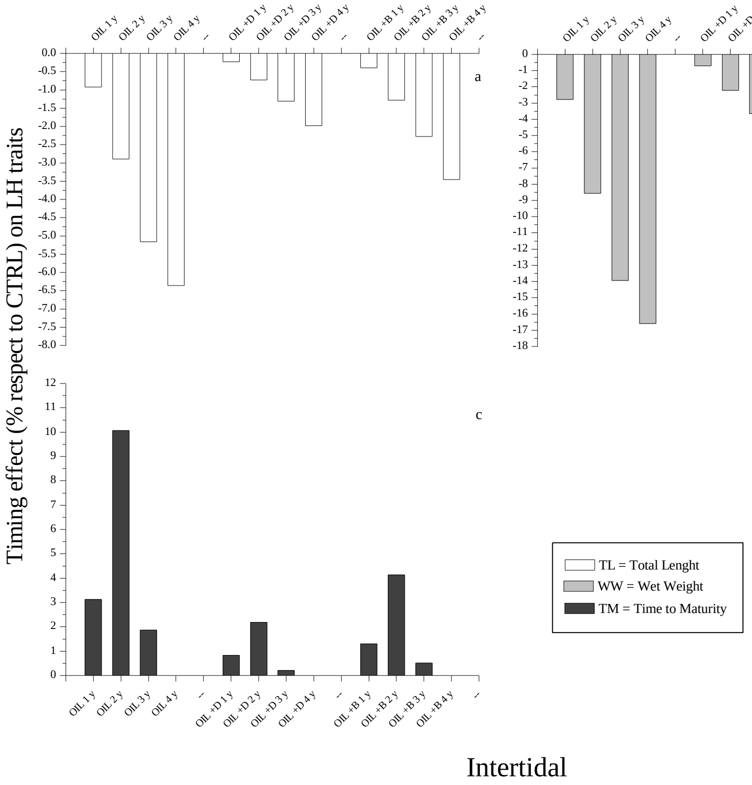

Figure 3 Timing effect on LH-Traits of intertidal populations from DEB simulations expressed as percentage variation of **(a)** Total Length (TL), **(b)** Wet Weight (WW) and **(c)** Time to Maturity (TM) in respect to CTRL.

Subtidal simulations showed that the crude oil addition (OIL) predicted an increasing negative effect on the total length (TL) from ~0.7% in the first year to ~1.7% in the fourth year, coupled with an increasing negative effect on the WW from ~2% in the first year to ~5% in the fourth year. The addition of dispersant (OIL+D treatment) predicted an increasing reduction of the total length (TL) from 0.2% in the first year to 1.7% in the last year, coupled with an increasing reduction of the WW from 0.5% in the first year to ~5% in the fourth year. The OIL+B treatment predicted an increasing reduction of the total length (TL) from 0.3% in the first year to ~1.7% in the last year, coupled with an increasing reduction of the WW from 0.9% in the first year to ~5% in the fourth year. In the system with only crude oil present (OIL) the total reproductive output (TRO) resulted between the second (−12%) and the third year (−8.5%). The OIL+D followed the same decreasing pattern (respectively −5.6 and −8.5%) as well as the OIL+B treatment (−9.4 and −8.4% respectively). Our simulation reported the highest effect on the maturation time in correspondence with the first year, with a +28% (OIL), a +5.8% (OIL+D) and a +10.5% (OIL+B) (Table 3; Fig. 4).

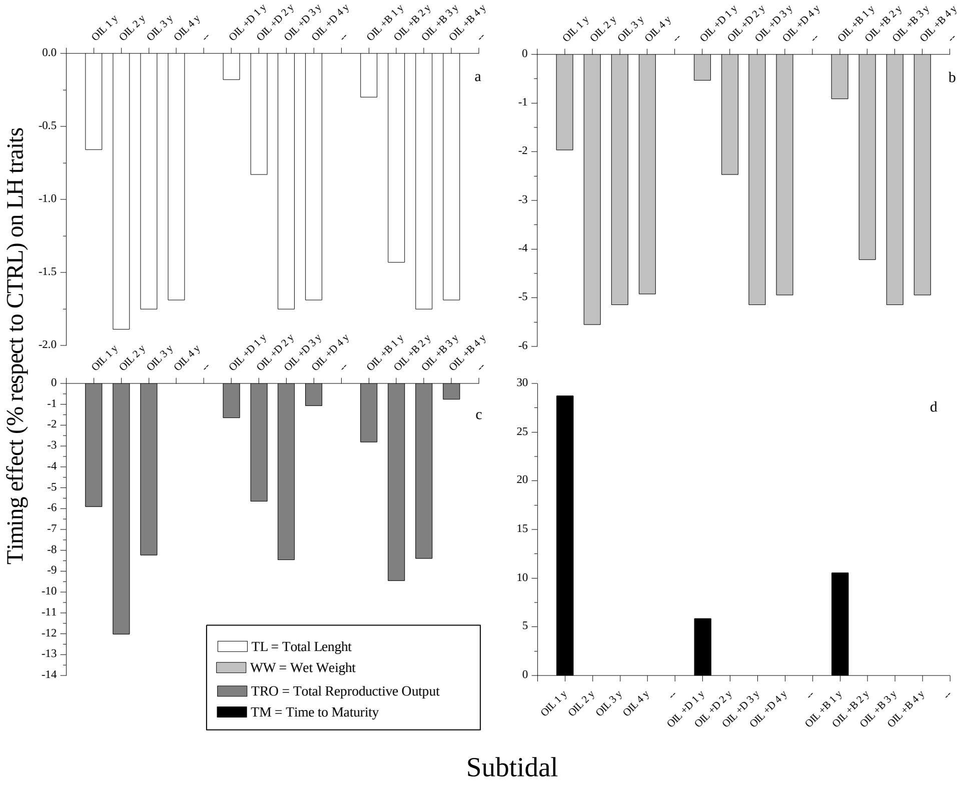

Figure 4 Timing effect on subtidal populations from DEB simulations expressed as percentage variation of **(a)** Total Length (TL), **(b)** Wet Weight (WW), **(c)** Total Reproductive Output (TRO) and **(d)** Time to Maturity (TM) in respect to CTRL.

Table 3. Timing effect (%) of a contaminant (OIL) and of two different oil recovery strategies (dispersant and nanobubbles, OIL+D and OIL+B respectively) on LH-traits of intertidal and subtidal individuals of *M. galloprovincialis* following a six-month spillover in the central Mediterranean Sea. (TL = Total Length, WW = Wet Weight, TM = Time to Maturity, disp = dispersant, nano = nanobubbles).

Population	**Treat**	**TL**	**WW**	**TM**	**Population**	**TL**	**WW**	**TRO**	**TM**
Intertidal	OIL 1 y	-0.92	-2.77	3.13	Subtidal	-0.66	-1.96	-5.9	28.71
Intertidal	OIL 2 y	-2.89	-8.57	10.06	Subtidal	-1.89	-5.55	-12.01	0
Intertidal	OIL 3 y	-5.16	-13.93	1.87	Subtidal	-1.75	-5.14	-8.23	0
Intertidal	OIL 4 y	-6.36	-16.6	0	Subtidal	-1.69	-4.92	0.18	0
Intertidal	OIL +D 1 y	-0.23	-0.7	0.83	Subtidal	-0.18	-0.53	-1.65	5.84
Intertidal	OIL +D 2 y	-0.73	-2.21	2.19	Subtidal	-0.83	-2.47	-5.64	0
Intertidal	OIL +D 3 y	-1.31	-3.64	0.21	Subtidal	-1.75	-5.14	-8.45	0
Intertidal	OIL +D 4 y	-1.98	-5.4	0	Subtidal	-1.69	-4.94	-1.06	0
Intertidal	OIL +B 1 y	-0.4	-1.22	1.3	Subtidal	-0.3	-0.91	-2.8	10.53
Intertidal	OIL +B 2 y	-1.28	-3.84	4.13	Subtidal	-1.43	-4.22	-9.45	0
Intertidal	OIL +B 3 y	-2.28	-6.3	0.51	Subtidal	-1.75	-5.14	-8.39	0
Intertidal	OIL +B 4 y	-3.46	-9.29	0	Subtidal	-1.69	-4.94	-0.75	0

4 DISCUSSION

Integrated modelling and experimental studies based on mechanistic simulations, to predict the effects of an oil spill on *M. galloprovincialis* populations, have never been performed before in a Mediterranean context, suggesting the importance of this tool in helping the near future remediation technique move a step forward to become the most developed integrated-monitoring-studies already successfully applied (Gorbi et al., 2008; Gomiero et al., 2011a, b). Interestingly, Gomiero and co-authors have recently performed a novel algorithm aiming at drawing up indices to rank the different stages of pollutant-induced stress syndrome, helping to translate the biomarker data into an actual health risk index to suggest to environmental managers (Mussel Expert System, MES; Gomiero et al., 2015).

Here, in order to assess the impact on shellfish fecundity we introduced 4 years' worth of hourly data of the seawater, recorded in four sites, with an average value of food, in order to exclude the interference derived from the natural resource variability. No reproductive events came out of most of our intertidal simulations, probably due to food limitations and temperature threshold. Although *M. galloprovincialis* is an autochthonous Mediterranean species, it is more frequent throughout the northern coasts of the Basin (Northern Tyrrhenian, Adriatic), while in the southern waters, it is represented by a patchy distribution except for highly eutrophic (e.g. Augusta Bay, Southern Sicily, Italy) or shallow waters (e.g. Bizerta Lake, Tunisia). A simulated six-month oil spill around Sicilian coasts through our models revealed a negative effect on growth and a stronger effect on the reproduction and the time needed to reach gonadic maturation. Model outputs revealed a twofold higher negative oil effect on the TL (−3.83 vs. −1.5%) and WW (−10.47 vs. −4.39%) of the intertidal populations in respect to subtidal ones. The latter were instead highly affected in the TRO (−6.49%) and in the TM (−7.18%). According to the DEB theory, when general environmental conditions deviate from common natural patterns, reproduction and growth can be consequently affected. Increasing maintenance costs as expressed by $[\dot{p}_M]$ is one among the most important cause driving the change in the growth and the reproductive performances of organisms. Here, we were able to demonstrate that both the remediation techniques, dispersant and nano-bubbles, showed not-significant differences in oxygen consumption rates. However, the calculation of the energetic cost of maintenance $[\dot{p}_M]$ indicates a lower increase for the OIL+D in respect to the OIL+B mesocosm (Fig. 1b) suggesting the dispersant as the best remediation technique. This was also confirmed by the model outputs, as long as they revealed a twofold higher negative OIL+B effect in TL (−1.86 vs.

−1.06%) and WW (−5.16 vs. −2.99%) in respect to the OIL+D treatment for intertidal populations. Outputs also revealed some effect of the OIL+B and OIL+D treatments on subtidal populations traits, such as TL (−1.29 vs. −1.11%) and WW (−3.8 vs. −3.27%), and a more important effect on TRO (−5.35 vs. −4.2) and on TM (−2.63 vs. −1.46). The gonadal development has previously been described in literature as one of the main proxy of increasing trends of heavy metal accumulation in proximity to oil platform extraction (Gomiero et al., 2011a; Mangano and Sarà, 2017). Moreover, the estimation of the fecundity potential of intertidal and subtidal populations, which is often omitted in other ecological studies, has great importance, both for ecological aquaculture and conservation management.

Our results can be explained by the faster physical effect of dispersant with respect to the slower nano-bubbles initial contribution. The dispersant is able to break down the slick of oil into smaller droplets and facilitates their transfer throughout the water column determining a rapid dilution reducing the oil exposure of the organisms. The ability to target the thickest part of the oil slick in a timely manner, before oil weathering reduce the efficiency of the action is one of the key success factors of dispersant application in oils spill response. The other fundamental advantage is their easiness of application through a variety of methods (e.g. spraying dispersant from vessels and small aircraft or helicopters) in the case of oil spills. In contrast, nano-bubbles tend initially to favor the oil water accumulation in the water upper layers by contrasting the mixing effects of the water turbulence. Over a longer period, the increased amount of oxygen and organic load at the surface can stimulate biodegradation activity of hydrocarbonoclastic bacteria (Ohnari, 1997; Moriguchi and Kato, 2002). Due to the limited duration of the mesocosm experiments, we assumed that the performance of the two applied techniques, in terms of reducing the availability of the contaminant to the organisms, was higher for the dispersant than nano-bubbles according to the faster physical effect of the first on the oil concentration. However, over a longer observation period, the efficiency of the two selected approaches should be more comparable due to the increased contribution of microbial activity. Nevertheless, recent research showed that in marine open water, the application of nano-bubbles may be not easily practicable due the “turbulence effect” which facilitate the dispersion. Thus the application of nano-bubbles should be mainly focused in closed basins or in oilywastewater treatment plants (Ohnari, 2001; Li and Tsuge, 2006).

4.1. Implications for biodiversity

Biodiversity is widely recognized as one of the fundamental provider of ecosystem services (Loreau et al., 2001; Tilman et al., 2014) affecting ecosystem productivity, nutrient cycling, stability and resistance to perturbations (Naeem et al., 1994; Smith et al., 2006; Mazancourt et al., 2013). On the other hand, anthropogenic disturbance is one of the most powerful driver capable to shape biodiversity (Miller et al., 2011) through a driving action on individual “life histories” (Denny et al., 2009; Sarà et al., 2018a). Looking at our case study, an oil spill can act not only at individual level but hierarchically, through the effect on hundreds of mussel-beds associated species (Suchanek, 1979), alter the ecosystem functioning in terms of provided good and services (Sarà et al., 2014a, b; Mangano and Sarà, 2017). Although modelling is an extremely specific tool, it is possible to generalize the effect of an oil spill as largely dependent on species' mobility and the possibility of escaping toxicity (Loureiro et al., 2006), resulting in a higher vulnerability of sessile and aquaculture species. By tailoring this model to other model species in the future it may be possible to detect the cumulative effect of oil spill on specific sectors (e.g. fishery, aquaculture) and aquatic environments (e.g. marine, freshwater, saltmarsh). An expansion of the knowledge on the needed degree of recovery could increase our understanding of how oil spill detrimental and extreme events can be absorbed by biota. In the future, recovery would need to include integrated strategies more specific based on various remediation techniques (i.e. chemical-physical compounds and/or mechanism such as oil skimmer, boom floating, sorbant and dispersant methods) to adapt to species responses and preserve the associated biodiversity functioning.

5 CONCLUSIONS

Our simulations finally led also to predict the timing effect of the disturbance that demonstrated a differing pattern between intertidal and subtidal populations. From the comparison of model outputs, it has been demonstrated that the disturbance strongly affected the growth of the intertidal populations from the second to the fourth year, and maturation mostly in the first and second year. The disturbance instead acted on subtidal population with a lower intensity on growth across the period from the second to the fourth year, but with a higher intensity on maturation during the first year, affecting the reproduction mostly in the second and third year.

There are actually no data in the current literature regarding the timing of disturbance to refer to, or compare with, so this actually represent the first exercise to assess and predict the differing effects of a contaminant across the life-span of a model species through mechanistic models. Our pilot experiment, coupled with bioenergetics models predicting the growth and the potential fecundity of a model species proposes a new approach in testing, on a broad spatial and time scale, the effects of any anthropogenic pollutant, and as a potential tool that will lead stakeholders and policy makers to evaluate current, and propose future, remediation strategies to achieve the Good Environmental Status. On a small scale, this method could be easily applied even to a single drilling platform at sea, in order to investigate the potential impact on nearby coasts and to design in advance the most effective technique to be adopted in case of an environmental emergency or oil-spills during the platforms' maintenance and dismiss phases.

Supplementary Data Chapter 3. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2018.06.094

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Supplementary Data: Chapter 3

Table S1. Effects (%) of a contaminant (OIL) and of two different oil recovery strategies (dispersant and nanobubbles, OIL+D and OIL+B respectively; Treat = Treatment) on LH-traits of intertidal individuals of *M. galloprovincialis* following a six-month spillover in four different Mediterranean sites (CT = Catania, LAMP = Lampedusa, PA = Palermo, PEMP = Porto Empedocle). (TL = Total Length, WW = Wet Weight, TRO = Total Reproductive Output, TM = Time to Maturity).

Site	Treat	Frequency	Timing	TL	WW	TRO	TM
CT	oil	6-months	1 year	-0.98	-2.94	0	3.50
			2 year	-2.97	-8.73	0	10.24
			3 year	-5.02	-13.32	0	0.00
			4 year	-6.52	-16.95	0	0.00
	oil + D	6-months	1 year	-0.25	-0.75	0	0.99
			2 year	-0.75	-2.26	0	2.57
			3 year	-1.27	-3.49	0	0.00
			4 year	-1.94	-5.29	0	0.00
	oil + B	6-months	1 year	-0.43	-1.30	0	1.55
			2 year	-1.31	-3.92	0	4.47
			3 year	-2.22	-6.05	0	0.00
			4 year	-3.40	-9.10	0	0.00
LAMP	oil	6-months	1 year	-0.98	-2.94	0	4.35
			2 year	-3.03	-8.93	0	12.83
			3 year	-5.51	-14.59	0	0.00
			4 year	-7.23	-18.71	0	0.00
	oil + D	6-months	1 year	-0.25	-0.75	0	1.25
			2 year	-0.77	-2.28	0	2.39
			3 year	-1.40	-3.86	0	0.00
			4 year	-2.61	-7.06	0	0.00
	oil + B	6-months	1 year	-0.43	-1.30	0	1.83
			2 year	-1.35	-3.99	0	5.09
			3 year	-2.44	-6.67	0	0.00
			4 year	-4.54	-12.07	0	0.00
PA	oil	6-months	1 year	-0.87	-2.70	0	2.14
			2 year	-2.37	-7.21	0	6.02
			3 year	-4.09	-11.92	0	7.49
			4 year	-6.26	-16.44	0	0.00
	oil + D	6-months	1 year	-0.22	-0.67	0	0.37
			2 year	-0.59	-1.83	0	1.38
			3 year	-1.02	-2.97	0	0.85
			4 year	-1.57	-4.32	0	0.00
	oil + B	6-months	1 year	-0.38	-1.18	0	0.78
			2 year	-1.04	-3.20	0	2.55

			3 year	-1.79	-5.20	0	2.03
			4 year	-2.75	-7.47	0	0.00
PEMP	oil	6-months	1 year	-0.84	-2.51	0	2.51
			2 year	-3.20	-9.43	0	11.15
			3 year	-6.02	-15.88	0	0.00
			4 year	-5.43	-14.32	0	0.00
	oil + D	6-months	1 year	-0.21	-0.64	0	0.71
			2 year	-0.82	-2.45	0	2.42
			3 year	-1.53	-4.22	0	0.00
			4 year	-1.80	-4.93	0	0.00
	oil + B	6-months	1 year	-0.37	-1.11	0	1.05
			2 year	-1.42	-4.25	0	4.40
			3 year	-2.67	-7.28	0	0.00
			4 year	-3.15	-8.50	0	0.00

Table S2. Effects (%) of a contaminant (OIL) and of two different oil recovery strategies (dispersant and nanobubbles, OIL+D and OIL+B respectively; Treat = Treatment) on LH-traits of subtidal individuals of *M. galloprovincialis* following a six-month spillover in four different Mediterranean sites (CT = Catania, LAMP = Lampedusa, PA = Palermo, PEMP = Porto Empedocle). (TL = Total Length, WW = Wet Weight, TRO = Total Reproductive Output, TM = Time to Maturity).

Site	Treat	Frequency	Timing	TL	WW	TRO	TM
CT	oil	6-months	1 year	-0.57	-1.68	-12.75	27.25
			2 year	-1.45	-4.25	-16.97	0.00
			3 year	-1.45	-4.23	-4.87	0.00
			4 year	-1.44	-4.10	0.19	0.00
	oil + D	6-months	1 year	-0.16	-0.46	-3.66	4.09
			2 year	-0.74	-2.17	-8.99	0.00
			3 year	-1.45	-4.23	-5.52	0.00
			4 year	-1.44	-4.17	-0.02	0.00
	oil + B	6-months	1 year	-0.27	-0.79	-6.17	7.94
			2 year	-1.26	-3.70	-14.83	0.00
			3 year	-1.45	-4.23	-5.36	0.00
			4 year	-1.44	-4.15	0.03	0.00
LAMP	oil	6-months	1 year	-0.64	-1.91	-3.39	29.21
			2 year	-2.15	-6.29	-10.91	0.00
			3 year	-1.80	-5.28	-9.19	0.00
			4 year	-2.11	-6.18	0.06	0.00
	oil + D	6-months	1 year	-0.17	-0.51	-0.90	7.97
			2 year	-0.84	-2.48	-4.37	0.00
			3 year	-1.80	-5.28	-9.19	0.00
			4 year	-2.11	-6.19	-1.09	0.00
	oil + B	6-months	1 year	-0.29	-0.87	-1.55	13.94
			2 year	-1.44	-4.25	-7.43	0.00
			3 year	-1.80	-5.28	-9.19	0.00
			4 year	-2.11	-6.19	-0.81	0.00
PA	oil	6-months	1 year	-0.67	-1.99	-3.48	27.04
			2 year	-1.97	-5.79	-9.96	0.00
			3 year	-1.83	-5.38	-9.27	0.00
			4 year	-1.83	-5.38	-0.45	0.00
	oil + D	6-months	1 year	-0.18	-0.53	-0.93	5.25
			2 year	-0.81	-2.39	-4.17	0.00
			3 year	-1.83	-5.38	-9.27	0.00
			4 year	-1.83	-5.38	-2.11	0.00

	oil + B	6-months	1 year	-0.31	-0.91	-1.61	10.16
			2 year	-1.39	-4.09	-7.09	0.00
			3 year	-1.83	-5.38	-9.27	0.00
			4 year	-1.83	-5.38	-1.70	0.00
PEMP	oil	6-months	1 year	-0.76	-2.25	-3.99	31.34
			2 year	-1.99	-5.85	-10.20	0.00
			3 year	-1.93	-5.67	-9.60	0.00
			4 year	-1.37	-4.03	0.94	0.00
	oil + D	6-months	1 year	-0.20	-0.61	-1.10	6.06
			2 year	-0.96	-2.84	-5.03	0.00
			3 year	-1.93	-5.67	-9.81	0.00
			4 year	-1.37	-4.04	-1.02	0.00
	oil + B	6-months	1 year	-0.35	-1.05	-1.87	10.09
			2 year	-1.64	-4.83	-8.47	0.00
			3 year	-1.93	-5.67	-9.75	0.00
			4 year	-1.37	-4.04	-0.54	0.00

CHAPTER 4

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Predicting effective aquaculture in subtropical waters: A dynamic energy budget model for the green lipped mussel, *Perna viridis*

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ABSTRACT

The green lipped mussel, *Perna viridis*, is an important aquaculture species throughout the Indo-Pacific region where production is often impacted by environmental degradation. To predict the impacts and mitigate against environmental problems due to various kinds of anthropogenic pollution, such as heavy metals and eutrophication, on *P. viridis* aquaculture a Dynamic Energy Budget (DEB) model was constructed. By integrating species-specific parameters and regional-specific environmental data the DEB model determined how the life history traits of *P. viridis* respond to changing environmental conditions. Using various levels of basal maintenance costs and food availability to elucidate the energetic costs due to environmental pollution, the DEB model predicted that a 20% increase in basal maintenance cost due to environmental pollution such as heavy metals will result in a subsequent decrease in both lifetime reproductive output and ultimate body size of *P. viridis* by ~18% and ~8%, respectively. Increasing food availability can, however, mitigate the energetic constraints due to increased basal maintenance cost. The time to reach commercial size, for example, will be longer by 13% and 3% under lower and higher food availability conditions, respectively when there is a 20% increase in maintenance cost due to environmentally induced stress, which would significantly increase the operational cost of an aquaculture facility. In light of the increased importance of *P. viridis* as an aquaculture species, the *P. viridis* DEB model can, therefore, be used to illustrate the effects of varying environmental conditions on *P. viridis* life history traits which are relevant to the success of aquaculture facilities, and contribute towards better management of this species.

Key words: DEB model, *Mytilus galloprovincialis*, Oil pollution, Remediation, Mediterranean sea, Good environmental status.

1 INTRODUCTION

Marine bivalves are important both in terms of their ecological roles and increasingly in terms of being aquaculture species (Gutiérrez et al., 2003). Shellfish aquaculture is becoming a more important component of the world's food supply (FAO, 2016), given the high nutrient content of bivalves (Gopalakrishnan and Vijayavel, 2009). The green lipped mussel, *Perna viridis*, which is widely distributed throughout the Indo-Pacific region (Sidall, 1980), is among the most important aquaculture species in Southeast Asian countries (Rajagopal et al., 2006) with a 43% increase (~650,000 t) in production over the past two decades (FAO, 2015). A major concern for the future of this industry in Southeast Asia is the impact of coastal water degradation, as a result of multiple anthropogenic activities at both local and regional scales (Williams et al., 2016; Martinez et al., 2018; Sarà et al., 2018), which impact the viability and hence production of aquaculture species (Handisyde et al., 2006).

Mussel farming is often operated along coastal areas which are susceptible to anthropogenic pollution such as heavy metals and eutrophication, which impact the commercial production of shellfish resources (Chalermwat et al., 2003; Cheevaporn and Menasveta, 2003; Siah et al., 2003; Islam and Tanaka, 2004; Casas and Bacher, 2006). Given the increasing demand for shellfish production (FAO, 2015), understanding how environmental changes may affect a species' life history (LH) traits such as ultimate size and total egg production is crucial when designing aquaculture management strategies (Béjaoui-Omri et al., 2014; Rinaldi et al., 2014). The success of aquaculture practice will be dependent on environmental characteristics which fundamentally affect growth of species, which is characteristically the most important trait to determine profitability of such practices (Sarà et al., 2018). Site selection for optimal environmental conditions for shellfish culture is, therefore, a key step to optimize profit and minimize the environmental impacts brought by the industry (Longdill et al., 2008).

Although the physiology of *Perna viridis* has been well studied (reviewed by Rajagopal et al., 2006), such information is based on short term experiments under specific environmental conditions. In reality, however, organisms live in a multi-dimensional ecological space (*sensu* Hutchinson, 1957), including the complex interactive influences of various environmental factors which can be additive, synergistic or antagonistic (Gunderson et al., 2016; Sarà et al., 2018). It is, therefore, difficult to disentangle the effects of environmental changes on organisms when there is no integration between how multiple environmental factors affect species' functional traits such as physiology (Howard et al., 2013; Todgham and Stillman, 2013), energy acquisition and allocation (Smolders et al., 2005; Sokolova, 2013), all of which are needed to predict consequences to LH traits (e.g., growth and reproduction; Petes et al., 2007, 2008). Physiological studies are generally unable to integrate these effects and, therefore, limit our ability to predict the responses of species to varying environmental conditions. Dynamic Energy Budget theory (DEB; Kooijman, 2010) has been developed to provide an integrative approach to link basal physiological information with environmental conditions, in a mechanistic bioenergetic framework. In essence, the DEB model captures the processes of energy acquisition and utilization in an organism under varying environmental conditions based on First Principles (Nisbet et al., 2000; Van der Meer, 2006; Filgueira et al., 2011) as applied to a suite of parameters that describe the physiology responses of a species to environmental variation. The DEB model, therefore, integrates the energy allocation strategies adopted by species, and allows subsequent prediction of LH traits (e.g., growth and reproductive potential) in response to changes in environmental variables such as temperature and food density (Van der Meer, 2006; Filgueira et al., 2011; Sarà et al., 2013). As such, by varying environmental parameters, one can estimate performance of a species under differing environmental conditions, which can inform aquaculture management practices. Here, we first parameterize *Perna viridis* and then use the mechanistic, predictive power of the DEB model to investigate the effects of changing somatic maintenance costs on the main LH end-points (ultimate size, growth as expressed in time to reach commercial size, reproductive potential etc.) using different scenarios. Such an approach allows us to predict *P. viridis* performance under different aquaculture conditions. Specifically, we investigated how environmental pollution may affect the mussel's energy allocation strategies (by varying the somatic maintenance DEB parameter), and this approach can be used to provide quantitative baseline information when assessing issues dealing with aquaculture management for *P. viridis* by scientists, policy makers and stakeholders.

2 MATERIALS AND METHODS

2.1. Approach and animal collection

To create the DEB model and then use the model to predict mussel LH traits and production under different scenarios, the study employed two approaches. The first involved collection of eco-physiological parameters, both through a systematic analysis of the current literature (Mangano and Sarà, 2017) and empirically, which are necessary for DEB model parameterization. The second used a modeling approach to simulate the ultimate size, reproductive output and the time to reach commercial size of *Perna viridis* under varying levels of energetic constraints and trophic conditions as might be experienced in an aquaculture context.

To obtain species-specific parameters required to populate the DEB model *Perna viridis* were collected between Feb 2014 and Jun 2015 from an aquaculture farm located at Tolo Harbour, Hong Kong (22°26′N, 114°16′E). Individuals of *P. viridis* were held at 25 °C and 30‰ salinity in a closed seawater system at The Swire Institute of Marine Science (Cape d'Aguilar, Hong Kong). Seawater was changed every two days and mussels were fed twice a day with *Isochrysis galbana* (~100,000 cell mL^{-1}) prior to experimentation.

2.2. Eco-physiological parameters

2.2.1. Arrhenius temperature

To obtain a set of temperatures to integrate into the DEB covariation method (Lika et al., 2011a, b), we estimated the Arrhenius temperature through mussel heart rate which has been shown to be a suitable proxy for measuring stress in *Perna viridis* in response to environmental changes (Nicholson, 2002; Nicholson and Lam, 2005; Ma, 2013). Mussels of different sizes were selected (shell length 2–2.99 cm, 3–3.99 cm, 4–4.99 cm and 5–5.99 cm, representing the population size range usually cultivated), with five replicates for each size class. Heart rates (HRs) were measured at five temperatures (i.e., 15 °C, 20 °C, 25 °C, 30 °C and 35 °C) within the temperature range that *P. viridis* experiences in its natural environment (Σn = 4 size classes × 5 temperatures × 5 replicates = 100). To achieve the desired experimental temperatures, a ramping rate of 1°C change every 30 min from ambient temperature (25 °C) was used, following Luk (2014). Animals were held at each temperature for 24 h after which heart beats were recorded for > 5 min following Burnett et al. (2013) and heart rates (HRs, in Hertz, Hz) calculated from the captured traces. Within a species' tolerance range, changes in HRs with temperature were illustrated by Arrhenius plots (i.e., ln (HR)

vs 1/temperature (in Kelvin)) and Arrhenius temperature (T_A) was calculated from the slope of the linear regression from the plots (Kooijman, 2010). The lower and upper tolerance temperatures were 15 °C and 35 °C which covered the lowest and highest temperature that *P. viridis* experiences in the natural environment (Tolo Harbour, data from 2013, Environmental Protection Department, HKSAR Government). The lower (T_{AL}) and upper (T_{AH}) boundaries of the Arrhenius temperature were obtained from the slope of the linear equation from the Arrhenius plots using natural-log HR data measured at optimal temperature and lower and upper tolerance temperatures respectively.
Slopes of linear equations (i.e. T_A, T_{AL} and T_{AH}) derived from Arrhenius plots of *Perna viridis* of different size classes were compared using Analysis of Covariance (ANCOVA, Zar, 1999). If the slopes were not significant, T_A, T_{AL} and T_{AH} obtained from different size classes were pooled.

2.2.2. Shape coefficient

Body volume and surface area play crucial roles in DEB energetic exchanges and fluxes and can be described using the shape coefficient, an abstract quantity that allows conversion of the organism's shell length to surface area and structural volume. To estimate this parameter, we collected mussels with spent gonads (to minimize the effects of reproductive tissues on estimations of the shape coefficient). Shell length was measured (± 0.1 mm) and then animals dissected, blotted dry and tissue wet weights (without shells) measured (± 0.001 g) to estimate the structural volume (n = 465). Tissue wet weight was most strongly related to shell length of *Perna viridis* (regression coefficient = 2.6; coefficient of determination, $r^2 = 0.943$) and the resultant shape coefficient was, therefore, derived using these two variables.

2.2.3. Ingestion and assimilation rates

To estimate food acquisition rates of *Perna viridis* collected mussels (shell length 4–5 cm) were held in well-aerated and filtered (0.7 µm; Whatman GF/F) seawater at 25 °C. A monoculture of the alga, *Isochrysis galbana* was added into beakers with predetermined volumes to achieve seven chlorophyll a concentrations (i.e., 1.27, 1.90, 4.70, 5.97, 11.57, 19.07 and 28.10 µg L^{-1}) to represent variation in food availability based on Hong Kong environmental conditions where *P. viridis* is intensively cultivated (Cheng, 2016) in order to calculate the mussel's surface area-specific maximum ingestion ($\{\dot{J}_{Xm}\}$) and assimilation rates ($\{\dot{p}_{Am}\}$ (see Sarà et al., 2013). Seven replicates of the seven concentrations were established together with four controls (i.e., beakers without animals) ($\Sigma n = 7$ concentrations × (7 replicates + 4 controls) = 77). During experiments, the algal cells were mixed using magnetic stirrers and 20 mL algal solutions were sampled at the beginning of the experiment and at 30-minute intervals over 2 h. The density of algal cells at each time was determined from counts using a haemocytometer. Mussels were then left for at least 18 h in 500 mL seawater without algae for defecation. The remaining algal food and faeces of each individual were collected under vacuum filtration (0.7 μm; Whatman GF/F), dried and combusted to obtain dry weight (DW) and ash free dry weight (AFDW) values for the determination of assimilation efficiency (AE) as described by Conover (1966). Algae from control replicates were also collected, dried and combusted to determine particulate organic matter of each algal concentration. To obtain the energy conversion factor (J mg^{-1}) of the algal food, the energy of 40 mg algal pellets concentrated from the algal stock were measured using an oxygen bomb calorimeter (6765, Parr Instrument Company).

2.2.4. Estimation of energy for somatic maintenance, growth and reserve density

Perna viridis (shell length 4–4.5 cm) were fed with a monoculture of *Isochrysis galbana* over two weeks prior to experimentation. Subsequently the mussels were evenly distributed into eight tanks (25 × 25 × 25 cm) and held in a closed aquarium system using filtered (0.22 μm) and aerated seawater maintained at 24 ± 0.5 °C and fed for two weeks. The tanks were then allocated to two treatments: either starved or well-fed (control) with four replicates for each group. Mussels were randomly selected every week to measure oxygen consumption (Pyroscience, FireStingO2, Germany) in respiratory chambers. After measurement, the tissue dry weight (TDW) and AFDW of each individual were recorded. Measurements were taken until the decrease in TDW of starved individuals levelled off prior to a second decrease in TDW (see Sarà et al., 2013), and the volume-specific maintenance cost ($[\dot{p}_M]$) was determined from the corresponding oxygen consumption rates. The somatic mass index, which reflects the amount of energy investment in the soma, was also derived from the TDW and AFDW of starved and control animals for determination of energy for growth ($[E_G]$) and reserve density ($[E_m]$) following methods described in Cardoso (2007).

Derivation of DEB parameters from data obtained from the experimental procedures described above, and how the DEB model generates the output of LH traits, are discussed in Kearney (2012), Montalto et al. (2014) and Sarà et al. (2013).

2.2.5. Derivation of DEB parameters for Perna viridis using the covariation method

Experimental values, together with zero- and uni-variate data, such as lengths at birth and puberty, age-specific size and the relationship between tissue wet weight and size, obtained from the literature and other field measurements (see Table 1; Cheng, 2016) were introduced into the DEB covariation procedure (a set of Matlab routines available online at http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/entries_web/Perna_viridis/Perna_viridis_res.html) to estimate the DEB parameters (Table 2) which cannot be derived empirically (Lika et al., 2011a, b). The estimation procedure ensures that the species parameter values fall within a biologically realistic range. The covariation procedure also gives a ‘goodness of fit’ value (defined as 10 × (1 − mean relative error)) and completeness metric (described in Lika et al., 2011a, b; Matzelle et al., 2014) to evaluate the reliability of the DEB parameters obtained from the procedure.

Table 1 Observed and predicted values of the dataset from DEB parameter estimation procedures for *Perna viridis* (for the observed value*: 1 = field observation, 2 = Laxmilatha et al., 2011; 3 = Tan, 1975; 4 = Rajagopal et al., 2006; 5 = Appukuttan, 1977; 6 = McFarland, 2015).

Parameter	Unit	Definition	Observed*	Predicted	
a_b	d	Age at birth	0.83	0.727	2
a_j	d	Age at metamorphosis	12	11.96	3
a_p	d	Age at sexual maturity	60	81.07	4
a_m	d	Life span	1825	1828	1
L_b	cm	Physical length at birth	0.007	0.006	2,4
L_j	cm	Physical length at metamorphosis	0.04	0.04029	2
L_p	cm	Physical length at sexual maturity	1.5	1.605	4
L_i	cm	Ultimate physical length	23	15.18	5
W_p	g	Wet weight at sexual maturity	0.1	0.1	1
W_i	g	Ultimate wet weight	84.2	84.43	5
R_i	# d^{-1}	Maximum reproduction rate	1.59×10^5	1.589×10^5	6

Table 2 DEB primary parameters for *Perna viridis* after estimation procedure for model simulation.

Parameter	Unit	Definition	Value
T_{ref}	K	Reference temperature	298
T_{A}	K	Arrhenius temperature	7,805
T_{L}	K	Lower tolerance temperature	288
T_{H}	K	Upper tolerance temperature	308
T_{AL}	K	Lower boundary of Arrhenius temperature	8215
T_{AH}	K	Upper boundary of Arrhenius temperature	17,280
δ_{M}	-	Shape coefficient	0.1912
AE	-	Assimilation efficiency	0.94
$\{\dot{J}_{Xm}\}$	J cm^{-2} d^{-1}	Surface area-specific maximum ingestion rate	182.7
$\{\dot{p}_{Am}\}$	J cm^{-2} d^{-1}	Surface area-specific maximum assimilation rate	171.74
$[\dot{p}_M]$	J cm^{-3} d^{-1}	Volume-specific maintenance cost	20.85
$[E_G]$	J cm^{-3}	Volume-specific cost for growth	2603
$[E_{\mathrm{m}}]$	J cm^{-3}	Reserve density	7052
κ	-	Fraction of energy for somatic maintenance and growth	0.44
κ_{R}	-	Reproductive efficiency	0.95

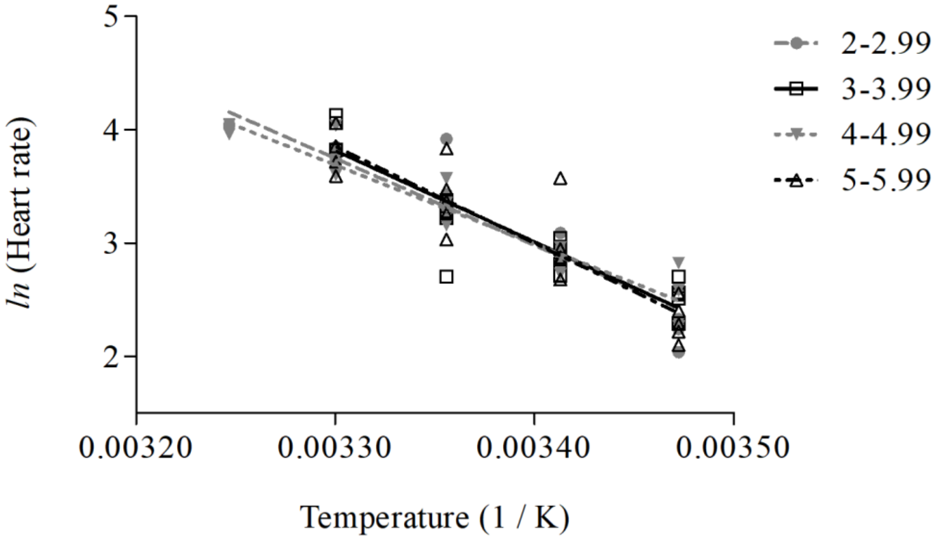
2.3. Model simulation: performance of Perna viridis under anthropogenic stressors and possible impact on aquaculture

To increase realism in the DEB simulations and to provide useful information to address management options for *Perna viridis* aquaculture, we formulated different scenarios of local pollution effects and trophic enrichment, as these are major threats in Hong Kong waters and in Southeast Asia (reviewed by Williams et al., 2016). To achieve this, we generated scenarios by varying the $[\dot{p}_{\mathrm{M}}]$ parameter and the functional response to study the potential differential effects due to both pollution and altered local trophic status on LH traits and harvesting time (time for mussels to reach commercial size, i.e., 5 cm; Vakily, 1989) of *P. viridis*. Pollutant stressors such as heavy metals have been recorded to elicit additional metabolic costs in bivalves increasing maintenance costs by 20% (Cherkasov et al., 2006) and we, therefore, varied the $[\dot{p}_{\mathrm{M}}]$ value from 5% to 20% following a scheme already used by Carrington et al. (2015) to represent the potential effects on LH traits exerted by pollutant stressors. We further varied the functional response (*f*) from 0.2 to 0.85 to explicitly include the potential effects of varying food availability on mussel performance (note that when $f = 1$ food is unlimited, when $f = 0$ food is not available) as described by Pouvreau et al. (2006). Following the approach by Sarà et al. (2012, 2013), simulations were run using hourly seawater temperatures obtained from the Hong Kong Observatory (HKO).

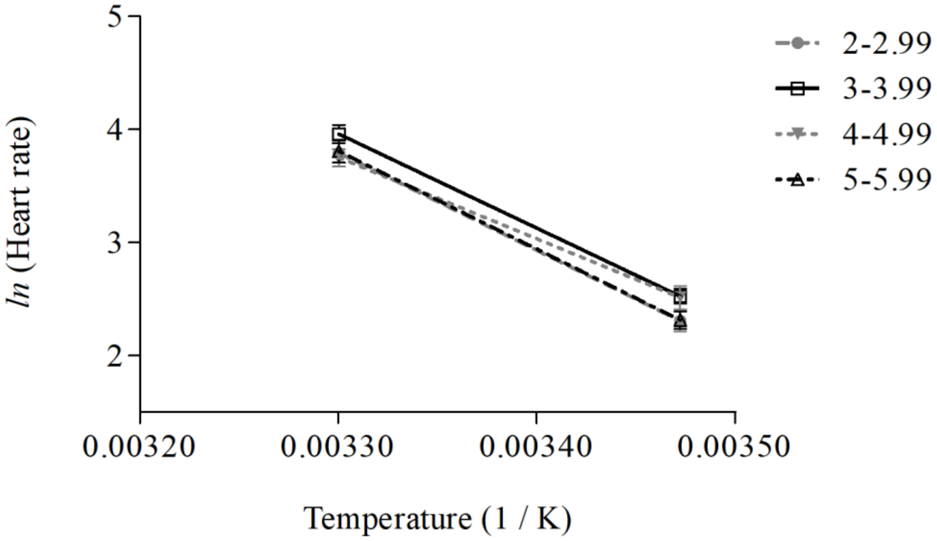
3. RESULTS

3.1. DEB model parameters

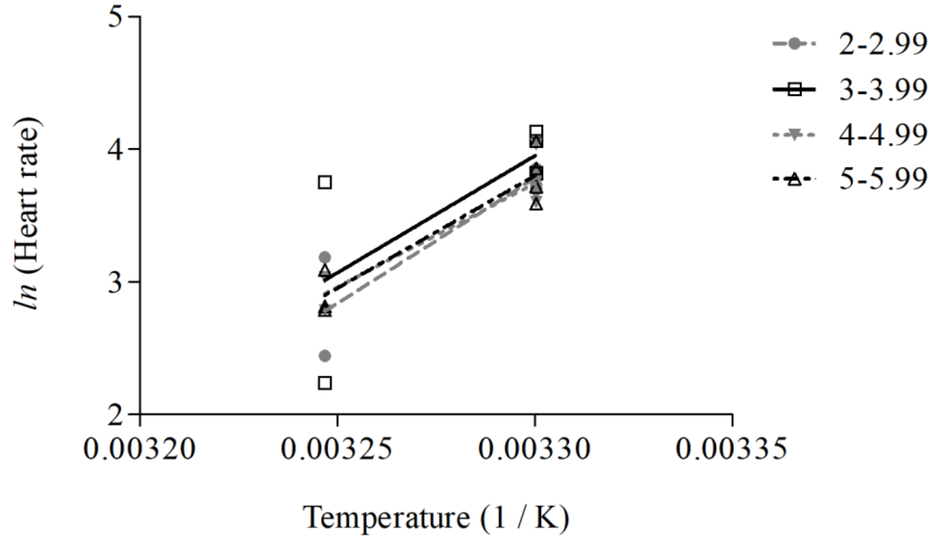
There was no significant effect of size on Arrhenius temperature (Fig. 1a; $F_{3,71} = 0.88$, $p > 0.05$) and, as a consequence, size classes were pooled. T_{AL} (Fig. 1b; $F_{3,30} = 0.07$, $p > 0.05$) and T_{AH} (Fig. 1c; $F_{3,19} = 0.07$, $p > 0.05$), similarly, did not differ between size classes and were also pooled, giving T_L and T_H of 288 K (i.e., 15 °C) and 308 K (i.e., 35 °C) respectively. The experimental shape coefficient was 0.2795 while experimental AE was 0.94 ± 0.08 (mean ± SD), $\{\dot{J}_{Xm}\}$, and $\{\dot{p}_{Am}\}$ were 174.87 ± 73.04 J cm−2 d−1 and 166.24 ± 74.44 J cm^{-2} d^{-1} respectively. The dry weight of starved individuals leveled off between weeks 12–14 (Fig. 2). The experimental mean $[\dot{p}_M]$ of starved mussels during week 12–14 was 1.17 ± 0.38 J cm^{-3} h^{-1} and the somatic mass indices (SMI) of fed and starved mussels were 1.80 ± 0.38 and 0.71 ± 0.20 mg cm^{-3}, respectively. All these empirically parameters were entered into the Matlab routine for estimation procedures (data summarized in Table 2). The DEB model for *P. viridis* had a goodness of fitness of 8.9 and completeness of 2.6 resulting in good overall performance, as it generated a good fit between observed and predicted data: for example, length at birth and length at puberty were accurately predicted by the model (Table 1) as well as the growth curve and the relationship between tissue wet weight and shell length, which closely fitted the observed data (Fig. 3). The input data and model are available online (http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/entries_web/Perna_viridis/Perna_viridis_res.html).



(a)



(b)



(c)

Fig. 1 Arrhenius plots showing variations in heart rates of *Perna viridis* of different size classes 2-2.99cm (● with dash line), 3-3.99cm (□ with solid line), 4-4.99cm (▼ with dotted line) and 5-5.99cm (Δ with dash-dotted line) over different experimental temperatures for the calculation of **a** T_A, **b** T_{AL} (b) and **c** T_{AH}. There were no significant differences among sizes in T_A, T_{AL} and T_{AH}.

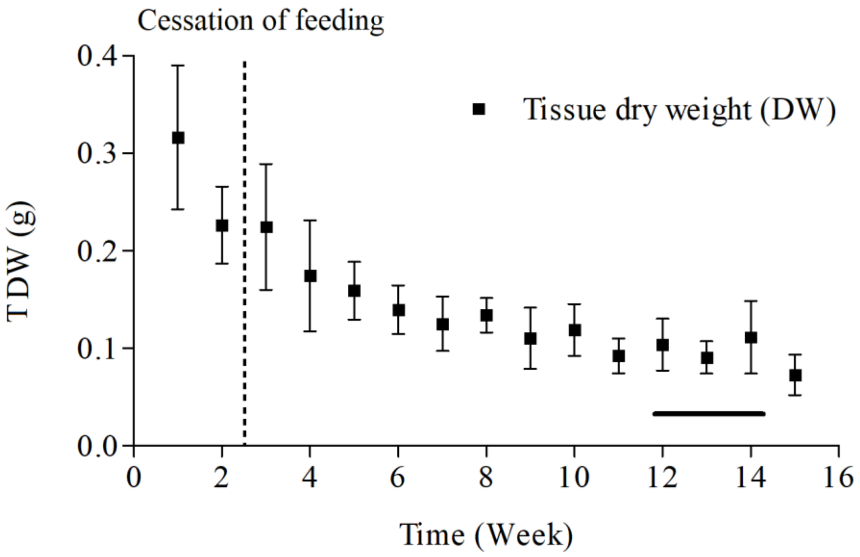
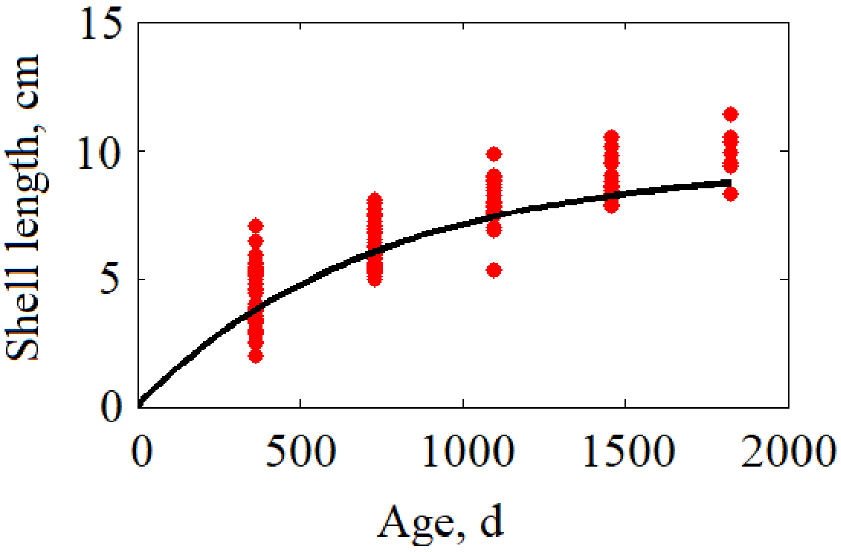
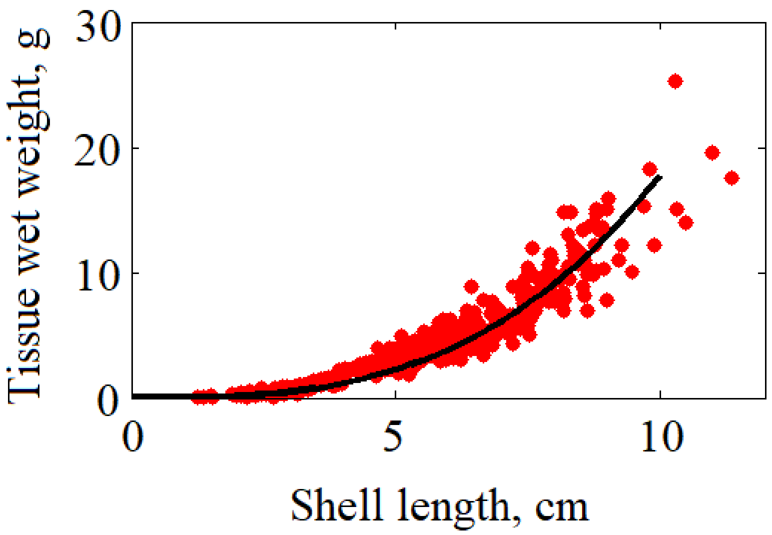


Fig. 2 Change (mean ± SD) in tissue dry weight (TDW) of *Perna viridis* which levelled off from week 12-14 (underlined), indicating the complete depletion of the energy reserve of the *P. viridis* at this time.



(a)



(b)

Fig. 3 Predicted (black lines) and observed (red dots) data in **a** shell length and age relationship and **b** tissue wet weight shell length relationship. Data from shell length age relationship and tissue wet weight shell length relationship were obtained from direct examination of mussel shell growth ring (Cheng, 2016) and shape coefficient experiment respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. DEB model simulations

Eighty-four spatially-contextualized models were run, crossing six levels of maintenance costs and 14 levels of food conditions (Fig. 4a, b). Our scenario analysis demonstrated that the increased basal metabolic demand negatively affected the LH outcomes by diminishing the reproductive output and growth of *P. viridis* at almost all levels of food density (Fig. 4a, b). When food density was low (represented by a low value (0.3) of *f*, which corresponds to 0.47 μg chl a L^{-1}), *P. viridis* did not have sufficient energy for reproduction regardless of maintenance costs, and when food density increased, lifetime egg production was negatively related to maintenance costs (Fig. 4a). Even though *P. viridis* received sufficient food supply (i.e., $f = 0.85$, chl a = 6.23 μg L^{-1}), a 20% increase in energy investment for basal maintenance also reduced lifetime egg production by 18%. The ultimate shell length was also dependent on both food density and increase in maintenance cost, with 7.5% reduction in growth at high food density (i.e., $f = 0.85$) as a result of the 20% increase in maintenance costs. Increasing maintenance costs also extended the time needed for mussels to achieve commercial size (Fig. 5). Increasing food availability, however, mitigated the effects of the increase in maintenance cost on harvesting time, with the time to reach commercial size increasing by 13% and 3% under lower (i.e., $f = 0.4$) and higher food availability (i.e., $f = 0.85$) respectively

(Fig. 5). When $f < 0.4$ (i.e., chl a = 0.73 µg L^{-1}), *P. viridis* could not, however, reach commercial size throughout its lifespan under various trophic conditions.

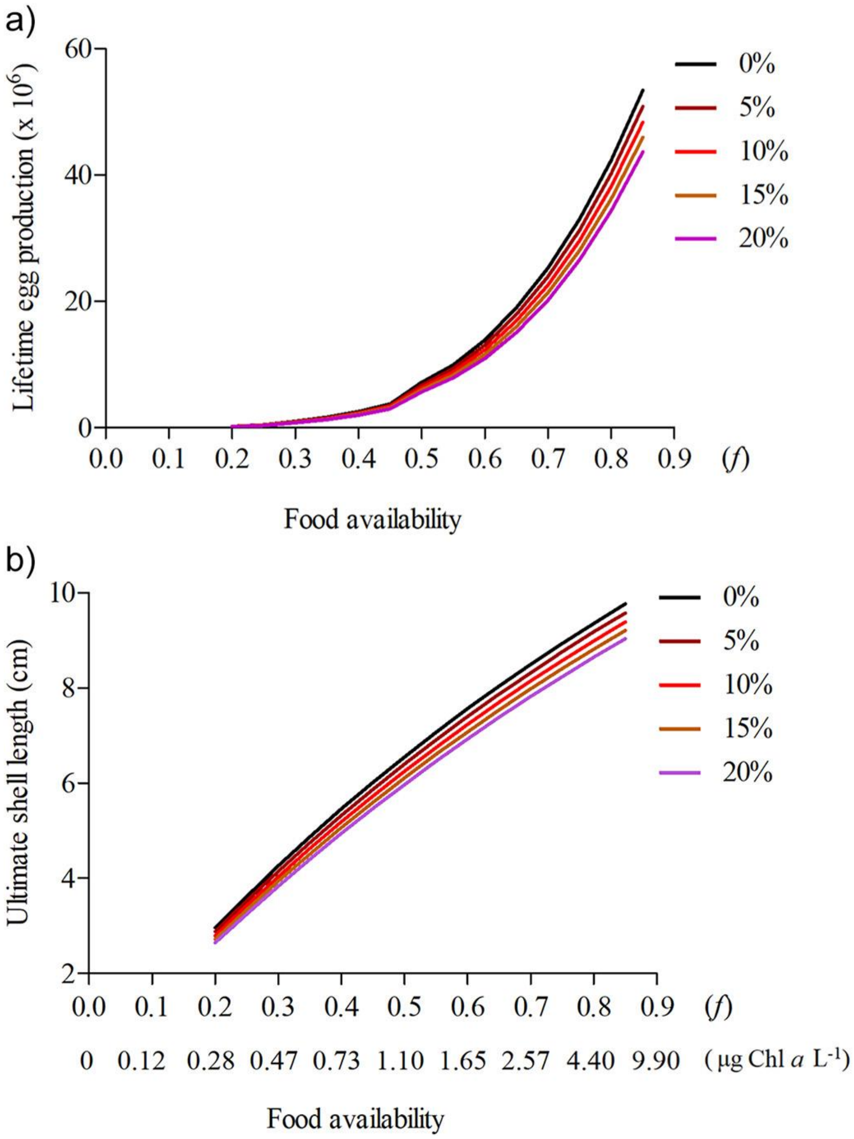


Fig. 4 Output of dynamic energy budget (DEB) model for *Perna viridis* illustrating the effects of increase in energy cost for basal maintenance on **(a)** lifetime egg production and **(b)** ultimate shell length. Holling type II functional response (*f*; Holling, 1959) was used to describe food availability. $f = X/(X + X_K)$ where X is the environmental food density and X_K is the half-saturation coefficient (1.1 µg chl *a* L^{-1}; McFarland, 2015). The maintenance cost ([$\dot{p}_M$] with present day value 20.85 J cm^{-3} d^{-1}) varied between 5% to 20%.

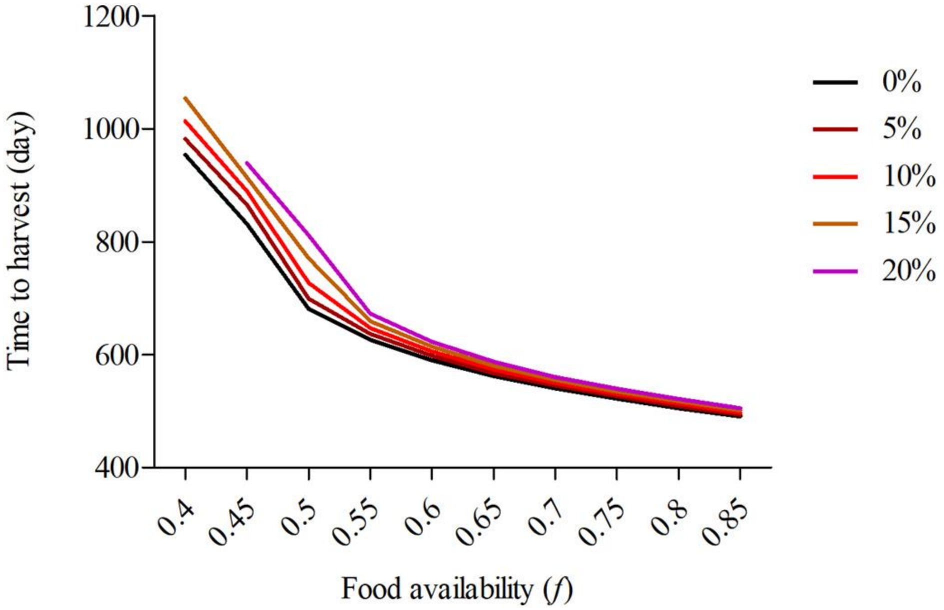


Fig. 5 Output of dynamic energy budget (DEB) model for *Perna viridis* illustrating the effects of increase in energy cost for basal maintenance on time to harvest (i.e., time when *P. viridis* reaches 5cm in shell length). Holling type II functional response (*f*; Holling, 1959) was used to describe food availability. $f = X/(X + X_K)$ where X is the environmental food density and X_K is the half-saturation coefficient (1.1 µg chl *a* L^{-1}; McFarland, 2015). The maintenance cost ($[\dot{p}_M]$ with present day value 20.85 J cm^{-3} d^{-1}) varied between 5% to 20%.

4. DISCUSSION

4.1. Energy allocation strategy of Perna viridis

The parameter set derived through this study can be considered reliable for predictions (Lika et al., 2011b; Matzelle et al., 2014) due to the high goodness of fit and completeness expressed by the model (see Section 3.2) and manifested by the close fit between predicted model outputs and observed data. The only anomaly was between observed and predicted age at puberty (Table 1) which may be due to the simulated relatively slower, with respect to field conditions, growth rate of *Perna viridis*. Although there was a difference between predicted and observed ultimate physical length, the observed value is variable according to environmental conditions and the largest size encountered in the field sampling was ~11 cm (Cheng, pers. obs.) which falls within the predicted value. Given such a close match between observed and predicted values, there is a high degree of confidence that the energy allocation strategy of *P. viridis* is reliably depicted from the DEB model.

Using the covariation method, maintenance cost and kappa (κ), the vital parameters governing the energy for LH traits such as growth and reproduction (Kooijman, 2010; Burton et al., 2011), were estimated. Maintenance cost ($[\dot{p}_M]$) plays an important role in governing the ability of organisms to cope with varying environmental conditions and their persistence over time under various biotic (such as varying food availability) and abiotic (such as changes in salinity) conditions (Sarà, 2007; Sarà and De Pirro, 2011). The maintenance cost of *Perna viridis* was estimated to be 20.85 J d^{-1} cm^{-3}, which is higher than other recorded mytilids (e.g., *Mytilus californianus*, 15.15 J d^{-1} cm^{-3}; Matzelle et al., 2014 and *Brachidontes pharaonis*, 14 J d^{-1} cm^{-3}; Montalto et al., 2014). A higher maintenance cost reflects higher basal metabolic demands of organisms for survival (see Sokolova and Lannig, 2008). The DEB model also revealed that *P. viridis* allocates more reserve energy for maturity maintenance and reproduction (56%, $1-\kappa$) than somatic maintenance and growth (44%, κ). The energy investment in reproduction for *P. viridis* is much greater than recorded in other mytilids species such as *Mytilus californianus* (1.1%; Matzelle et al., 2014) and *Brachidontes pharaonis* (1.7%; Montalto et al., 2014), which agrees with the observed high Gonadosomatic Index (~30%) of *P. viridis* measured by Cheung (1993) in Hong Kong and further supports the accuracy of the DEB model.

4.2. Chlorophyll-a as a food proxy for model simulations

There are various kinds of food sources in open coastal waters such as detritus and phytoplankton which could form part of a bivalve's diet. The combination of different food sources into the DEB model has, however, proved difficult as current formulations of the model can only take into account one food proxy (Rosland et al., 2009; Duarte et al., 2012). As a result, a single proxy for food, phytoplankton (expressed as chlorophyll-a, chl-a), was chosen for the model simulation. Chlorophyll-a has previously been shown to be a reliable quantifier for derivation of DEB ecophysiological parameters for shellfish (Pouvreau et al., 2006) as well as for a variety of bioenergetic studies which have accurately simulated organismal responses under fluctuating conditions (e.g. Widdows and Staff, 2006; Ren and Schiel, 2008; Sarà et al., 2008; Ezgeta-Balić et al., 2011). Using chl-a as a proxy for the bivalves food source also has the added advantage of being able to run DEB models with chl-a data from remote sensing satellite imagery, which can allow predictions at larger scales (Sarà et al., 2011, 2012, 2013; Thomas et al., 2011). Such data can also be used to address aquaculture management solutions such as site-selection and monitoring of aquaculture operations as satellite chl-a data is now an open resource on many web sites (see Sarà et al., 2012, 2018).

4.3. Potential effects of pollution on energy balance

Since basal metabolic demand has priority over other physiological processes, any change of basal metabolism will impact important LH traits such as growth and reproduction (Kooijman, 2010). Understanding how maintenance cost varies can, therefore, be beneficial for studying the effects of environmental changes on species' fitness (Burton et al., 2011). Environmental pollutants such as heavy metals, for example, have been shown to increase the standard metabolic rate (i.e., rate reflecting the energy demand for basal maintenance) of various species (Baghdiguian and Riva, 1985; Lannig et al., 2006; Ivanina et al., 2008; Sokolova and Lannig, 2008). The scenarios employed in this study demonstrated the effects of increasing maintenance cost of *Perna viridis* due to varying environmental conditions which affected its LH traits, with a reduction in reproductive output and growth. Energy balance is, therefore, vital for stress tolerance, which involves trade-offs between maintenance costs and fitness-related traits (Sokolova et al., 2012). The negative energetic constraints due to increased basal maintenance costs can, however, be buffered with increasing food density (Carrington et al., 2015) which permits higher energy supply to fulfill basal metabolic needs without sacrificing contributions to other LH traits.

4.4. Implication of model outputs for aquaculture management

Under varying environmental conditions, mussels can alter the balance of their bioenergetic budgets, and the outcomes of such energetic decisions are important for managing shellfish culture (Rinaldi et al., 2014; Montalto et al., 2017). To evaluate the success of shellfish cultivation, one can assess the growth of the target species (Pogoda et al., 2011). The DEB model simulations for *Perna viridis* can, therefore, provide primary information on how environmentally induced increased basal maintenance costs may affect the growth rates and reproductive output of the mussel which will govern the ultimate harvesting time (Rinaldi et al., 2014). Traditionally, mussels are harvested when they reach the minimum commercial size (i.e., 5 cm for *P. viridis*; Vakily, 1989). With reduced growth, mussels take longer time to reach such commercial size, therefore, extending the harvesting time. Such extension in harvesting time subsequently increases operational costs of a mussel farm which can contribute up to 43% of total operating cost (Kripa and Mohamed, 2008). Prolonged cultivation also increases the risk of loss in biomass due to higher chances of exposure to environmental disturbances such as storms, phytotoxins, hypoxia and sudden salinity changes (Spencer, 2002; Sarà et al., 2018). Increases in operational cost and risk of losses, therefore, make such mussel aquaculture operations less profitable, which may reflect poor decisions in site selection (Spencer, 2002).

Indeed, the main objective of modern management strategies such as ecosystem based management is to provide tailored management measures based on ecological functioning principles at a sufficient scale to reduce impacts on economic yield. The DEB model can quantify species' LH traits as function of potential temporal fluctuations in local environmental variables (Sarà et al., 2012). Such an approach, therefore, makes the model extremely useful for prediction of time for the mussel to reach commercial size and subsequently estimating the operational cost for a selected site (Sarà et al., 2018). Our model shows that *Perna viridis* exerts extra energy to overcome additional energetic costs due to environmental fluctuations. Such energetic constraint diminishes the species' growth rate, one of the most important traits for an aquaculture facility which, as a consequence, extends cultivation time prior to harvesting. Another important trait for aquaculture practitioners is reproductive potential. Since the gametes contribute 30% of the total weight of *P. viridis* (Cheung, 1993), the amount of gametes can have a significant impact on the price of harvested mussels. Both traits, therefore, will affect the overall economic success of aquaculture facilities. Since traditional aquaculture farming practices usually results in higher primary productivity around aquaculture facilities (Dalsgaard and Krause-Jensen, 2006), and adequate food supply is able to alleviate the effects of energetic constraints caused by environmental stress and shorten harvesting time, this implies the possibility of integrated multi-trophic aquaculture involving the utilization of waste nutrients produced from higher trophic level organisms (e.g., fishes) for lower trophic levels (e.g., filter feeders, Sarà et al., 2012; Troell et al., 2009), an area in which the DEB model has been successfully applied. Such approaches, however, have yet to be applied to *P. viridis*, which may be a novel direction for better management of this aquaculture species.

5. CONCLUSIONS

With growing human populations in the past decades, aquaculture is becoming increasingly important to meet the increasing demand for seafood. Such practice, however, requires strategic management as aquaculture is vulnerable to deterioration in environmental quality which ultimately affects success of an aquaculture system and this is especially true in Southeast Asia. The present experimental and modelling approach paves the way towards understanding the energy allocation strategy of the important aquaculture species, *Perna viridis*, and can be used to illustrate the effects of changing environmental conditions on the species LH traits throughout its life span. Using this approach, the effects of various stressors on the target species can be explicitly revealed through changes in DEB parameter values which affect species' LH traits. With such flexibility, the DEB model becomes increasingly important for predicting species performance under varying environments, providing important information for the management and the increasing competitiveness of aquaculture to meet future demands.

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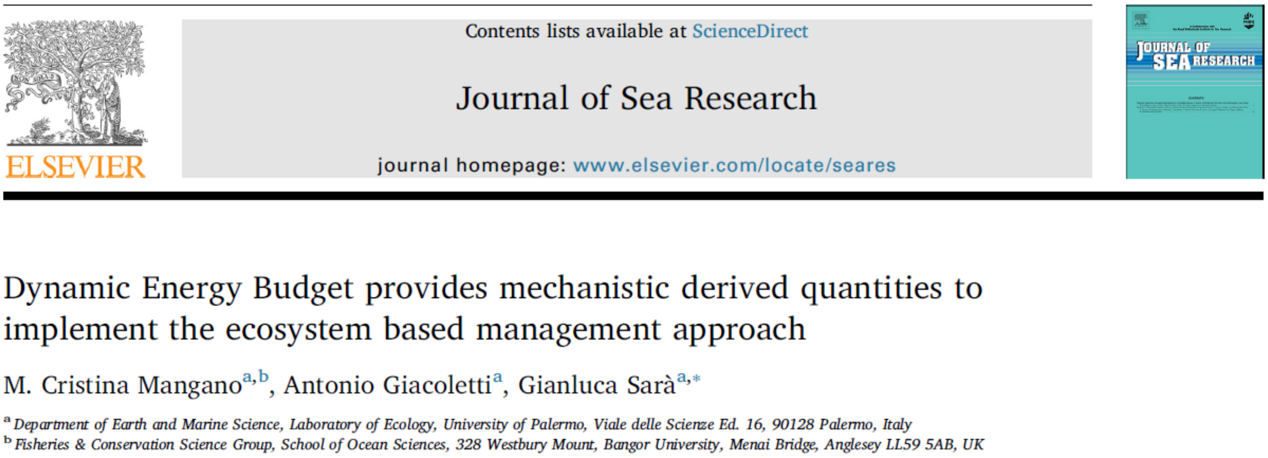
CHAPTER 5



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Dynamic Energy Budget provides mechanistic derived quantities to implement the ecosystem based management approach

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ABSTRACT

The on-going climate change threats are rapidly growing at both global and local scales, affecting ecosystems, societies and economies by altering natural distribution and productivity of key commercial species. Although the ecosystem based management (EBM) focuses on ecosystem equilibria, to provide realistic management measures for important activities at sea such as fisheries and aquaculture, there is a need of quantities; mechanistic approaches are suggested as reliable solutions. Here, a Dynamic Energetic Budget (DEB) application studies the link between environmental change (temperature forecasted increasing scenario in a context of COP 21 [Paris climate conference Agreement] and food density increase) and life-history traits of some Mediterranean fishery and aquaculture target species (*Engraulis encrasicolus*, *Dicentrarchus labrax*, *Mytilus galloprovincialis*, *Crassostrea gigas*). A sensitivity analysis was applied to simulate the effects of future environmental change on the time needed to reach the commercial size and the length at first maturity. We also explored the efficiency of Integrated Multitrophic Aquaculture (IMTA) as a potential management solution in a context of an adaptive EBM. The worst scenario of rising temperatures (+2°C) seems to reduce the time needed to reach the commercial size in most species and IMTA potentiates the thermal effect on it. A spatial contextualisation of model outcomes allowed disentangling potential conflicts among human activities at sea. The DEB based life history traits approach can provide quantities to inform the management of marine activities at local scale additionally allowing translating complex results into useful figurative representations for stakeholders.

Keywords: adaptive Ecosystem-Based Management; Dynamic Energy Budget; Climate change; Fisheries; Aquaculture; IMTA

1 INTRODUCTION

Climate change represents an ongoing and rapidly growing threat at both global and local scale equally affecting the environment, societies and economies by forcing shifts in the distributional range and productivity of key commercial species (Lam et al. 2016). The magnitude of these effects on marine species (both in the wild and captive conditions) and their replies (e.g. increase or decline of abundance; Pecl et al., 2017) can vary and the possible changes can depend on: i) species life-histories traits, ii) local environmental conditions and iii) contextual presence of more than one anthropogenic related stressor (Gunderson et al., 2016; Pecl et al., 2017; Sarà et al. 2018a, b, c). The effect of increasing temperature pushes species distribution shifts at large scales, such as the poleward shift, which seems concurrent across the current literature (Poloczanska et al., 2013; Mieszkowska et al., 2014; Rutterford et al., 2015; Sarà et al. 2018b). What is still poorly recognised and understood is how the effect of multiple drivers of environmental change vary in space and time at local scale. Additionally and contrary to what happened so far, the future management of socio-economic important activities at sea, such as fishery and aquaculture, as well as the corresponding systems of governance, should be informed by observations and predictions made at local scale. Only a more adaptive management that integrates approaches that involve the full array of interactions within an ecosystem, including humans, rather than considering single issues in isolation, starting from an appropriate knowledge of species biological traits, will allow the translation of the effects of environmental change into realistic management measures (Carpenter and Folke, 2006). Thus, strategies of dynamic adaptation should be designed taking into account new more ongoing favourable environmental conditions affecting the optimisation of the species' biological traits (Sarà et al. 2018a, b, c) to avoid unrealistic or inapplicable management measure. Oftentimes, this leads to measures that generate conflicts in between the different anthropogenic activities that foster tension among coastal stakeholders (Baudron and Fernandes, 2015; *sensu* Sarà et al. 2018a).

The Dynamic Energy Budget Theory (Kooijman 2010) feeding the functional-based approach (Schoener, 1986; Kearney and Portner 2009; Kooijman 2010; Sarà et al. 2014; 2018c) may represents an effective and powerful mechanistic tool in providing those kinds of quantities to inform effective and flexible fisheries and aquaculture management plans. Spatially contextualised quantities based on functional traits of fished and cultivated species (e.g. Sarà et al. 2018a) can help us in disentangling the effects of increasing temperature due to climate change. Such kind of information can preserve and reinstate the socio-economic integrity of marine regions in shifting, dynamic and changing ecosystems (UNEP/MAP, 2016).

Here, we introduce a comprehensive approach that integrates quantities generated by a mechanistic DEB based application, with the aim to operationalize, inform and support a more adaptive management of marine resources and the related development (e.g. fisheries ad aquaculture). Our framework involves a spatially-explicit module where, by mapping our outcome, we were able to contextualise potential management issues. In this regards, Sicily, due to the cross-border central position in the Mediterranean Sea, coastal socio-economies based on sea-related products (e.g. seafood, oil and gas extraction) and peculiar physiography and oceanography, can represent an ideal case study area to test the effectiveness of integrated approaches and to explore possible benefits coming from a quantitative mechanistic analysis based on species traits (Mangano and Sarà 2017; Mangano et al. 2017; Capodici et al. 2018). In doing so, we identified and quantified the spatio-temporal shifts under climate change in terms of time to reach the both the commercial size for three selected aquaculture model species (*Mytilus galloprovincialis*, *Crassostrea gigas*, *Dicentrarchus labrax*) and the length at fist maturity for a fishery model species, *Engraulis encrasicolous*, (hereafter TIME). All these species are common and highly valuable in the Mediterranean fisheries and aquaculture and are among the most consumed species in the Basin. We used the power of the functional-based approach to generate at regional level the kind of site-specific mechanistic predictions of species shifts resulting from climate change (in terms of increasing temperature from 0.5 to 2.0 °C in respect of the current worst scenario increasing temperature - *sensu* COP 21 Paris climate conference Agreement; Hulme 2016). In the specific case of two out three farmed bivalves (*M. galloprovincialis* and *C. gigas*), we crossed the thermal outcomes with another potential source of variation such as the trophic enrichment (in terms of chlorophyll-a increase), simulating the presence of an Integrated Multi-Trophic Aquaculture (IMTA; Sarà et al. 2012) when more species belonging to different trophic levels are cultivated together (Sarà et al 2012; Sarà et al. 2018a). Thus, we performed an analysis crossing both temperature increase (from current up to +2°C) and trophic condition scenarios (normo-trophic conditions vs. IMTA trophic enrichment conditions).

Quantities produced by our approach were mapped allowing to spatially visualize a quantitative informational baseline of climate effects on our target species. These can be used in helping scientists to inform policy makers and stakeholders with the final aim to produce more tailored management strategies and plans based on local scale outcomes.

2 MATERIALS AND METHODS

The DEB model represents the "quantitative from scratch" framework which mechanistically investigates the fluxes of energy that one organism activates in order to optimize fitness during the life span (Marquet et al. 2014; Kearney et al. 2015). The mechanistic properties rely on energy and matter flows from habitat through organisms. Flows of energy and matter (and time) through habitats and organisms are subjected to conservation laws (Charnov and Krebs 1974) and, consequently, they are traceable (and "budgetable" accountable) processes. We can use these principles to mechanistically predict the functioning of each species and thereby the magnitude and variability of life-histories traits (Loreau 2010; Kearney 2012; Pethybridge et al. 2013). The standard DEB model (Kooijman 2010; Kearney 2012) incorporates whole-organism bioenergetics, allowing the connection between the individual behaviours to population growth *via* the description of how energy and mass are managed by organisms and how metabolic trade-offs are involved in response to local environmental change. The mechanistic nature of the standard DEB model allows that the bioenergetics features of any organism may be related to environmental conditions, so that growth rate and the ultimate fitness can be predicted. This is only feasible if the organismal body temperature and food density available are known, as for our four studied target species and when all DEB parameters of that species have been estimated (Pethybridge et al. 2013). Here we selected two fishes, the European anchovy, *Engrauils encrasicolus* and the European seabass, *Dicentrarchus labrax,* and two bivalve molluscs, the Japanese oyster, *Crassostrea gigas* and the Mediterranean Blue mussel, *Mytilus galloprovincialis* (Figure 1). Metabolic rates of these ectotherms such as all living organisms depend on body temperature and the available food density of the environment where organisms live. DEB describes the dynamics of these processes and the possibility of modelling the effects of body temperature and food density assumes an important role to predict where, when and with what magnitude species' persistence evolves over time (Montalto et al. 2014) with potential repercussions on biodiversity and local economies. The amount of energy from food available to biological processes is regulated, in the DEB theory, by Holling's functional responses (Holling 1959). Once food is ingested, the amount of energy from food flows through the organism at some extent depending on physiological rates. Body temperature represents an important constraint in the DEB theory especially in ectotherms in which it approximates that of the mean temperature (*e.g.* seawater as in fish). The effect of temperature on metabolism follows the Arrhenius relationship (1889) (Kooijman 2010). Arrhenius temperature and the lower and upper boundaries of the body temperature tolerance range can be extrapolated from literature data or estimated by a direct calculation of physiological rates at different temperatures (Pethybridge et al.

2013; Sarà et al. 2013). The DEB model allows us to quantify some of the most important life history traits (Sarà et al. 2011; 2014). For the purpose of this study, we extrapolated and presented the duration of the grow-out phase as expressed in terms of days (TIME), needed to reach the minimum “commercial size” for the three aquaculture target species and the minimum “length at first maturity” for the fisheries species. These size were gathered from literature (FAO CASIP 2004; http://www.fao.org/fishery/culturedspecies; https://www.fishbase.org/; http://www.sealifebase.org/). In Table 1 and Figure 1, we both reported DEB parameters for all species and a graphical description of the applied approach on the model target species.

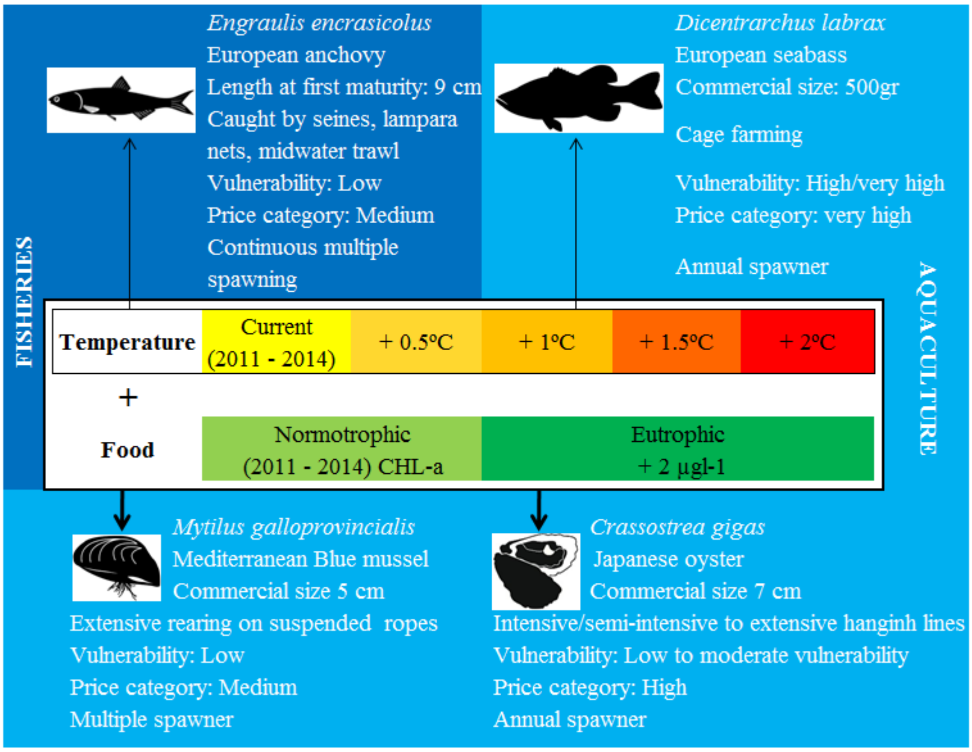


Figure 1: Conceptual framework showing the simulated scenario of “temperature” increase (from current scenarios, years 2011 – 2014 to +2ºC, step 0.5ºC) and “food” increase (from a current “normotrophic” condition, 2011 – 2014 CHL-a, to “eutrophic” condition + 2 µgl-1). Variation of the commercial size, proxy of the duration of the grow-out production phase, have been modelled along a temperature and food increasing scenarios for the two bivalve aquaculture target species (*Mytilus galloprovincialis* and *Crassostrea gigas*) in order to text the Integrated Multi-Trophic Aquaculture, IMTA, effects as a potential management solution in a context of an adaptive ecosystem based approach. Variations of the commercial size of the farmed fish, *Dicentrarchus labrax*, and length at first maturity on the fisheries target species, *Engraulis encrasicolus*, have been modelled along a temperature-increasing scenario only. **Notes:** the + 2ºC temperature increase value reflects the foreseen worst temperature increasing scenario in COP21 (Paris climate conference Agreement, 2015); the + 2 µgl-1 food increase value (Chlorophyll-a, CHL-a) reflects an eutrophic condition simulating the trophic enrichment of a typical IMTA, condition. Data on the farming or fisheries methods, selected sizes, vulnerability, price and spawning have been reported to better characterise the selected target species (FAO CASIP 2004 http://www.fao.org/fishery/culturedspecies; https://www.fishbase.org/; http://www.sealifebase.org/).

Table 1 DEB parameters for *Mytilus galloprovincialis, Crasssostrea gigas, Dicentrarchus labrax and Engraulis encrasicolus* (1 = Kooijman 2010; 2= van der Veer et al. 2006; 3 = Sarà et al. 2011; 4 = Thomas et al. 2006; 5 = Schneider 2008; 6 = Rico-Villa et al. 2010; 7 = Cardoso et al. 2006; 8 = Pouvreau et al. 2006; 9 = Sarà et al. 2018c; 10 = Freitas et al. 2010; 11 = Eroldoğan et al. 2008; 12 = Dalla Via et al. 1987; Claireaux & Lagardere 1999; Person-Le Ruyet et al. 2004; Claireaux & Lefrançois 2007; 13 = Pethybridge et al. 2013; 14 = Teal et al. 2012); * denotes parameters governing physiological rates based on experimental data at 25°C, the others are given at the respective reference temperature.

Symbol	Description	Units	*Mytilus galloprovincialis*		*Crassostrea gigas*		*Dicentrarchus labrax*		*Engraulis encrasicolus*	
			Value	Ref	Value	Ref	Value	Ref	Value	Ref
Vb	Structural volume at birth	cm^3	0.0000013	1	0.00000015	6;7	-	-	0.000225	13
Vs	Structural volume at seeding	cm^3	-	-	-	-	4.17	9	-	-
Vp	Structural volume at puberty	cm^3	0.06	2	1.40	7	419.60	10	1.73	13
δ_M	Shape coefficient	-	0.225	-	0.175	-	0.217	10	0.169	-
$\{\dot{J}x_m\}$	Maximum surface area-specific ingestion rate	$J\ cm^{-2}h^{-1}$	8.2	4	23.3	7*	58.5	9	13.5	13
ae	Assimilation efficiency	-	0.88	3	0.75	8	0.89	11	0.71	13
X_K	Saturation coefficient	$\mu g\ l^{-1}$	2.1	3	9.5	8	0.034	9	33.0	13
$[E_G]$	Volume-specific cost of growth	$J\ cm^3$	5993	5	1900	6	5600	10	4000	13
$[E_m]$	Maximum storage density	$J\ cm^3$	2190	2	2295	6	3850	10	2700	13
$[\dot{p}_M]$	Volume-specific maintenance cost	$J\ cm^{-3}\ h^{-1}$	1	2	24	6*	1.71	10	2	13
κ	Fraction of utilized energy spent on maintenance and growth	-	0.7	2	0.8	free fit	0.8	9	0.7	13
κ_R	Reproduction efficiency	-	0.8	3	0.7	8	0.95	12	0.95	13
T_A	Arrhenius temperature	°K	7022	-	5800	-	6228	12	9800	-
T_L	Lower boundary of tolerance range	°K	275	2	281	6	279	12	278	14
T_H	Upper boundary of tolerance range	°K	296	2	305	6	303	12	305	14
T_{AL}	Rate of decrease at lower boundary	°K	45430	2	75000	6	7333	12	50000	14
T_{AH}	Rate of decrease at upper Boundary	°K	31376	2	30000	6	3961	12	100000	14

2.1 Environmental variables to run DEB models.

To study the potential overlap among activities at sea, aquaculture and fisheries, we spatially contextualised our analysis in the central Mediterranean, around Sicily. Sicilian coasts provide an ideal model area to test the power of this mechanistic approach as there are many conflicts at sea to be solved and local economies strongly rely on fisheries and aquaculture products. Thus, we downloaded 4-year datasets (2011-2014) from the Environmental Marine Information System (EMIS) maintained at the European Joint Research Centre website (http://emis.jrc.ec.europa.eu/) and MyOcean (http://www.myocean.eu) of, respectively, daily chlorophyll-a (CHL-a), weekly Net Primary Production (NPP) and daily Sea Surface Temperature (current SST). These datasets were used to feed DEB models of present 4 target species under current conditions (2011-2014). Data have been downloaded with a spatial resolution of 0.11 degrees to obtain 68 coastal pixels covering about 850 km of the Sicilian coastline. SST and CHL-a were obtained for all 68 pixels and CHL-a was used as a proxy of available food to bivalves target species as commonly carried out in the current literature and in several companion studies (*e.g.* Sarà et al. 2011; 2012; 2013b; 2014; 2018b). Instead, for the European anchovy which was the only wild fishery species, we extrapolated a spatially continuous dataset of food density throughout the study area and across time (Stromberg et al. 2013). This method transforms the local Net Primary Production into wet mass of zooplankton (mg m^{-3}) starting from values of Carbon per Unit Volume expressed as grams of Carbon per cubic metre and after having converted it into the wet mass of zooplankton by the coefficient of conversion provided by Cushing et al. (1958). Food for anchovy was obtained only for 45 pixels due to the lack of consistent Net Primary Production data in 23 pixels out 68. For the only intensive cultivated captive species (seabass; i.e. not relying on natural food under wild conditions; Sarà et al. 2018a), the effect of food was expressed through calibrating the functional response using growth and environmental data coming from Sicilian farms (Sarà et al. 2018c). Thus, we adjusted the half-saturation coefficient (which is a measure of how much organism are able to adapt to local food conditions) to fit the observed fish length reached in Sicilian farms.

To simulate the future increasing temperature scenarios forecasted by COP 21 (Hulme 2016), we performed a simulation study (here after sensitivity analysis). Thus we ran DEB models pixel by pixel and increasing the current temperature from 0.5°C to 2.0°C (0.5° step), we obtained 4 increasing temperature DEB scenarios (current +0.5°C, +1.0°C, +1.5°C, +2.0°C; Figure 1). The procedure was stopped at +2.0°C which represents the worst COP 21 scenario expected in the coming years (Hulme 2016). We decided to apply this type of procedure as it is defined more reliable than using IPCC (AR4 or AR5; Montalto et al. 2016) simulations from current up to the year 2050-2075 based on the Representative Concentration Pathways, RCPs (Moss et al. 2010; Cheung et al. 2017).

One objective of this study was to explore the efficiency of IMTA as a potential management solution in a context of an adaptive ecosystem based approach. Thus, we ran pixel by pixel models for bivalves both under current "normotrophic" conditions – as expressed by current CHL-a data (2011-2014) – and under "eutrophic" conditions as expressed by a CHL-a increment of 2.0 μg l^{-1} (according to Sarà et al. 2012 observations of the fish culture trophic aided-enrichment on *Crassotrea gigas* and *Mytilus galloprovincialis* life-histories traits; Gulf of Castellammare, Sicily).

In total, we ran 1925 simulations and data have been presented through percentile maps in the attempt to express the model outcome through a synthetic indicator (Figure 2-3). We subsequently calculated the overall mean TIME for each species, defined by averaging the TIME values of all pixels and we grouped 6 classes of percentiles in order to study the temporal deviation of every pixel in respect to the overall mean. Class 0 = 97.5% percentile (*i.e.* $\mu+2\sigma$) represented the "Pessimum" condition (black pixels); Class 1 = 84.1% percentile (*i.e.* $\mu+1\sigma$) represented the "Pejus" condition (pink pixels); Class 2 = 50% percentile (*i.e.* μ) represented the "Mean" condition (yellow pixels); Class 3 = 15% percentile (*i.e.* $\mu-1\sigma$) represented the "Good" condition (green pixels); Class 4 = 2.5% percentile (*i.e.* $\mu-2\sigma$) represented the "Optimal" condition (light blue pixels) and Class 5 = 0.1% percentile (*i.e.* $\mu-3\sigma$) represented the "Best" condition (blue pixels) (Figures 2, 3). In so doing, we classified all pixels and we made all species comparable. This kind of analysis would show, for instance, that if one pixel of a certain target species fall into Class 0, the environmental conditions supporting growth are the worst and the time to reach the commercial size is the greatest. At the other extreme, if a pixel fall into Class 5, the time needed to reach the commercial size is the shortest in respect to the mean and so on.

3 RESULTS

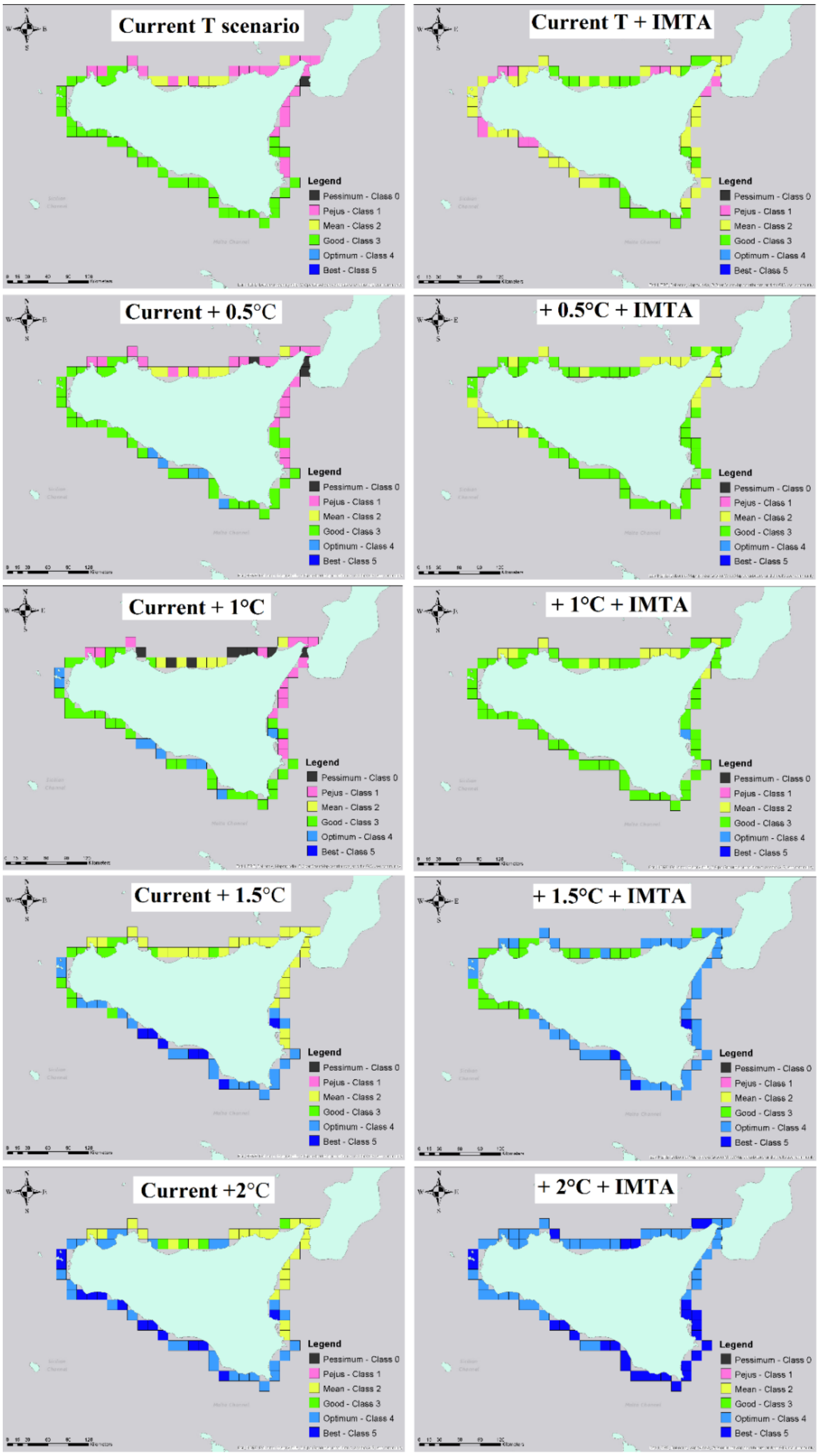
All DEB models generated outcome which were in line with already observed data and we are therefore confident that our analysis generated realistic results (see Sarà et al. 1997; 1998; 2012 for *M. galloprovincialis* and *C. gigas* model validation; Sarà et al. 2018c for *D. labrax;* Basilone et al. 2006 for *Engrauils encrasicolus*).

Overall, the increasing temperature from current up to +2°C generated conditions for reducing the time to reach the commercial size in most species of this study. Seabass was the only species that underwent a fluctuating behaviour, in that TIME reduced up to +1.0°C (i.e. 679 days) and later started to increase again reaching 710 days on average in all 68 Sicilian pixels. Bivalves, when not cultivated under IMTA conditions and then separately in respect to fish farms, reduced their TIME of about 4% and 6% (Table 2), for *Mytilus* and *Crassostrea*, respectively. However, if both bivalves are cultivated under IMTA enriched conditions, as those commonly measured close to the European fish farms, the trophic enrichment generated a sort of synergistic acceleration of TIME of at least 2-3% more than normo-trophic conditions (no IMTA). While aquaculture species benefited from increasing temperature, to a certain extent, within 10%, TIME values of the European anchovy decrease to almost 20% (17.8%; Table 2) in respect to the current; this translates into a temporal anticipation of reaching the puberty size of almost two months (49 days fewer than current). The spatial contextualization of what we observed at single level species follows the same line: the warmer the temperature, the faster the growth. Thus, as shown in figure 2, it is easy to observe that when the temperature increases, aquaculture species not cultivated together (Fig. 1), reach the commercial size before current, with some exceptions when temperature was +1°C (black pixels in the northern areas). This spatial pattern becomes more robust when species are combined in IMTA, in that the trophic enrichment seems to potentiate the effect of increasing temperature on TIME. Consequently, under a COP21 worst case scenario, under IMTA conditions, many Northern Sicilian pixels change their TIME and all fall into Class 4 and 5 (i.e. the TIME is smaller than 2 and 3 standard deviation units in respect to the mean, meaning that the species grow much faster). In figure 3, the reported maps also combine fishery (*Engraulis encrasicolous*) pixels. Unfortunately, due to lack of NPP data, we can show only 45 pixels out 68 but this is sufficient for the purpose of this study, as it shows that increasing temperature affects the spatial suitability of most areas around Sicily. Specifically, under the worst case COP 21 scenario, only some northern areas will be less suitable for both activities, both with and without IMTA.

Table 2 a) The estimated time to reach the commercial size (TIME) for the whole study area in all scenarios and **b)** the percentage difference between TIME under current conditions and other scenarios.

a) TIME	Current	Current + 0.5°	Current + 1.0°	Current + 1.5°	Current + 2.0°
Engraulis encrasicolous	273	260	247	235	224
Dicentrarchus labrax	700	685	679	685	710
Mytilus galloprovincialis	967	957	947	938	929
Crassostrea gigas	1135	1114	1098	1082	1068
M. galloprovincialis IMTA	538	529	519	509	498
C. gigas IMTA	205	201	197	193	188

b) TIME Diff%	Current	Current + 0.5°	Current + 1.0°	Current + 1.5°	Current + 2.0°
Engraulis encrasicolous	-	4.7	9.3	13.7	17.8
Dicentrarchus labrax	-	2.2	3.0	2.2	-1.4
Mytilus galloprovincialis	-	1.1	2.1	3.0	3.9
Crassostrea gigas	-	1.9	3.3	4.7	5.9
M. galloprovincialis IMTA	-	1.8	3.6	5.5	7.4
C. gigas IMTA	-	2.1	4.1	6.1	8.2



Current T scenario

Current T + IMTA

Current + 0.5°C

+ 0.5°C + IMTA

Current + 1°C

+ 1°C + IMTA

Current + 1.5°C

+ 1.5°C + IMTA

Current +2°C

+ 2°C + IMTA

Figure 2: Aquaculture target species – *D. labrax*, *C. gigas* and *M. galloprovincialis* – maps showing the percentile class in which each pixel around Sicily falls. On the left panel of each rows the mapped outcome obtained by cultivating species separately; on the right panel the mapped outcome obtained when species were combined in IMTA. From top to bottom mapped outcome under increasing temperature conditions from current up to current +2°C (step 0.5°C; the first panel of each column is the current, the last panel current +2°C). Black = "Pessimum" condition (Class 0, 97.5% percentile, *i.e.* $\mu+2\sigma$); Pink = "Pejus" condition (Class 1, 84.1% percentile, *i.e.* $\mu+1\sigma$); Yellow = "Mean" condition (Class 2, 50% percentile, *i.e.* μ); Green = "Good" condition (Class 3, 15% percentile, *i.e.* $\mu-1\sigma$); Light blue = "Optimal" condition (Class 4, 2.5% percentile, *i.e.* $\mu-2\sigma$); Blue = "Best" condition (Class 5, 0.1% percentile, *i.e.* $\mu-3\sigma$).

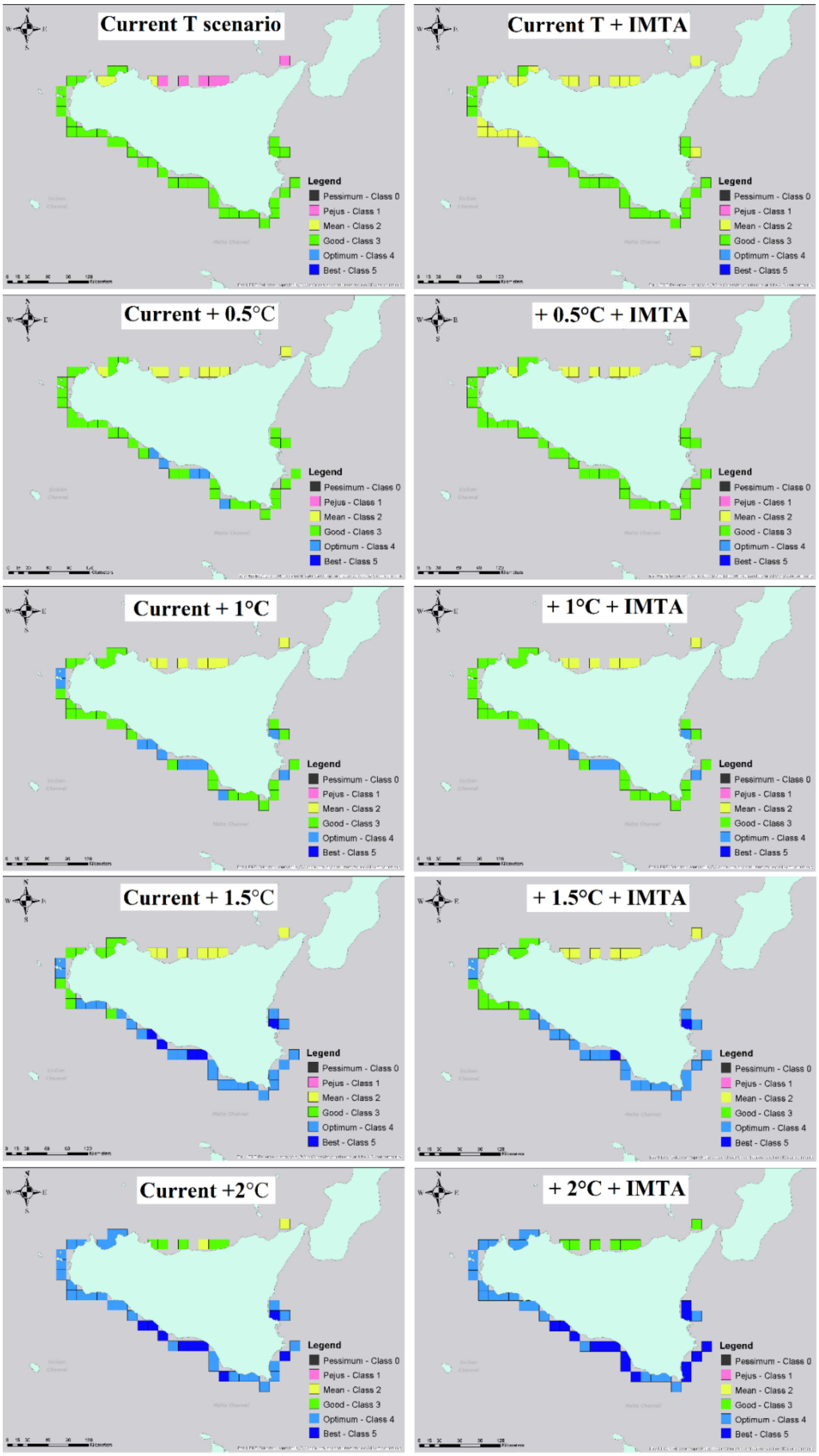


Figure 3: Maps showing the percentile class in which each pixel around Sicily falls when combining fishery and aquaculture target species. On the left panel of each rows the mapped outcome obtained by cultivating species separately; on the right panel the mapped outcome obtained when species were combined in IMTA. From top to bottom mapped outcome under increasing temperature conditions from current up to current +2°C (step 0.5°C; the first panel of each column is the current, the last panel current +2°C). Black = "Pessimum" condition (Class 0, 97.5% percentile, *i.e.* $\mu+2\sigma$); Pink = "Pejus" condition (Class 1, 84.1% percentile, *i.e.* $\mu+1\sigma$); Yellow = "Mean" condition (Class 2, 50% percentile, *i.e.* μ); Green = "Good" condition (Class 3, 15% percentile, *i.e.* $\mu-1\sigma$); Light blue = "Optimal" condition (Class 4, 2.5% percentile, *i.e.* $\mu-2\sigma$); Blue = "Best" condition (Class 5, 0.1% percentile, *i.e.* $\mu-3\sigma$).

4 DISCUSSION

Our integrated experimental and modelling approach shows that the increasing temperature within the current thermal species-specific boundaries of present target organisms will cause a general shortening of both cultivation time and length at first maturity (TIME) with an effect which was variable among present target species. The fishery species will be subjected to a larger reduction, up to two months (about 20% under the scenario +2°C) in the time to reach the puberty size, while the effect of increasing temperature on the aquaculture species will be much smaller. While it is not the purpose of this study to comment on the potential adaptive and economic implications induced by our highlighted climate change temporal shifts, we notice that there is a growing body of research showing that climate change induces spatial shifts in terms of distributions towards the poles (Poloczanska et al. 2013), to deeper waters (Dulvy et al. 2008) or following temperature velocity (Pinsky et al. 2013). Also, current literature reports numerous cases of phenological alterations which make species more vulnerable to increasing temperature (Helmuth et al. 2014) and other climate stressors. Our analysis aims to show how a DEB functional-based approach is able to provide quantities to operationalize the management of some marine activities at local scale. Nevertheless, up to date, DEB has not applied to assess the potential role of phenotypic plasticity in pushing adaptation of species under climate change; this will be a fruitful research ground for DEB scientists, evolutionary biologists and ecologists in the near future. Moreover, we preferred to perform a sensitivity analysis which is more useful than climate projections as those provided by IPCC. While these projections are routinely applied and provide a picture of the potential availability of thermal habitats, they still involve a recognized uncertainty around climate projections and are not able to provide absolute estimates of change in biological traits or range shifts (Payne et al. 2015; Kleisner et al., 2017).

Our modelling outcomes under the current scenario agrees with the common cultivation time for European seabass at these latitudes (about 22-24 months *i.e.* from 668 to 740 days; FAO CASIP 2004; FAO 2014; Sarà et al. 2018c). DEB outcomes for cultured bivalves was in agreement with the reality (*e.g.* Sarà et al. 2012; FAO 2014; Martinez et al. 2018) such as that of the European anchovy, whose predicted values fell very well within those reported in the current literature for Sicilian Channel anchovy (Basilone et al. 2006; FAO 2014).

Our outcomes from the two bivalves aquaculture target species showed that the trophic enrichment due to IMTA potentiates the effects of increasing temperature on TIME in a synergistic combination (*sensu* Gunderson et al. 2016; Sarà et al. 2018a, b, c). A similar effect is not new across the current literature that shows trophic augmented status (i.e. the trophic enrichment due to eutrophication) can work as a buffering factor being able to reduce the detrimental effects of stressor disturbance such as sea acidification (*e.g.* Connell et al. 2017; 2018). The trophic enrichment due to the IMTA practice produces a comparable effect on our tested aquaculture target species (*M. galloprovincialis* and *C. gigas*), although it is not possible to evaluate the economic effects of the further shortening of time to reach the commercial size, it might not necessarily represent a positive outcome.

When the DEB functional-traits outcome is translated at spatial level, by a spatially-contextualized and mapped analysis, it generates easy-to-read maps which are useful to engage with the stakeholders. They can easily identify and proactively implement adaptive site-specific management strategies tailored to target species. By adopting a spatial resolution of 0.11°, which corresponds to about 13 km pixels, we are able to underline some spatial bottlenecks where both fisheries and aquaculture will be not supported by local conditions or where they can be a win-win solution and become successful drivers for local economies. Our analysis allowed to highlight as the Northern Sicilian areas will become unsuitable in the future to both aquaculture activities of *D. labrax* - our target species combined in IMTA - and for the fisheries species, *E. encrasicolus,* both are weakened by the increasing temperature (*e.g.* black pixels under current + 1°C scenario). Such an analysis can help stakeholders and decision-makers to visualize those areas to be devoted to alternative activities at sea favoring the development of other sectors rather than fisheries and aquaculture. On the other hand, designing specific downscaled management measures can be easy at this or higher spatial resolutions. Nonetheless, having seen the difficulty of gathering data at a sufficient scale to feed an adaptive EBM, the objective of oceanographers and climatologist should be that of increasing the resolution of their scenarios to increase the accuracy of the local-scale tailored management measures. This should be one of the most important priorities for an adaptive EBM to be able to provide tailored management measures based on ecological functioning principles at a sufficient scale to be realistic in order to reduce future socio-economical conflicts when preserving the ecosystems. The use of mechanistic-derived quantities and high resolution spatial analysis in ecology and resource management science can help us to adopt a medical analogy: the “actual patient care should be highly individualized, and patient treatment should not be based on the results of broad-scale generalizations, without considering the patient’s history, risk factors and other medications” (*literally* Helmuth et al. 2014). Thus predictions of environmental effects at local scale on biological responses should not be based on only mean conditions of environmental regimes (*e.g.* monthly, annually), but should rely on higher resolution data (at least daily). A broad-brush approach could be appropriate if high resolution data are lacking, but the present-day technology (*e.g.* satellite and remote sensing; Capodici et al. 2018) and recent scientific advancement (*e.g.* DEB theory) offer impressive improvement of the temporal and spatial resolution of many types of data needed to feed regional management strategies. Thanks to DEB, we are now able to incorporate such kind of "*patient tailored*" information needs to develop appropriate tailored marine resource management.

In conclusion, our final maps can be read as a quantitative informational baseline indicator of climate risk that can be shared and discussed by scientists, policy makers and stakeholders when producing management plans at local level under pressures of climate change. The quantitative mapping of changes in species' thermal habitats and growth performance are an easy-to-communicate-tool that allows to enlarge the common people's understanding while narrowing the science-policy communication gap and ensuring a more interactive science-policy interface (Kearney & Porter, 2009; Hickey *et al.*, 2013; Shelton, 2014; Pacifici *et al.*, 2015; Payne *et al.*, 2015; Gluckman, 2016; Mangano and Sarà 2017). Our analysis, focused on a single fishery species and one aquaculture fish, is reductive although our main aim was to show the feasibility of the approach and the effectiveness of the message. Thus, we suggest extending this mechanistic approach to other fishery and aquaculture species, exploiting the power of species-specific biological traits (*sensu* Courchamp *et al.*, 2015). This would help generate predictions about multispecies trade-offs in space and time as well as identify winners and losers in the face of climate change (Sarà et al. 2018c). Freely available multispecies trade-off maps may represent a desirable tool to drive decision-makers, stakeholders and public opinion in developing adaptation and mitigation solutions at biologically- and ecologically-relevant spatio-temporal scales. Our DEB functional-based approach and the provided scenario-based quantitative maps, showing different simulation outcomes, can represent a tool to analyze and help narrow the field of action to be taken in place (e.g. Decision Support Tools; *sensu* Punt et al. 2016) and to discuss possible future activities on which to build local socio-economies (Mullon et al., 2016; Fernandes et al., 2017). This will allow the fostering of the resilience of the socio-economic environment (i.e. more adaptive, flexible to change) when based on marine resources that respond to rapid changes in a climate change context (Ogier et al. 2016). An adaptive management based on multiple species mechanistic quantities - according to the innovative Ecosystem-Based Fisheries Management (EBFM) - are what European coastal countries need today.

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CHAPTER 6

Predicting the multiple effects of acidification and hypoxia on *Mytilus galloprovincialis* (Bivalvia, Mollusca) life history traits

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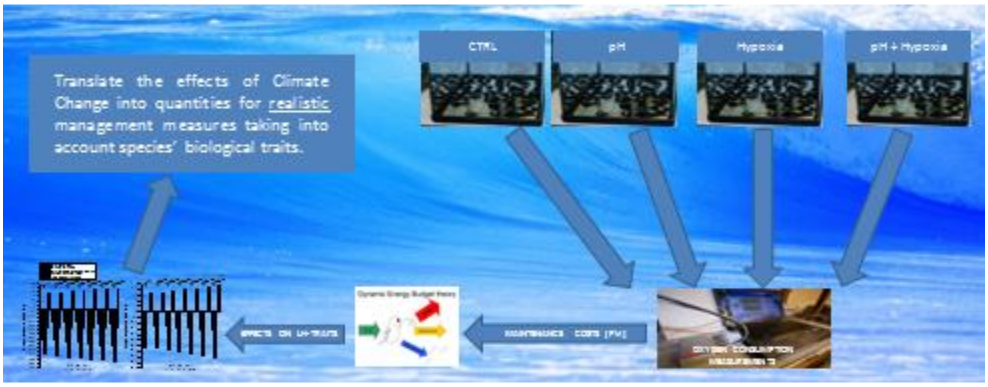

Translate the effects of Climate Change into quantities for <u>realistic</u> management measures taking into account species' biological traits.

ABSTRACT

Many recent studies have revealed that the majority of environmental stressors experienced by marine organisms occur at the same time and place, and that their interaction may complexly affect a number of ecological processes. Here, we experimentally investigated the effects of pH and hypoxia on the functional and behavioural traits of the mussel *Mytilus galloprovincialis*, we then simulated the potential effects on growth and reproduction trough a Dynamic Energy Budget (DEB) model under a multiple stressor scenario. Our simulations showed that hypercapnia had a remarkable effect on growth and reproduction differentially as a function of the trophic conditions. This study showed the major threat represented by the hypercapnia and hypoxia phenomena under the current climate change context, and that a mechanistic approach can illustrate complex and site-specific effects of environmental change, producing that kind of information useful for management purposes, at larger temporal and spatial scales.

Key words: Acidification; Climate change; DEB Model; Hypoxia; *Mytilus galloprovincialis*; Multiple-Stressor; Mussel.

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Abstract

Many recent studies have revealed that the majority of environmental stressors experienced by marine organisms occur at the same time and place, and that their interaction may complexly affect a number of ecological processes. Here, we experimentally investigated the effects of pH and hypoxia on the functional and behavioural traits of the mussel Mytilus galloprovincialis, we then simulated the potential effects on growth and reproduction trough a Dynamic Energy Budget (DEB) model under a multiple stressor scenario. Our simulations showed that hypercapnia had a remarkable effect on growth and reproduction differentially as a function of the trophic conditions. This study showed the major threat represented by the hypercapnia and hypoxia phenomena under the current climate change context, and that a mechanistic approach can illustrate complex and site-specific effects of environmental change, producing that kind of information useful for management purposes, at larger temporal and spatial scales.

Keywords	Acidification; Climate change; DEB Model; Hypoxia; Mytilus galloprovincialis; Multiple-Stressor; Mussel
Taxonomy	Functional Ecology, Ecological Modeling, Marine Ecosystems, Global Ecological Change, Ecology
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1 INTRODUCTION

Since the dawn of research investigating the possible effects of ocean acidification (OA) on aquatic organisms (e.g. Bamber, 1990), most studies have shown that elevated pCO_2 levels, as predicted for the next century, may affect to some extent the functional traits (Schoener, 1986; Koehl, 1989) of marine organisms (Feely et al., 2004; Navarro et al., 2013). Referring to functional traits, we consider all those specific traits that define each species in terms of their ecological roles (Diaz & Cabido, 2001), and thereby the species' identity. In marine ectotherms such as bivalves, crabs, sea urchins and fish, these traits include tolerance and sensitivity to environmental conditions (e.g. physiological tolerance limits - Kearney & Porter, 2009) defining the ability of each species to support their own metabolic machinery (Sokolova et al., 2012; Sarà et al., 2014). They further include the ability to obtain energy from food, the so-called functional response (Holling, 1959) or those behavioural (e.g. swimming behaviour, habitat use, mating system) and morphological (e.g. shape, thickness) traits which led to optimise the energetic income (Krebs & Davies, 1992) and lastly to reach the ultimate fitness (Roff, 1992).

Research performed over the last decade and summarized in the recent IPCC (2014) report clearly shows that OA will affect marine organisms and ecosystems (Connell et al., 2017, 2018) in the coming decades, and such projections have stimulated new research that aims to understand the impact on calcifying marine organisms. Reductions in growth and calcification rates are just those kinds of the physiological impacts of OA (Thomsen et al., 2013; Byrne, 2012; Beniash et al., 2010). While much research showed that low pH may impair most functional traits (e.g. respiration, ingestion, absorption and excretion; Pörtner et al., 2004), functions connected with energy uptake such as feeding and assimilation seem to be reduced at a larger extent in many species with expected implications for the amount of energy available for growth and reproduction (Kurihara et al., 2008; Appelhans, 2012; Navarro et al., 2013; Zhang et al., 2015; Sui et al., 2016). Such information has been obtained through both acute and chronic exposure to OA but no studies are yet available to assess the potential effects of OA on the magnitude of other Life History (LH) traits, such as maximum habitat body size, fecundity, time to reach maturation and the number of spawning events under future conditions of environmental change (*sensu* Kearney and Porter, 2009; Sarà et al., 2011; 2013b). Thus, apart from long term experiments carried out in few field sites worldwide (e.g. Ischia [Hall-Spencer et al., 2008] and Vulcano [Duquette et al., 2015] islands) in the Southern

Mediterranean Sea and in other Seas (Maug Island [Pala, 2009] or SW Pacific [Connell et al., 2017]) where lowered pH seawater is naturally available, the recent introduction of mechanistic functional trait-based (FT) models based on the Dynamic Energy Budget theory (DEB; Kooijman, 2010) can offer a reliable opportunity for disentangling the effect of OA on LH traits.

Functional trait-based DEB (FT-DEB) approach (Kearney and Porter 2009; Sarà et al., 2011, 2012, 2013a, b, 2014, 2018c; Mangano et al., 2018) relies on the quantitative prediction of organismal functional traits and fecundity within the fundamental niche limits of one particular species (Hutchinson, 1957). Such an approach aims to exploit mechanistic rules to connect environmental human-induced variability to functional traits (Schoener, 1986; Diaz & Cabido, 2001) and in turn functional traits to species LH (Stearns, 1992) traits. The novelty of the FT-DEB approach relies on its intrinsic mechanistic nature deriving from the fact that it is based on flux of energy and mass through an organism which are traceable processes that are subject to conservation laws (according to the new posited concept of ecomechanics; Denny & Helmuth, 2009; Denny & Benedetti-Cecchi, 2012; Carrington et al., 2015). This provides an exceptionally powerful tool to predict organismal functional traits, capturing variation across species to solve a very wide range of problems in ecology and evolutionary biology (Lika et al., 2011; Kearney, 2012; Pouvreau et al., 2006; Pequerie et al., 2010; Sarà et al., 2011; 2013b; 2014). FT-DEB could provide information about the effect of OA on the fecundity (as expressed by the number of gametes per life span, the so-called Darwinian fitness; Bozinovic et al., 2011) and the degree of reproductive failure of species providing theoretical predictions about LH traits having implications on population dynamics and community structure throughout the species range (Sarà et al., 2013a).

Here, we specifically exploited the FT-DEB model spatially and explicitly contextualised along the Italian coasts under subtidal conditions, using four-year thermal series and satellite chlorophyll-a (CHL-a) concentrations, to test the multiple effect due to the combination of pH and hypoxia on the physiological and behavioural traits of our target species, the bivalve *Mytilus galloprovincialis* (Lamarck 1819). Recent insights obtained by the experimental research have shown that OA mainly affects feeding rates (FR), assimilation efficiency (AE) and volume-specific somatic maintenance costs $[\dot{p}_M]$ of marine organisms (Appelhans, 2012; Navarro et al. 2013; Kroeker et al. 2014; Zhang et al., 2015; Jager et al. 2016). Here, we translated the combined effects of hypoxia and hypercapnia on assimilation and oxygen consumption rates as measured under different treatments into effects on AE and $[\dot{p}_M]$. Somatic maintenance is a suite of functional traits used in recent bioenergetics based on the

DEB theory that mechanistically can be used to investigate the role played by multiple stressors on LH traits of organisms by using first principles (Sarà et al., 2014). We further documented the effects of those stressors on *M. galloprovincialis* shells through the use of a scanning electron microscope (SEM) and compared the maximum shell breaking load of treated *vs.* control specimens. A behavioural analysis completed the frame concerning the individual's response to both single and combined stressors. Carried out in a context of OA, this exercise comprises a first step in linking the fields of ecomechanics and climate change ecology, which should yield a more mechanistic understanding of how biodiversity will respond to environmental change (*sensu* Buckley et al., 2012).

2 MATERIALS AND METHODS

This study consisted of three steps: 1) laboratory investigation on the effects of pH and hypoxia on functional (both behavioural and physiological) traits of *Mytilus galloprovincialis*; 2) collection of water temperature data and CHL-a data from two Mediterranean sites (Trieste and Palermo), as a further forcing variable in the DEB model and lastly 3) model running to simulate growth and fitness of *M. galloprovincialis* under stressful conditions by using DEB parameters estimated by activities in the first step.

2.1 Experimental set-up. Specimens of *M. galloprovincialis* (45 - 55 mm) were provided by the Ittica Alimentare company (Soc. Coop., Palermo) and transferred within 30 minutes to the laboratory. Mussels were then carefully cleaned and placed in a 300L tank filled with natural seawater at room temperature (18-20°C), field salinity (37-38) and fed *ad libitum* with cultured *Isochrysis galbana* (Sarà et al., 2011). Mussels were acclimated for two weeks to reduce stress generated by manipulation and transport (Sarà et al., 2013a) and once acclimated, 200 specimens were randomly divided in groups of 25 organisms, transferred to 8 independent rectangular glass tanks of 120L capacity (100 cm long, 30 cm deep, 40 cm wide) and kept in a conditioned room at 21°C for 4 weeks according to common protocol with bivalves (Braby & Somero 2006; Fields et al., 2012; Kittner and Riisgård 2005). Tanks 1 to 4 were filled with sea water and continuously aerated through air pumps, while Tanks 5 to 8 were not aerated and covered with a plastic film disposed on the water surface, in order to avoid gas-exchanges between air and water. Tanks 1-2 were used as a control (CTRL), while hypercapnia was imposed in Tanks 3-4 (Tr1), hypoxia (2 ppm) in Tanks 5-6 (Tr2), and both factor (pH 7.5 and hypoxia) in Tanks 7-8 (Tr3) (see Table 2).

Table 2. Seawater carbonate chemistry parameters (mean ± se). Seawater pH on the NBS scale (pH_{NBS}), temperature (T; °C), and salinity were used to calculate CO_2 partial pressure (pCO_2; µatm) as well as aragonite and calcite saturation states (respectively Ωar and Ωca), for a total alkalinity of 2500 mmol kg^{-1}.

	Measured				Calculated			
	Temperature (°C)	pH_{NBS}	O_2 mg/l	Salinity (PSU)	pCO_2 (µatm)	CO_3^-	Ωca	Ωar
CTRL	20.77 ± 0.01	8.01±0.001	7.29±0.02	37.18±0.11	624.31±4.9	167.93±0.95	3.95±0.02	2.58±0.01
Tr1	20.77 ± 0.01	7.53±0.002	7.30±0.02	37.12±0.05	2151.17±22.02	62.05±0.73	1.46±0.02	0.95±0.01
Tr2	20.77 ± 0.01	8.01±0.001	2.44±0.02	37.07±0.04	729.88±18.24	152.53±1.51	3.59±0.04	2.34±0.02
Tr3	20.77 ± 0.01	7.53±0.002	2.44±0.02	37.21±0.17	2238.83±20.72	59.59±0.42	1.40±0.01	0.91±0.01

Mussels were acclimated to two different nominal pH treatments: (i) pH 8.0 in Tanks 1-2 (CTRL) and 5-6 (Tr2), corresponding to present average pH at the sampling site; and (ii) pH 7.5 in Tanks 2-3 (Tr1) and 7-8 (Tr3), deviating from present range of natural variability and relevant for 2100 OA scenarios (see Table 2 for calcium carbonate saturation status) (Melzner et al., 2011; Gazeau et al., 2013). The carbonate system speciation (*p*CO2, HCO_3^-, CO_3^{2-}, ΩCa and ΩAr) was calculated from pH_{NBS}, temperature, salinity and alkalinity (T_A = 2.5 mM; Rivaro et al., 2010) using CO2SYS (see Table 2; Lewis and Wallace, 1998) with dissociation constants from Dickson & Millero (1987). Previous studies reported that excretion of ammonium and phosphate may be able to alter total alkalinity, but this variation was showed to be minimal and can be neglected (Gazeau et al. 2007; Mingliang et al. 2011, Waldbusser et al. 2011), specially at the low mussel densities as those adopted in our experimental setup. The pH was manually controlled 8 times a day by an electronic pH-meter (Cyberscan 510, Eutech Instruments; accuracy = ± 0.01 pH) and gaseous CO_2 was injected directly into the aquarium through a commercial ceramic diffusor, when required. Oxygen concentration and temperature were monitored with the same frequency through the PiroScience FirestingO2 oxygen logger equipped with a dedicated temperature sensor. Water movement and recirculation were assured by water pumps. Tanks were siphoned at the end of each day, removing all the faecal material in order to avoid the accumulation of waste products, and 20% of water was weekly changed with specific pre-conditioned sea water for each treatment. Each tank was further equipped with biological filter and a skimmer to remove organic compounds such as food and waste products potentially affecting water quality.

2.2 Behavioural observations. The valve gape of mussels was recorded by means of the two simplest behavioural categories reported in Jørgensen et al. (1988): closed valves and opened valves. Each observation was carried out by an operator with the aim to record changes in the behavioural repertoire of bivalves in response to the exposure to a single stressor (pH or hypoxia) and to both pH and hypoxia, compared to individuals kept in normal environmental conditions. All experiments were conducted at environmental (37-38) salinity and with well-aerated sea water through a gentle flow (Ameyaw-Akumfi & Naylor, 1987), except for specimens of Tank 5-6 and 7-8, that were not aerated in order to maintain the hypoxia level set through the gaseous nitrogen. Behavioural observations were repeated six times a day at the end of week 1 and 4 of exposure, involving 5 random specimens for each treatment.

2.3 Oxygen consumption. The rate of oxygen consumption was determined twice (week 1 and week 4) in a respirometric glass chamber (0.3L) inside a temperature-controlled water bath, in order to investigate the effects of multiple stressors on metabolic somatic maintenance costs and to integrate it in the standard DEB model. Volume-specific somatic maintenance costs, as expressed by the [$\dot{p}_M$] parameter (J cm^{-3} h^{-1}), represent the amount of energy needed to fuel basal metabolism ($\dot{p}_M$) scaled with the organisms' volume ([$\dot{p}_M$] = $\dot{p}_M/V$). All determinations were performed at 21°C using filtered seawater with the same pH and oxygen content as that of the respective treatment, stirred with a magnetic stirrer bar beneath a perforated glass plate supporting each individual (Ezgeta-Balic et al., 2011). The decline in oxygen concentration was measured by a PiroScience FirestingO2 respirometer, capable of four sensor connections. We used a total of n = 64 mussels per week, 16 for each treatment (8 for each tank) acclimated as above, fed *ad libitum* until the day before the experiment. The decline was continuously recorded for at least 1 h, excluding an initial period (~ 10 min) when usually there is a more rapid decline in oxygen caused by a disturbance of the sensor's temperature equilibration. Respiration rate (RR, μmol O_2 h^{-1}) was calculated according to (Sarà et al., 2013b): $RR = (C_{t0} - C_{t1})x\, Vol_r x 60(t_1 - t_0)^{-1}$, where C_{t0} is oxygen concentration at the beginning of the measurement, C_{t1} is the oxygen concentration at the end of the measurement, and Vol_r is the volume of water in the respirometric chamber. Volume-specific somatic maintenance costs were then calculated by converting oxygen consumption rates expressed in μmol h^{-1} in J h^{-1} through a conversion factor (Kooijman 2010) and then in J cm^{-3} (van der Veer et al., 2006; Ren and Schiel 2008) (for the calculation of dry weights refer to the end of section 6.2.4).

2.4 Assimilation efficiency. Assimilation efficiency (AE) was measured through the Conover ratio (1966) $\mathrm{AE} = (\mathrm{F} - \mathrm{E})/[(1 - \mathrm{E})\mathrm{F}]$, where F is the ratio between ash-free dry weight (AFDW) and dry weight (DW) for food, and E is the same ratio for the faeces; this represents the efficiency with which organic material is absorbed from the ingested food. Here, after oxygen consumption measurement, the same specimens were placed into separate beakers containing 1L of filtered seawater (specific for each treatment) and a magnetic stirrer bar. In order to allow the mussels to open their valves and start their filtration activity, they were given 15 minutes before the introduction of food with an initial concentration of ~ 15,000 *Isochrysis galbana* cells ml^{-1}. After a period of 2 h mussels were moved to cleaned 1L glass beakers with filtered seawater for a period of 12 h, after that the water contained in each beaker was filtered on pre-ashed and weighted GF/C fibreglass filters. Once filtered, filters were washed with 0.5 M ammonium formate (purest grade) to remove adventitious salts (Sarà et al., 2013a), dried in the oven (95°C for 24 h) and then incinerated in a muffle furnace (450°C for 4 h). After each step, the samples were weighted using a balance (Sartorius BL 120S ± 1µg). For the calculation of AE, together with the faeces collected from the mussels, filters containing algal food were dried and incinerated as above. After respirometric measurement and the collection of faeces each animal was killed by gentle freezing and dissected, and the shells were separated from the body tissue to calculate the condition index according to Davenport & Chen (1987) (CI = (body weight/shell weight) × 100), and their individual dry weights to standardize respiration rates.

2.5 Water temperature data. The main forcing driver of shellfish LH inside DEB models is represented by seawater temperature (Pouvreau et al., 2006; Kearney et al., 2010; Kooijman, 2010; Sarà et al., 2011; 2013). DEB simulations were run under subtidal conditions (body temperature was expressed by the mean seawater temperature; Montalto et al., 2014) with 4 years-hourly data (Jan 2006 - Dec 2009) of seawater temperature measured about 1 m below the surface at the closest meteo-oceanographic station held in Trieste (LAT 45° 38′ 57.81″; LONG 13° 45′ 28.58″) and Palermo (LAT 38° 07′ 17.08″; LONG 13° 22′ 16.79″). The period of 4 years is consistent with the normal life span of most Mediterranean shellfishes (Sarà et al., 2012; 2013b). Both sites were chosen as they represent two opposite temperature and food conditions for mussel growth in Italy, with Trieste as representative of lower temperature (average 16.98 ± 6.19 °C) and higher food levels (average 1.36 ± 0.37 CHL-a), and Palermo of higher temperatures (average 20.19 ± 4.64 °C) and lower food (average 0.19 ± 0.09 CHL-a). Data are available online from the Italian Institute of Environmental Research (ISPRA) web page (http://www.mareografico.it/).

2.6 CHL-a dataset. CHL-a was derived from satellite imageries (µg L^{-1}; http://emis.jrc.ec.europa.eu/) and adopted as a reliable food quantifier for suspension feeders (Kearney et al., 2010; Sarà et al., 2011; 2012)

2.7 Model description. The Dynamic Energy Budget (DEB) theory provides a general framework that allows to describe how physiological mechanisms are driven by temperature and food availability, and influences growth and the reproductive performances in marine organisms (Sousa et al., 2010; Monaco et al., 2014; Jusup et al., 2017). Following the κ-rule (Kooijman, 2010) a fixed energy fraction (κ) is allocated to growth and somatic maintenance, while the remaining fraction (1-κ) is allocated to maturity maintenance plus maturation or reproduction (Fig. 1a).

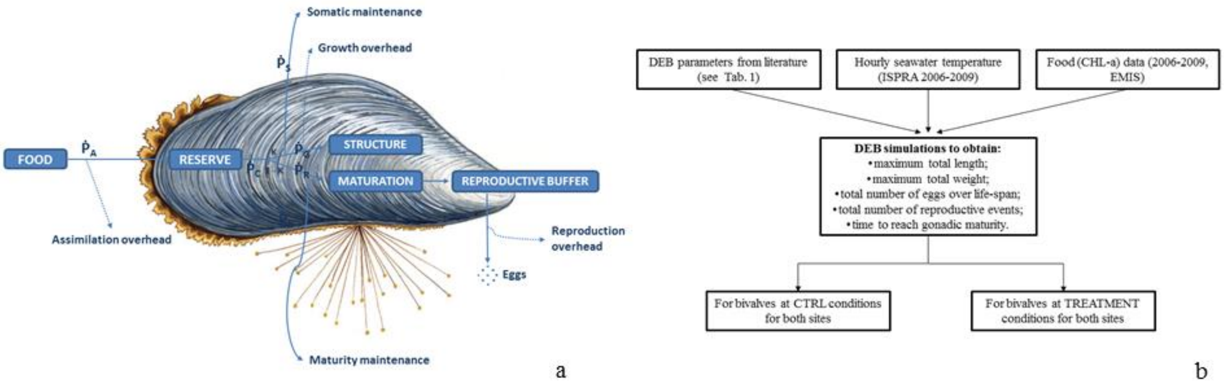

Fig. 1a) Schematic representation of the standard Dynamic Energy Budget model (Kooijman 2010) and the flux of energy through an organism coming from the environment; $\dot{P}_A$ = assimilation flux, $\dot{P}_C$ = mobilisation flux, $\dot{P}_G$ = growth flux, $\dot{P}_R$ = reproduction flux, $\dot{P}_S$ = somatic maintenance flux; and **1b)** flow diagram of study approach (modified from Sarà et al., 2012).

If the general environmental conditions deviate from common natural patterns (i.e. changes in temperature, food availability etc.) reproduction and growth are consequently affected. According to the DEB theory, a reduction in growth can be caused either by reduced food assimilation ($\dot{p}_A$), enhanced maintenance costs ($\dot{p}_M$), or enhanced growth costs ($\dot{p}_G$). Using this approach, and through the DEB parameters reported in Table 1, except for the variation in the maintenance costs [$\dot{p}_M$] and in the assimilation efficiency of food (AE) which were experimentally estimated throughout this study, we performed simulations using a standard version of the DEB model (Nisbet et al., 2010) aimed at investigating the potential variations in growth and fecundity of our model species (Fig. 1b).

Table 1. DEB parameters for *Mytilus galloprovincialis* (1 = Kooijman, 2010; 2= van der Veer et al., 2006; 3 = Sarà et al., 2011; 4 = Thomas et al., 2006; 5 = Schneider, 2008).

Symbol	Description	Units	*Mytilus galloprovincialis*	
			Value	Ref
V_b	Structural volume at birth	cm^3	0.0000013	1
V_s	Structural volume at seeding	cm^3	-	-
V_p	Structural volume at puberty	cm^3	0.06	2
δ_m	Shape coefficient	-	0.225	3
$\{J_{Xm}\}$	Maximum surface area-specific ingestion rate	$J\ cm^{-2}h^{-1}$	8.2	4
ae	Assimilation efficiency	-	0.88	3
X_K	Saturation coefficient	$\mu g\ l^{-1}$	2.1	3
$[E_G]$	Volume-specific cost of growth	$J\ cm^3$	5993	5
$[E_m]$	Maximum storage density	$J\ cm^3$	2190	2
$[\dot{p}_M]$	Volume-specific maintenance cost	$J\ cm^{-3}\ h^{-1}$	1	2
κ	Fraction of utilized energy spent on maintenance and growth	-	0.7	2
K_R	Reproduction efficiency	-	0.8	3
T_A	Arrhenius temperature	°K	7022	2
T_L	Lower boundary of tolerance range	°K	275	2
T_H	Upper boundary of tolerance range	°K	296	2
T_{AL}	Rate of decrease at lower boundary	°K	45430	2
T_{AH}	Rate of decrease at upper Boundary	°K	31376	2

The idea of quantitatively assess the effect of a stressor including it as a modification of a specific parameter was first introduced by Jager et al. (2016) with the *stress factor* “s” applied to assimilation, maintenance and costs of growth. Thus, after estimating the effect induced by a treatment on the oxygen consumption, in our case expressed as percentage variation, we summed/subtracted the energetic amount due to the effect of a stressor (Giacoletti et al. 2018) to the species-specific $[\dot{p}_M]$ parameter of *M. galloprovincialis* (Sarà et al. 2011), then we run our models. Previous proposed approaches, taking into account starvation for $[\dot{p}_M]$ estimation, wouldn’t be realistically applicable for testing and quantifying the effect of a stressor on the energy budget, without adding a further stressor. Jager et al. (2016) was therefore the first to adopt this concept, although using a simplified DEB model (DEBkiss; Jager et al. 2013) that did not involve the concepts of reserve and maturity that play a central role in DEB theory. Although this may not be considered a reliable measure of maintenance costs but a simpler proxy of metabolic effect, negligible costs for growth and gonadic development stand on the assumption of constant protein turnover throughout the experimental range (Hawkins et al. 1989).

To run DEB simulations, local thermal series of selected sites were used together with satellite CHL-a concentrations, obtaining a first model with environmental conditions. A second model was run with the $[\dot{p}_M]$ calculated from the oxygen measurements on specimens of *M. galloprovincialis* from Tanks 3-4 (pH 7.5) simulating a chronic hypercapnia condition for the full cycle (4 years) and the relative estimated AE. Subsequently, a sensitivity analysis was applied to simulate the effect of one random hypoxia event (duration = 30 days) for each of the four years of the cycle, then simulating two yearly events, and so on up to six hypoxia events. The starting month of each event was randomly chosen for every year with the use of a table of random digits. The $[\dot{p}_M]$ calculated from the oxygen consumption rate measurements on specimens from Tanks 7-8 (pH 7.5 and hypoxia) was used in substitution to $[\dot{p}_M]$ from pH 7.5 tanks 3-4, coupled with the relative estimated AE, when simulating both stressors. Simulations were performed using the R routine and the MS excel spreadsheet for Standard DEB model developed by Kearney (2012), and further modified (for use in bivalve modelling) by Sarà et al. (2013a). Outputs of the DEB models were: the maximum theoretical total length of shellfish (TL), the maximum total weight (TW), the total number of eggs (TRO) produced during a life-span of 4 years, the total number of reproductive events (RE) and the time needed to reach gonadic maturity (TM) for each treatment.

2.8 Effects on shell: mechanical strength and SEM pictures. The functional impact of exposure to pH, to validate the effect on morphological structure of valves, was tested on mussels exposed to the two nominal pHs for 4 weeks. Twice (week 1 and week 4), 16 mussels for each treatment were collected and dissected, and both valves were cleaned and dried with absorbent paper. The left valve was then sliced transversely using a circular saw (Dremel® 300 series) to section the whole length of the shell. Age was estimated using the analysis of shell rings proposed by Peharda et al. (2011) by counting the number of rings with the use of a stereo microscope (Leica EZ4). The right valves were instead evaluated for their breaking properties through crushing tests (maximum breaking load in N) as previously done in Martinez et al. (2018). The effect of low pH exposure was also documented by the use of a scanning electron microscope (SEM; Zeiss LEO 440) that led to a thorough investigation on the integrity of the mussels' external protein layer (*periostracum*) and on the underlying mineral layer, rich in calcite and aragonite.

2.9 Statistical analysis. The assumption of normal distribution has been tested through the Anderson–Darling test using Past® software. In order to test for significant differences in respiration rate and the assimilation efficiency, ANOVAs were performed using Treatment (CTRL, Tr1, Tr2, Tr3) and Time (Week 1 and Week 4) as fixed factors, with respectively four and two levels. In order to test for significant differences in behavioural categories, ANOVAs were performed using Treatment (CTRL, Tr1, Tr2, Tr3) as fixed factors, while Breaking load was tested with Treatment (CTRL, Tr1, Tr2, Tr3) and Time (Week 1 and Week 4) as fixed factors. When significant differences were detected, the Student-Newman-Keuls (SNK) post-hoc pair wise comparison of means was used (Underwood, 1997). Cochran's test was used prior to ANOVA to test the assumption of homogeneity of variance (Underwood, 1997). When no homogeneous variances were rendered with any type of transformation, the significance level was set at 0.01 instead of 0.05, as ANOVA can withstand variance heterogeneity, particularly in large balanced experiments, thereby reducing the possibility of a Type I error (Underwood, 1997).

3 RESULTS

3.1 Valve gaping. During behavioural observations on *M. galloprovincialis*, specimens showed a significant difference in the behavioural categories, showing respectively 33.3 ± 11.2 % (CTRL), 50 ± 4.5 % (Tr1), 80 ± 8.9 % (Tr2) and 83.3 ± 6.1 % (Tr3) of opened valves (Fig. 2; Table 3, ANOVA, $p < 0.001$) at week 4 (Fig. 2). The percentage of closed valves can be easily calculated as 100 – open valves. No significant differences resulted between week 1 and 4 (ANOVA, $p > 0.05$), between CTRL and Tr1 and between Tr2 and Tr3.

Table 3 ANOVA table of results. Effect on valve gape and breaking load of *Mytilus galloprovincialis* (* = p < 0.05; ** = p < 0.01; *** = p < 0.001; ns = not significant).

Source	df	Valve gape			Source	df	Breaking load		
		MS	F	P			MS	F	P
Treatment (Tr)	3	19.24	17.97	***	Treatment (Tr)	3	3838.12	15.18	***
Time (Ti)	1	0.18.75	0.18	ns	Time (Ti)	1	777.19	9.22	**
Tr x Ti	3	0.076	0.07	ns	Tr x Ti	3	132.92	1.58	ns
Residuals	40				Residuals	56			
Cochran's C				ns	Cochran's C				ns

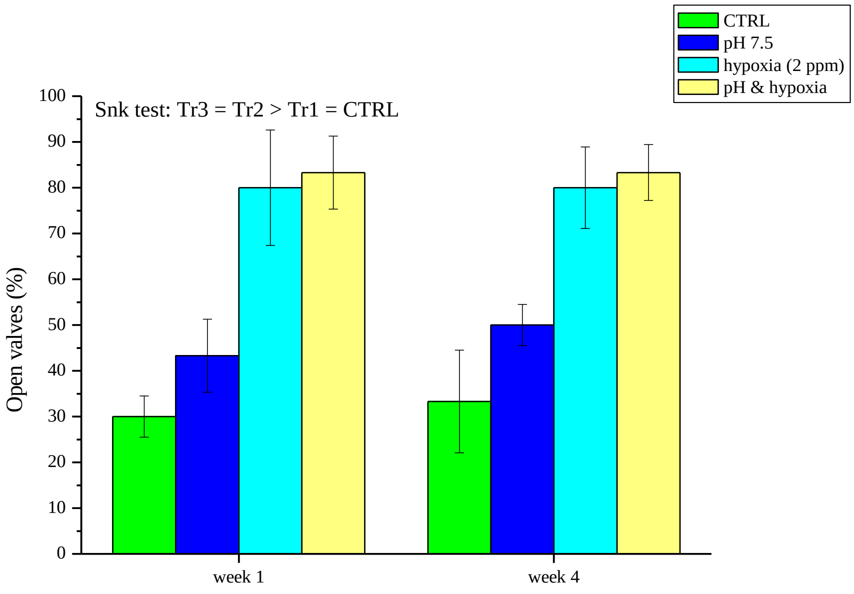

Fig. 2 Behavioural observations (± se) of *Mytilus galloprovincialis* under different treatments of oxygen (normoxia – hypoxia 2ppm) and pH (7.5 – 8.0).

6.3.2 Oxygen consumption. Results showed a significant reduction in the oxygen consumption rate by specimens of *M. galloprovincialis* exposed to treatments (Table 4, ANOVA, $p < 0.01$), although the SNK test revealed no significant differences among the various groups (Fig. 3a). No significant effects were highlighted for the time factor (Table 4, ANOVA, $p > 0.05$), so in Fig. 3a we reported only results for week 4. The rate of oxygen consumption was reduced by up to 42% in Tr1, to 35% in Tr2, and to 41% in Tr3, causing a decrease in the $[\dot{p}_M]$ by up to 29% in Tr1, to 47% in Tr2, and to 49% in Tr3 across the four weeks of exposure.

Table 4 ANOVA table of results. Respiration rate (RR) and assimilation efficiency (AE) of *Mytilus galloprovincialis* (* = p < 0.05; ** = p < 0.01; *** = p < 0.001; ns = not significant).

Source	df	RR st			AE		
		MS	F	P	MS	F	P
Treatment (Tr)	3	312.9183	6.95	***	0.2783	12.21	***
Time (Ti)	1	205.1325	4.56	*	0.0424	1.86	ns
Tr x Ti	3	40.7752	0.91	ns	0.0198	0.87	ns
Residuals	120	45.0271			0.0228		
Cochran's C				*			ns

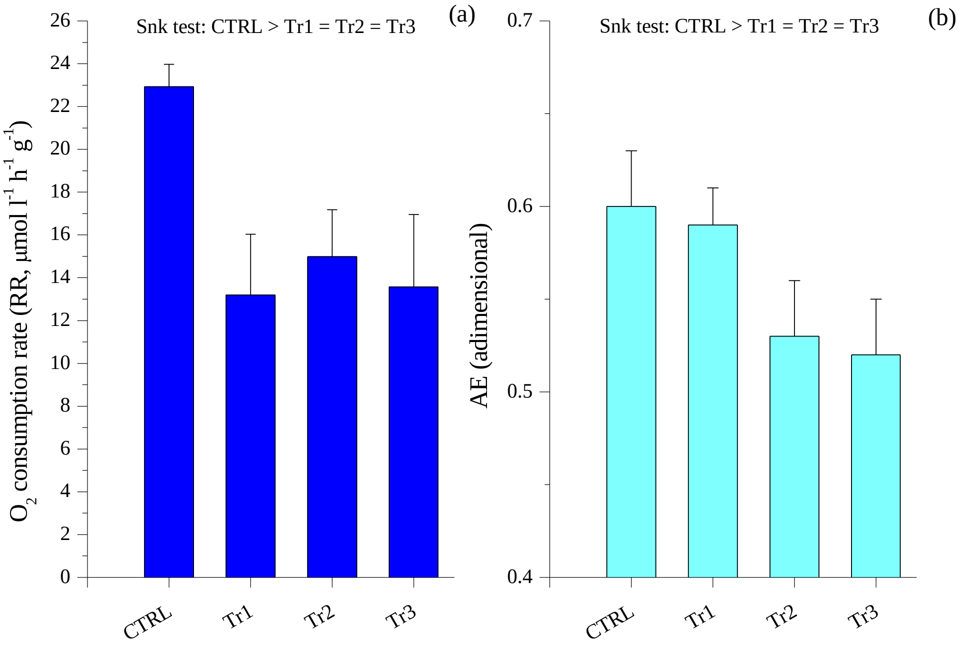

Fig. 3 (a) Oxygen consumption rates (RR) and **(b)** Assimilation efficiency (AE) ± se of *Mytilus galloprovincialis* under different treatments of oxygen (normoxia – hypoxia 2ppm) and pH (7.5 – 8.0) at week 4. CTRL = control; Tr1= pH 7.5; Tr2 = hypoxia 2ppm; Tr3 = pH 7.5 & hypoxia 2ppm.

6.3.3 Assimilation efficiency. Assimilation efficiency of food (AE) was significantly affected by treatments (Table 4, ANOVA, $p < 0.001$) after four weeks of exposure. No significant effects were highlighted for the time factor (Table 4, ANOVA, $p > 0.05$), so in Fig. 3b were reported only results for week 4. In particular, AE decreased of 2.4% in Tr1, of 12.4% in Tr2, and of 14.4% in Tr3, although the SNK test revealed no significant differences among treatments (Fig. 2b). At the end of the 4 weeks exposure, the BCI resulted comparable (35.16 ± 1.12 %) to the initial values (39 ± 1.9 %).

6.3.4 DEB simulation results. Once [$\dot{p}_M$] and AE were experimentally estimated under each treatment, those parameters were used to run DEB models in order to predict the effects on LH traits. Thus, DEB simulations were performed under local thermal conditions (as expressed by the thermal series recorded in Trieste and Palermo; see Section 6.2.5) and using satellite CHL-a concentrations (2006-2009) as a proxy of food. Results showed a remarkable effect exerted by hypercapnia and an increasing hypoxia contribution related to the intensity of disturbance (*i.e.* number hypoxic events *per* year) on LH traits of *M. galloprovincialis* by the end of 4th year (Table 5). Total length (TL) and total weight (TW) in Trieste and in Palermo were similarly reduced by hypercapnia (Fig. 4), with a progressive addictive effect of hypoxia (Table 5). Total length (TL) resulted unaffected by hypoxia up to a frequency of 2 hypoxia events (f = 0.17; 1/Time), then the Trieste site (representative of eutrophic conditions) reported a larger effect. On the opposite the total weight (TW) highlighted a larger effect of hypoxia on the oligotrophic site (Palermo) (Fig. 5). The total number of eggs produced (TRO) and the total number of reproductive events (RE) in Trieste were strongly reduced by hypercapnia (Fig. 4), with the same progressive hypoxia contribution (Table 5). Maturation time (TM) was affected both in Trieste and Palermo by hypercapnia, with the same hypoxia contribution previously shown. Palermo showed no reproductive events in the DEB simulations.

Table 5 DEB simulation outputs. Percentage variation of treatments from CTRL: Total length (TL), Total weight (WW), Total reproductive output (TRO), Total reproductive events (RE), Time to maturity (TM).

				DEB outputs (CTRL) after 4 years				
Site	Stressor	Hypoxia events (days)	Frequency (1/Time)	TL (cm)	WW (g)	TRO (n° egg)	RE	TM (days)
Trieste	CTRL	0	0	9.55	11.19	6.74e6	9	232
Palermo	CTRL	0	0	3.08	0.31	0	0	739
				Percentage contributing effect of Hypoxia				
Trieste	pH+hypoxia	30	0.08	-0.6	-1.4	-1.1	0	0.8
Trieste	pH+hypoxia	60	0.17	-1.3	-2.8	-2.8	0	2.1
Trieste	pH+hypoxia	90	0.25	-2	-4.3	-4.5	0	3.6
Trieste	pH+hypoxia	120	0.33	-2.7	-5.8	-6	0	4.4
Trieste	pH+hypoxia	150	0.42	-3.4	-7.2	-7.4	0	5.5
Trieste	pH+hypoxia	180	0.50	-4	-8.4	-8.8	0	6
Palermo	pH+hypoxia	30	0.08	-0.6	-1.6	0	0	0.8
Palermo	pH+hypoxia	60	0.17	-1.3	-3.3	0	0	2.1
Palermo	pH+hypoxia	90	0.25	-1.9	-4.6	0	0	3.8
Palermo	pH+hypoxia	120	0.33	-2.6	-6.1	0	0	4.5
Palermo	pH+hypoxia	150	0.42	-3.2	-7.4	0	0	5.7
Palermo	pH+hypoxia	180	0.50	-3.9	-8.9	0	0	7.6

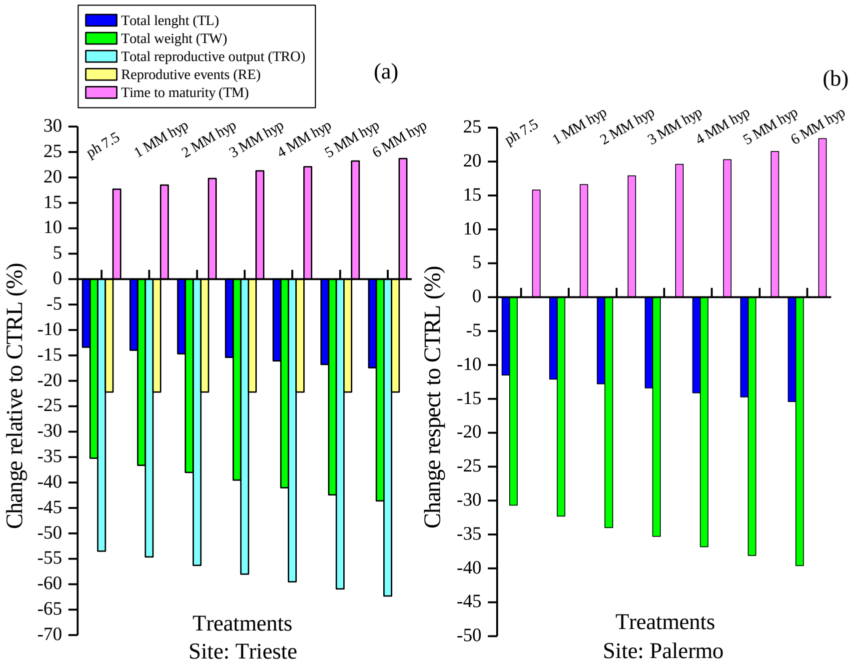

Fig. 4 Results from DEB simulation for (a) Trieste and (b) Palermo sites expressed as percentage variation of DEB outputs respect to CTRL. TL and TW were reduced by 13.4% and 35.2% in Trieste, and by 11.5% and 30.7% in Palermo by hypercapnia, with a progressive addictive hypoxia effect up to 8.9%. TRO and RE were reduced by 53.4% and 66.7% in Trieste by hypercapnia, with a progressive addictive hypoxia effect up to 8.8%. TM increased by 17.8% in Trieste and by 15.7% in Palermo with a similar hypoxia effect (up to 7.6%).

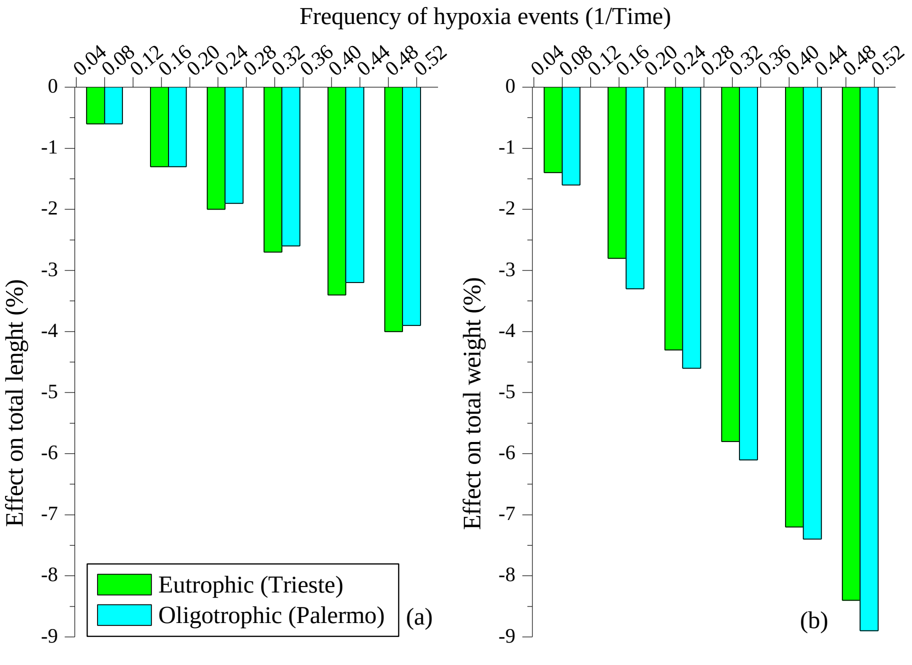

Fig. 5 Percentage effect of Hypoxia from DEB simulations on TL and TW considering the two different trophic conditions represented by Trieste (a) and Palermo (b).

6.3.6 Effects on shell. Results from the breaking load experiment revealed a significant effect of pH (58.8 ± 5 N) and of combined stressors on the breaking load (50 ± 2.7 N), compared to hypoxic (64.4 ± 3.7 N) and CTRL specimens (77.2 ± 2.2 N) (Fig. 6) (Table 3, ANOVA, $p < 0.001$). In addition, the effect was stronger at week 4 than after one week of exposure (Table 3, ANOVA, $p < 0.01$). Deeper investigations through scanning electron microscopy validated an effect by showing an increasing erosion of the shell after exposure to CO_2-induced acidification. The external dissolution pattern usually started from the umbonal region and progressed toward the margin of the shell, usually associated with some degree of damage to the *periostracum*. The damage was present at differing extensions in all specimens exposed to treatments, except in the control mussels (Fig. 7 b, c, d). The alteration of the underlying carbonate layer was instead visible only in Tr1 and Tr3, with details in Fig. 8 (b, d). This kind of alteration was never observed under control pH (Fig. 7a).

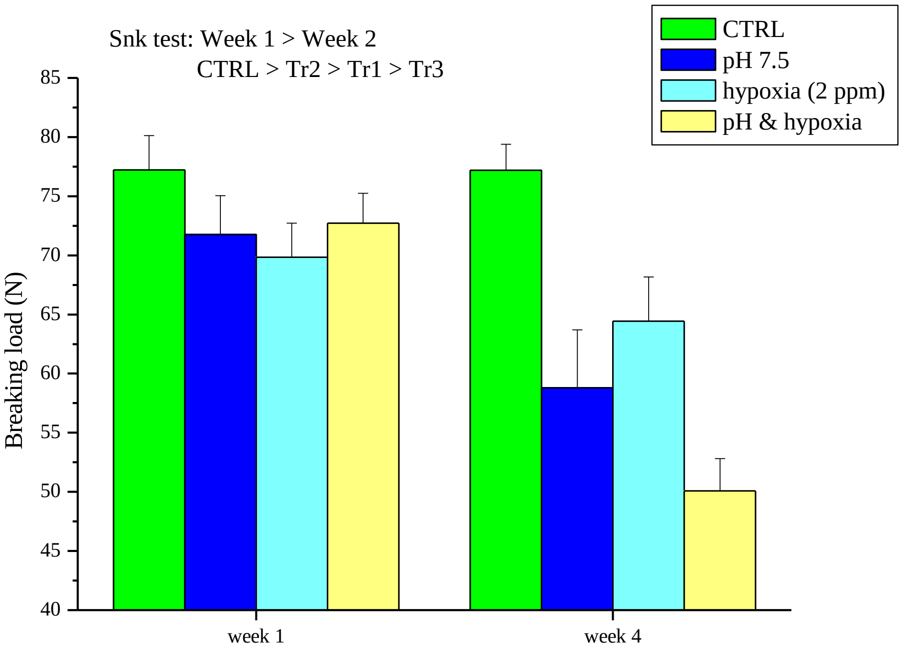

Fig. 6 Breaking load of valves (in Newton, N ± se) exposed to different treatments of oxygen (normoxia – hypoxia 2ppm) and pH (7.5 – 8.0) at week 1 and 4.

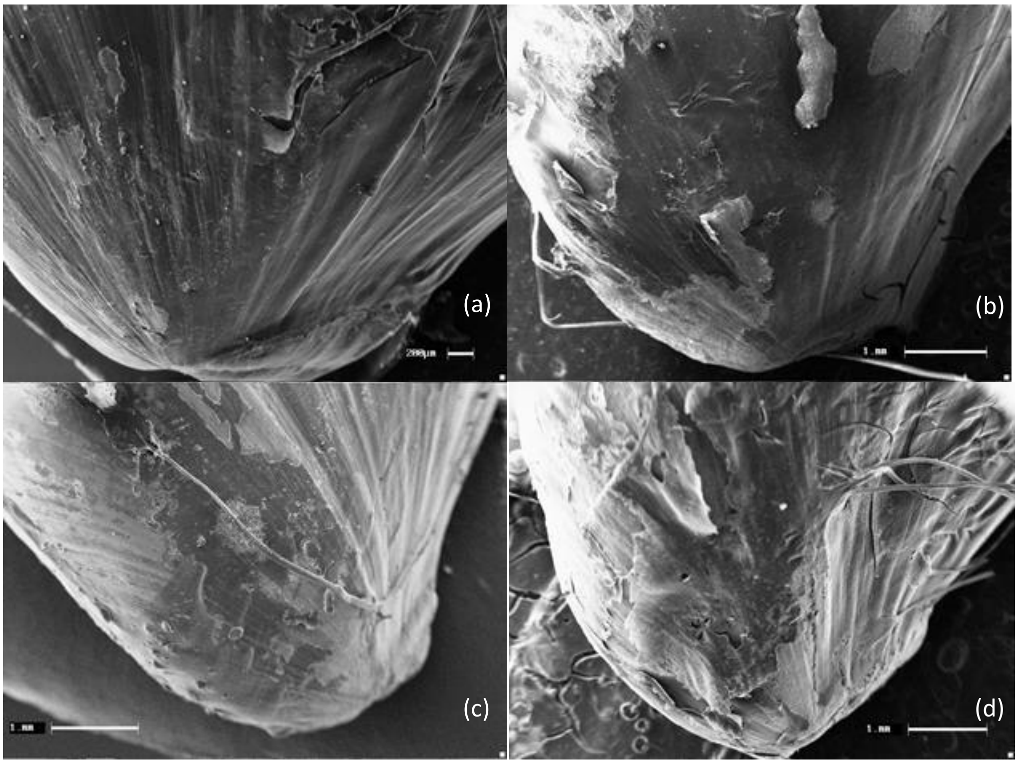

Fig. 7 SEM pictures of different shells exposed to (a) control condition (CTRL); (b) pH 7.5 and normoxia condition (Tr1); (c) normal pH and hypoxia condition (Tr2); (d) both pH 7.5 and hypoxia conditions (Tr3).

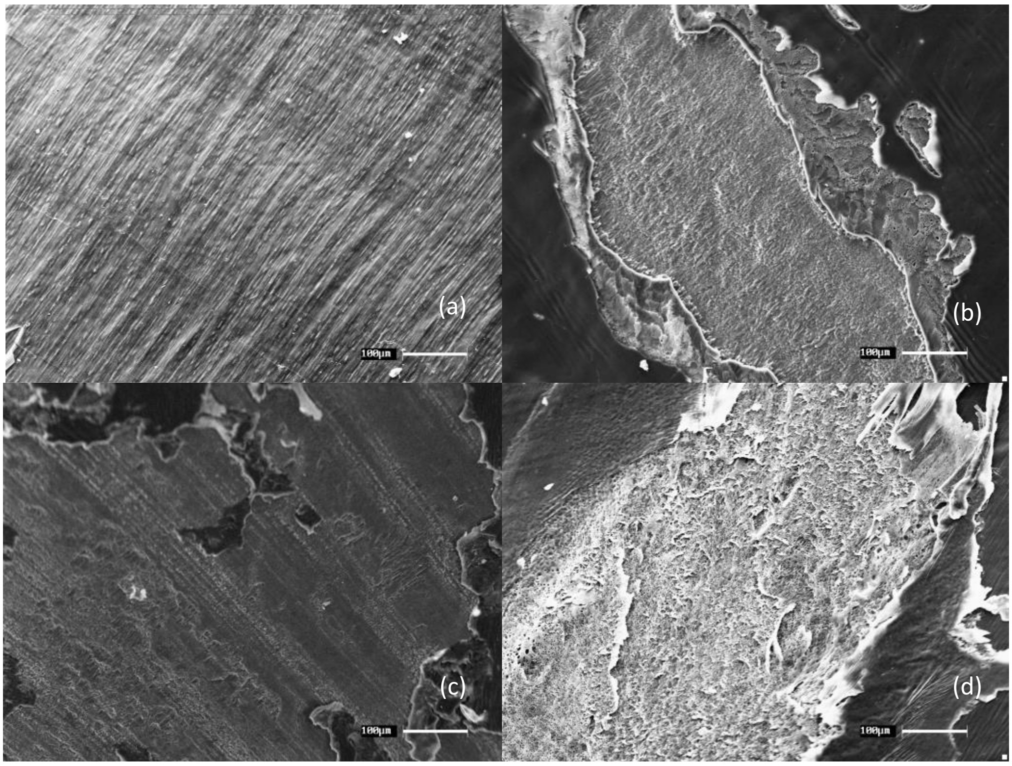

Fig. 8 Details of different shells exposed to (a) control condition (CTRL); (b) pH 7.5 and normoxia condition (Tr1); (c) normal pH and hypoxia condition (Tr2); (d) both pH 7.5 and hypoxia conditions (Tr3).

4 DISCUSSION

Marine organisms, and in particular intertidal species (Montecinos et al., 2009), have been recognized as being equipped with well-developed and conserved mechanisms to compensate OA such as (i) passive buffering of intra- and extracellular fluids; (ii) transport and exchange of relevant ions; (iii) transport of CO_2 in the blood in those species that have respiratory pigments; (iv) metabolic suppression to wait out periods of elevated CO_2 (e.g. Lindinger et al., 1984; Cameron, 1989; Walsh and Milligan, 1989; Hand, 1991; Heisler, 1993; Guppy and Withers, 1999; Pörtner et al., 2004). Several authors recorded suppressed of feeding activity and growth, depressed metabolism, increased N excretion and loss of tissue weight for marine bivalves exposed to reduced seawater pH (Bamber, 1990; Michaelidis et al., 2005; Berge et al., 2006; Gazeau et al., 2010).

Bivalves are in fact capable of maintaining a constant internal pH by decreasing their metabolic rates and/or dissolving their shell; the shell acting then as a source of CO_3^{2-} (Bamber, 1990; Michaelidis et al., 2005; Berge et al., 2006) counterbalancing the effect of dissolved CO_2 crossing biological membranes. Compensation of low pH, associated with anthropogenic increases in seawater pCO_2 (Fabry et al., 2008), through adjustments in ionic composition appears to be a trade-off that is not likely sustainable over longer time-scales.

Our behavioural observations showed that mussels exposed to low pH resulted in a higher, even if not significant, percentage of opened valves respect to CTRL individuals, with the highest significant difference relative to hypoxia exposition. This evidences a low-pH effect of the adductor muscle which is in line which was previously observed by Pynnönen & Huebner (1995) and after exposition to hypoxia (Sheldon & Walker 1989). In agreement with the current literature, showing deleterious effects of CO_2-induced acidification on several invertebrates (Barnhart & McMahon, 1988; Barnhart, 1989; Rees & Hand, 1990) and similarly to other studies by *M. galloprovincialis* (Gestoso et al., 2016; Michaelidis et al., 2005), our results showed how hypercapnia (pH reduced by 0.6 units, relative to the natural pH of the lower Tyrrhenian waters) was able to induce a decline in metabolic rates of mussels. This kind of decline has already been noticed by other authors as an adaptive strategy for survival under transiently stressful conditions (Michaelidis et al., 2005). According to Pörtner et al. (2004), metabolic reduction due to hypercapnia could be a result of acid-base disturbances and therefore be similar to the response of intertidal individuals to anaerobic conditions.

Direct effects of hypoxemia have been further proven to cause fatal decrements in an organism’s performance in growth, reproduction, feeding, immunity and behaviour (*sensu* Pörtner & Farrell, 2008). Combined effects by stressors such as OA and hypoxia narrow the thermal window of functioning according to species-specific sensitivities, modulating biogeographical distributions, coexistence ranges, community shifts and ecological interactions (Pörtner & Farrell, 2008). The mussel *Mytilus edulis* has been proven able to compensate both short- and long-term exposure to hypercapnia by dissolution of shells (Lindinger et al., 1984; Michaelidis et al., 2005), resulting in reduced growth and metabolism. A similar mechanism of release of inorganic molecules into the pallial cavity (as $CaCO_3$ from valves) has been documented during periods of anaerobic metabolism, to maintain the acid-base balance (Chaparro et al., 2009), determining further physiological and energetic cost such as decreased growth, respiration rate and protein synthesis (Pörtner et al., 2005).

During periods of environmental oxygen limitation, many organisms are able to suppress ATP demand, shut down expensive processes, such as protein synthesis (Hand, 1991), but at the same time limiting growth and the reproductive potential. Although suppression of metabolism under short-term experimental conditions is a sub-lethal reversible process, reductions in growth and reproductive output will effectively diminish the survival of the species on longer time-scales (Fabry et al., 2008).

The contemporary occurrence in our simulations, of monthly hypoxia events, revealed a growing contribution to what was already elicited by hypercapnia alone on growth and reproduction. Current literature has not currently explored the combined effects of multiple stressors on long-term experiments by modulating the intensity and duration of disturbance. This would probably translate as a very complex experimental set-up which would be hardly practicable, especially on long-term scales. On the other hand, mechanistic models offer a more practical alternative to long-term, in-field research when studying the effects of multiple-stressors, with the advantage of testing, at the same time, the magnitude and the duration of disturbance on LH traits of a model species. Our results highlighted the general hypoxia growing effect following the increasing duration of disturbance, with a particular focus in Trieste on TW and TRO, while in Palermo on TW and TM (Fig. 4).

A further important peculiarity of the mechanistic modelling deals with the possibility to spatially contextualise the effects of single and multiple stressors on selected outputs by integrating local thermal conditions and food concentrations (Sarà et al. 2018c). Comparing the effect of hypoxia across frequencies (Fig. 5), total length (TL) resulted unaffected up to a frequency of 2 hypoxia events in both sites, then the largest effect was recorded in the coldest but more trophically enriched site (Trieste). On the contrary a smaller effect of hypoxia was detected on TW in Trieste, suggesting a sort of food compensation capacity on the effect of environmental stressor (Mackenzie et al., 2014). Also the DEB model easily allowed the estimation of the fecundity potential of organisms, that is often omitted in other ecological studies, but that represents a crucial quantity for resource (e.g. aquaculture) (Sarà et al. 2018c) and conservation purposes. To verify impacts on shellfish fecundity, we contextualised our simulation by introducing Trieste hourly temperature series after those of Palermo, with the respective local actual CHL-a concentrations, as long as in the first site no reproductive events came out from our simulations, probably due to food limitations and temperature threshold. This is reflected by natural populations in Palermo colonising only substrates in trophic-enriched sites. A combined effect of the simultaneous stressors, such as those considered across this study, has proven in the present study to affect the organism's performance in growth, reproduction and behaviour. Our results highlighted an effect of pH alone and when combined with hypoxia on the breaking load of shells of our experimental mussels. Through a similar approach, Martinez et al. (2018) showed that temperature was a primary factor driving shell's fragility along a Mediterranean latitudinal gradient. Present findings corroborate that idea that fragility can be affected by both stressors through a combined effect.

Multiple stressors can narrow, especially when organisms are on the edge of their thermal tolerance range, the thermal window and this has a potential for generating repercussions on biogeographical distribution, coexistence ranges, community shifts, food webs and species interactions (*sensu* Pörtner & Farrell, 2008). Moreover, an appropriate knowledge of species' biological traits and a mechanistic understanding of the effect of each stressor, reached through an FT-based approach, will allow the translation of the effects of environmental change into realistic management measures taking into account the optimisation of the species' biological traits (Sarà et al. 2018a, b).

5 CONCLUSIONS

Additional research is still required to improve our knowledge of organismal response to multiple stressors, in particular, of many marine ectotherms with indeterminate growth amongst invertebrates (e.g. crustaceans, molluscs). Nevertheless, modelling the growth and reproductive potential (and failure) of species vulnerable to those stressors with predictive tools, such as bioenergetic models is a useful approach for management and protection purposes, but also for shellfish culture in general.

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CHAPTER 7

Moving towards a strategy for addressing climate displacement of marine resources

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ABSTRACT

Realistic predictions of climate change effects on natural resources are central to adaptation policies that try to reduce these impacts. However, most current forecasting approaches do not incorporate species-specific, process-based biological information, which limits their ability to inform actionable strategies. Mechanistic approaches, incorporating quantitative information on functional traits, can potentially predict species-specific responses resulting from the cumulative impacts of small-scale processes acting at the organismal level, and can be used to infer population-level dynamics. Here we present a proof-of-concept study using the European anchovy as a model species that shows how a trait-based, mechanistic species distribution model can be used to explore the vulnerability of marine species to environmental changes. We crossed scenarios of temperature and food to generate quantitative maps of selected mechanistic model outcomes (*e.g.* Total Length and Total Reproductive Output). These results highlight changing patterns of source and sink spawning areas as well as the incidence of reproductive failure. This study demonstrates that model predictions based on functional traits can reduce the degree of uncertainty when forecasting future trends of fish stocks. Such a sensitive and spatially-explicit predictive approach may be used to inform more effective adaptive management strategies of resources in novel climatic conditions.

Key words: Adaptive management, climate change, Dynamic Energy Budget model, *Engraulis encrasicolus*, temperature increase, Life-History traits, scenarios

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Substantive Area:	Population Dynamics and Life History < Population Ecology < Substantive Area, Climate Change < Ecosystems < Substantive Area, Resource Management (Wildlife, Fisheries, Range, Other) < Ecosystems < Substantive Area, Spatial Statistics and Spatial Modeling < Statistics and Modeling < Theory < Substantive Area

Organism:	Fishes < Vertebrates < Animals
Habitat:	Marine < Aquatic Habitat < Habitat
Geographic Area:	Europe < Geographic Area
Additional Keywords:	Adaptive management, climate change, Dynamic Energy Budget model, Engraulis encrasicolus, temperature increase, Life-History tra
Abstract:	Realistic predictions of climate change effects on natural resources are central to adaptation policies that try to reduce these impacts. However, most current forecasting approaches do not incorporate species-specific, process-based biological information, which limits their ability to inform actionable strategies. Mechanistic approaches, incorporating quantitative information on functional traits, can potentially predict species-specific responses resulting from the cumulative impacts of small-scale processes acting at the organismal level, and can be used to infer population-level dynamics. Here we present a proof-of-concept study using the European anchovy as a model species that shows how a trait-based, mechanistic species distribution model can be used to explore the vulnerability of marine species to environmental changes. We crossed scenarios of temperature and food to generate quantitative maps of selected mechanistic model outcomes (e.g. Total Length and Total Reproductive Output). These results highlight changing patterns of source and sink spawning areas as well as the incidence of reproductive failure. This study demonstrates that model predictions based on functional traits can reduce the degree of uncertainty when forecasting future trends of fish stocks. Such a sensitive and spatially-explicit predictive approach may be used to inform more effective adaptive management strategies of resources in novel climatic conditions.

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1 INTRODUCTION

Understanding and forecasting how ongoing climate change will likely alter the structure and functioning of ecosystems is one of the central challenges facing marine environmental managers (van de Pol et al. 2018). This task is especially challenging due to the high levels of spatial and temporal heterogeneity in climate- and non-climate-related drivers (Lohrer et al. 2015), the interaction of multiple stressors on organisms and ecosystems (Crain et al. 2008) and high variability in the vulnerability of different species to environmental change (Gunderson et al. 2016). Specifically, whilst climate change is a global phenomenon, species respond physiologically and behaviourally to local environmental conditions (Helmuth et al. 2014). Scaling up responses to forecast future responses of ecosystems and their component species requires an understanding of how key drivers will individually and collectively affect ecosystem composition, structure and function at local scales; however, large gaps still exist in our basic knowledge of most marine species (Fulton 2011; Pecl et al. 2014; 2017). Moreover, organisms are increasingly likely to experience novel suites of environmental conditions, and so their responses are also likely to be novel and difficult to predict (Solow 2017; van de Pol et al. 2018). As a consequence, we need new tools that, whilst being informed by knowledge of current distribution patterns, can also account for organisms' vulnerability to a broader range of conditions than those currently or previously observed.

To improve projections of the future status of individual species and ecosystems, and to effectively support the development of more sustainable policies that minimize expected impacts and maximize potential opportunities, novel combinations of modeling and field and laboratory experimentation are required. Such an integrated approach is necessary to produce realistic forecasts at spatio-temporal scales relevant to organisms and populations (Burrows et al. 2011; Helmuth et al. 2014, Pacifici et al. 2015; van de Pol et al. 2018; Queirós et al., 2018). While several existing predictive ecological modelling frameworks produce model outputs and scenarios with different fields of application depending on the question being addressed (Koenigstein et al. 2016), each have distinct strengths and weaknesses.

1.1 Predictive ecological modeling frameworks to management: the mechanistic model

The adoption of a risk-based approach and of process-based (hereafter mechanistic) models has been recently suggested (Pecl et al. 2014; Pacifici et al. 2015; Fordham et al. 2017). By using functional trait-based mechanistic bioenergetics (*sensu* Kooijman 2010; Sousa et al. 2008; 2010; Sarà et al. 2013; 2018) coming from experimental data, mechanistic models are able to incorporate the effects of environmental drivers at levels that exceed the range of values currently operating in nature (Teal et al. 2018). The spatially- and temporally-explicit quantitative predictions generated by these models are species-specific and based on Life History traits such as fecundity and body size (Pecquerie et al. 2009; Kearney et al. 2010; Pethybridge et al. 2013). Model outcomes such as these are critical to parameterize population-based models and are required if they are used to inform appropriate, proactive mitigation and adaptation strategies at scales relevant to spatial management and national and regional policy decision-making (van de Pol 2018; Sarà et al. 2018; Mangano et al. 2018). As a drawback, mechanistic models are more time-consuming to develop, as they require detailed information on species-specific dynamic responses to environmental change (Fennell et al. 2013). To date, however, relatively few examples exist of the application of such approaches over large geographic scales (Montalto et al. 2016) and they are seldom applied to commercially important species (Mangano et al. 2018; Sarà et al. 2018).

1.2 Future proofing marine resource management: a case study using Engraulis engrasicolus

In this study we explore a mechanistic physiological approach, based on Dynamic Energy Budget (DEB) theory (Kooijman 2010), to quantify the effects of future environmental change on the potential distribution and vulnerability of the European anchovy (*Engraulis encrasicolus*) population. By translating environmental change into biological effects through a downscaled approach, we compared the current status of this population in a core area of the Mediterranean distributional range (*i.e.* the Strait of Sicily, Southern Mediterranean, recognized hotspot for this species; Basilone et al. 2006) with its future responses to predicted temperature increases. A sensitivity analysis to simulate both temperature increase and trophic condition scenarios (food availability, *i.e.* oligo- and eutrophic conditions) allowed us to explore the robustness of the models' outputs (Pecquerie et al. 2009; Payne et al. 2015; Kleisner et al., 2017; Sarà et al. 2018).

We developed scenario-specific quantitative maps to show the different simulation outcomes, which allowed: *(1)* the identification of current source and sink areas and the detection of future temporal and spatial shifts and *(2)* the predictions of reproductive failure and size-structure shifts due to climate change of the anchovy. By providing critical insights into the effects of climate on this key species, independent of fishing pressure, our results may be used to inform novel policy targets for resilience and to help develop adaptive management strategies that enable a more sustainable exploitation of marine resources (Goh 2012; Queirós et al. 2018).

2 METHODS

2.1 The Dynamic Energy Budget (DEB) model

DEB theory (Kooijman 2010) provides a conceptual and quantitative framework to model metabolism at the whole organism level encompassing all life-stages. The standard DEB model (Kooijman 2010; Sousa et al. 2010; Kearney 2015) describes the rate at which an organism assimilates and utilises energy for maintenance, growth and reproduction as a function of parameters that characterise the species' physiology and its response to environmental forcing variables (*e.g.* food availability and temperature) taking into account metabolic trade-offs. In contrast to net-production models (*e.g.* scope for growth), which assume assimilated energy is partitioned between maintenance and both growth and reproduction, DEB theory assumes assimilated energy is first stored as reserves, and is then distributed among physiological processes (Filgueira et al. 2011). This storage effect thus permits the exploration of time history effects, specifically those related to energetic status (feeding history) and vulnerability to factors such as temperature (Kearney et al. 2010). DEB is, therefore, a reliable and powerful tool to mechanistically describe the whole life cycle of an organism and to make predictions of Life History (LH) traits (Pecquerie et al. 2009; Kearney et al. 2010; Pethybridge et al. 2013; Nisbet et al. 2012). According to the κ-rule a fixed energy fraction (κ) is allocated to growth and somatic maintenance, while the remaining fraction (1-κ) is allocated to maturity maintenance plus maturation or reproduction. Changes in environmental conditions (changes in temperature, food availability etc.) thus can be translated into effects on growth and reproduction. DEB theory therefore allows, through the explicit modelling of energy and mass fluxes through organisms, the derivation of individual performance in terms of the most important Life History traits of a species (Sarà et al. 2011; 2013a, b; 2014; 2018; Mangano et al. 2018). DEB also allows an understanding of the interacting time histories of exposures to environmental conditions. Thus, for example, increasing temperature can (up to a point) increase metabolic rates. These in turn can lead to faster rates of maturity and growth, but only in the presence of sufficient food. In contrast, increased metabolic demand in the absence of food can lead to rapid declines in growth.

2.2 Model outcome validation and mapping

We performed simulations to investigate potential variations in the ultimate body size and fecundity of *Engraulis encrasicolus* under different climate and food availability scenarios. Model outputs are expressed in terms of Total Length (TL, cm) and Total Reproductive Output (TRO, the total number of eggs per life span) and presented through scenario-based quantitative maps (Mangano et al. 2018). Twenty TL size classes (ranging from 6 to 16 cm TL) and twelve TRO classes (ranging from 0 to 1,200,000 eggs per life span) are represented (Figure 3 and 4). Minimum, maximum, mean and median values for each scenario are also reported. The changes of number of cells per TL and TRO size classes across the study area for each scenario, as predicted by the DEB model, were evaluated: increases or decreases from baselines are highlighted (Tables 1 and 2). DEB TL simulation have been validated by extracting data from the literature (Figure 1A). TRO simulation outputs were validated using data collected *in situ* during *ad-hoc* oceanographic surveys [see acknowledgement section] (Figure 1B).

To build these maps, knowledge of organismal body temperature (assumed to be similar to Sea Surface Temperature, SST) and environmental food densities is a prerequisite, together with the *Engraulis encrasicolus* species-specific DEB parameters [see Supporting Information Table S1, Supplementary Data Chapter 7]. To analyse the patchy distributions and trends of both TL and TRO size classes under the selected scenarios, z-score values and strength of clustering (positive = high clustering; zero no apparent clustering; negative = low clustering) were estimated through the Spatial Analyst tool of ArcGIS (Getis and Ord 1992; Getis 1995) and reported for each scenario and LH trait respectively (only significant, $p \leq 0.05$, values are reported).

2.3 Forcing variables: food density

All spatial analyses were performed using GIS procedures and tools, specifically ESRI ArcGIS 10.2 (and Spatial Analyst extension), GRASS GIS 7 and custom Python scripts. The Geographic Coordinate System WGS84 (EPSG 4326) was used. Our simulations were restricted to the continental shelf on the basis of depth (from 0 to 200 meters below sea level) identified through bathymetry data obtained from EMODnet (2016). A vector polygon grid feature class of 346 square cells (having a size of 0.11° [~12.5 km]) covering the study area was used. Food availability is an important forcing variable of the model and is expressed as density (wet mass mg m^{-3}), which for anchovy primarily comprise zooplankton (Tudela and Palomera 1997 and literature therein). Because locally collected data for zooplankton were fragmented spatially and temporally due to sampling, we judged it not to be sufficiently representative of actual food availability for anchovies throughout the study area (Torri et al. 2018). To obtain a spatially continuous dataset on the distribution of food throughout the study area and across time we followed the recent approach proposed by Strömberg et al. (2009) and applied by Mangano et al. (2018) [see Supporting Information, Supplementary Data Chapter 7]. To simulate future trophic changes, we carried out a sensitivity analysis by adding or subtracting (cell by cell) a fixed amount of 10% generating three future scenarios: oligotrophic (current NPP - 10%), eutrophic (current + 10%) and no-change (current NPP).

2.4 Forcing variables: temperature

Due to the short life span of the anchovy (~4 years), we extracted daily sea surface temperatures (SST; 1 km resolution) from JPL MUR SST data (2010) over a time range of 4 years (2011-2014) for each cell. To simulate the effect of future increases in temperature, as forecasted by COP 21 (*sensu* COP 21 Paris Climate Conference Agreement; Hulme 2016) we performed a sensitivity analysis by running DEB models cell by cell and by increasing the current temperature from 0.5°C to 2.0°C (0.5°C step) obtaining four increasing temperature DEB scenarios (current +0.5°C, +1.0°C, +1.5°C, +2.0°C).

3 RESULTS

DEB model outputs based on observed environmental conditions provided good predictions of the two selected LH traits in European anchovy, giving a high level of confidence in the forecasts generated (Figure 1A, B).

Validated DEB model outcomes of both the proxy of population size-structure (TL) and proxy of population fecundity (TRO) across the study area were positively affected by increased temperatures (but see the sensitivity analysis from current temperature to +2 °C scenario with + 0.5 increment intermediate scenarios) coupled with increases in trophic conditions. An increase in food availability had a greater effect on both LH traits, with the highest mean values being predicted under the higher food (eutrophic) scenario (*i.e.* 10% above current levels of nutrients, NPP; Figure 2A, TL and B, TRO). Scenario-based quantitative maps of LH traits represent the geographically explicit forecasts across the study area (model predictions in each cell; Figures 3, 4). By mapping the model predictions of each of the size classes, it was possible to disentangle the spatial heterogeneity of climate change effects (*i.e.* number of cells *per* size class gained or lost; Tables 1, 2; Figures 3, 4). TL and TRO were predicted to undergo a phenological shift under future conditions with scenario-specific response patterns *per* each size class (Tables 1, TL and 2, TRO).

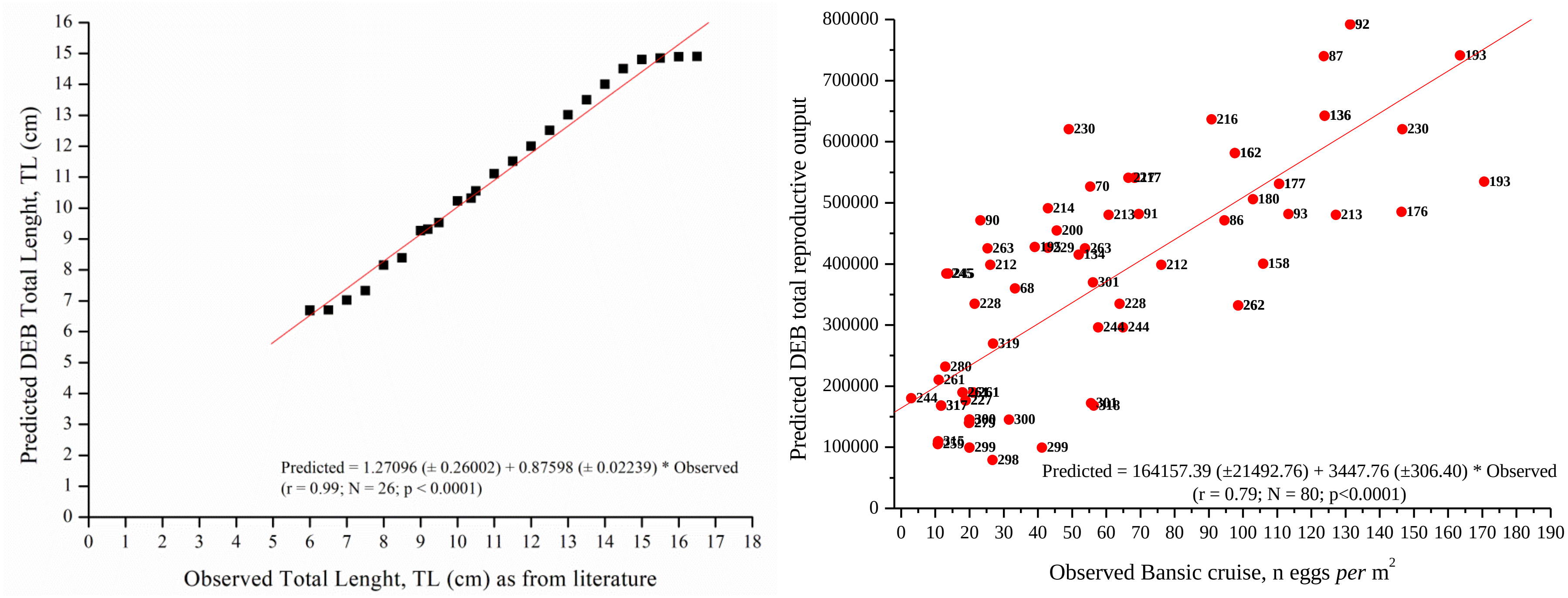


Figure 1. Model validation. Regression (red line) between observed and predicted data, equation and significant values. Left panel TL simulation outputs validation by using data extracted from literature (Basilone et al. 2004; 2006); right panel TRO simulation outputs validation by using data collected *in situ* during ad-hoc oceanographic surveys (oceanographic cruises in the framework of RITMARE project – MIUR, Ministero dell'Innovazione, Ministero Ambiente, Regione Sicilia; FAO MedSudMed GCP/RER/010/ITA; Patti et al. 2017)

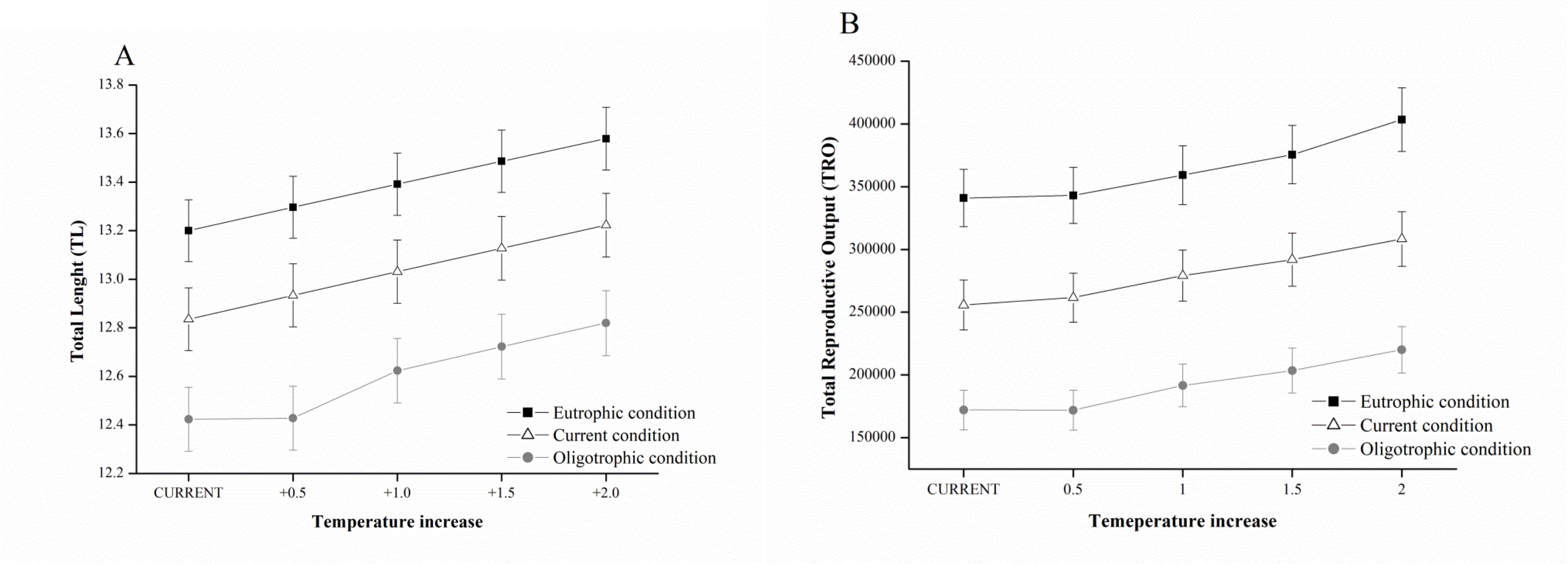


Figure 2. (A) Total Length (TL, cm) and (B)Total Reproductive Output (TRO) predicted trends per trophic scenario (current, oligo-. eutrophic) under increasing temperature scenarios (from current to + 2.0 °C).

Temperature increase crossed to food decrease (-10% oligotrofic condition) and food increase (+10% eutrophic condition)

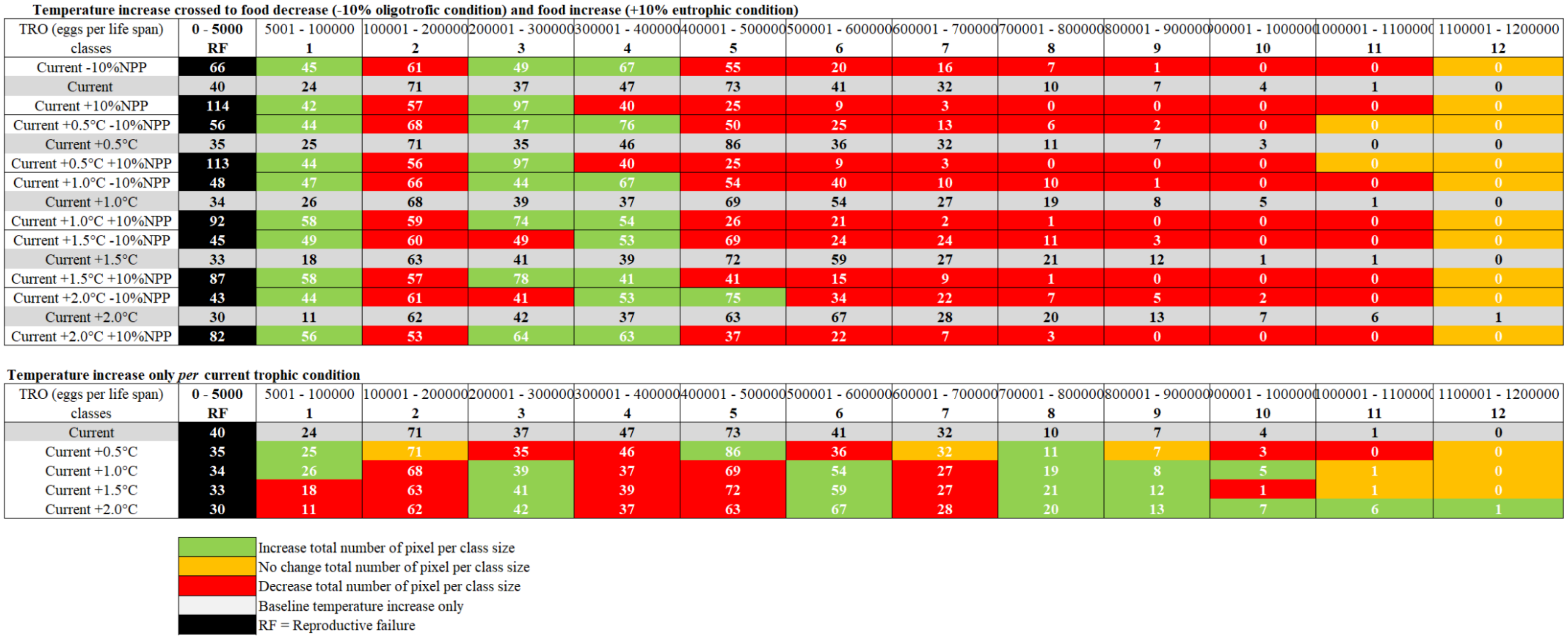
Class size TL (cm)	6.1-6.5	6.6-7.0	7.1-7.5	7.6-8.0	8.1-8.5	8.6-9.0	9.1-9.5	9.6-10.0	10.1-10.5	10.6-11.0	11.1-11.5	11.6-12.0	12.1-12.5	12.6-13.0	13.1-13.5	13.6-14.0	14.1-14.5	14.6-15	15.01-15.5	15.6-16
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Current -10%NPP	1	3	2	2	1	2	3	2	12	11	33	63	41	65	84	40	22	0	0	0
Current	0	2	4	0	2	1	3	2	2	12	16	33	65	38	88	74	32	13	0	0
Current +10%NPP	0	1	2	1	2	2	1	5	0	4	12	18	42	58	47	108	55	22	7	0
Current +0.5°C -10%NPP	1	3	2	2	1	2	3	2	11	12	32	64	41	65	84	40	22	0	0	0
Current +0.5°C	0	1	3	2	2	1	2	3	2	11	11	28	64	46	63	89	41	18	0	0
Current +0.5°C +10%NPP	0	0	2	2	2	2	1	3	2	2	12	16	35	66	41	98	66	27	10	0
Current +1.0°C -10%NPP	1	2	1	3	1	2	4	1	6	12	22	59	46	40	106	49	22	10	0	0
Current +1.0°C	0	1	3	2	2	0	3	3	2	7	12	23	64	44	51	100	45	21	3	0
Current +1.0°C +10%NPP	0	0	1	3	2	2	1	2	3	2	10	11	33	67	39	76	88	34	13	0
Current +1.5°C -10%NPP	0	3	1	3	1	1	5	1	4	12	17	61	43	39	93	67	24	12	0	0
Current +1.5°C	0	1	2	1	3	1	1	5	1	4	13	20	61	46	41	109	51	20	7	0
Current +1.5°C +10%NPP	0	0	1	3	2	2	0	3	3	2	8	10	25	65	44	59	97	44	19	0
Current +2.0°C -10%NPP	0	3	1	2	2	1	4	1	4	11	14	53	47	39	73	80	35	17	0	0
Current +2.0°C	0	1	2	1	2	1	1	4	1	4	12	16	60	43	42	98	64	23	10	0
Current +2.0°C +10%NPP	0	0	1	3	1	2	1	1	5	1	4	13	20	63	46	49	107	45	22	3

Temperature increase only per current trophic condition

Class size TL (cm)	6.1-6.5	6.6-7.0	7.1-7.5	7.6-8.0	8.1-8.5	8.6-9.0	9.1-9.5	9.6-10.0	10.1-10.5	10.6-11.0	11.1-11.5	11.6-12.0	12.1-12.5	12.6-13.0	13.1-13.5	13.6-14.0	14.1-14.5	14.6-15	15.01-15.5	15.6-16
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Current	0	2	4	0	2	1	3	2	2	12	16	33	65	38	88	74	32	13	0	0
Current +0.5°C	0	1	3	2	2	1	2	3	2	11	11	28	64	46	63	89	41	18	0	0
Current +1.0°C	0	1	3	2	2	0	3	3	2	7	12	23	64	44	51	100	45	21	3	0
Current +1.5°C	0	1	2	1	3	1	1	5	1	4	13	20	61	46	41	109	51	20	7	0
Current +2.0°C	0	1	2	1	2	1	1	4	1	4	12	16	60	43	42	98	64	23	10	0

🟩	Increase total number of pixel per class size
🟧	No change total number of pixel per class size
🟥	Decrease total number of pixel per class size
⬜	Baseline temperature increase only

Table 1. Changes of number of cells per Total Length (TL, cm) size classes across the study area for each scenario as predicted by the DEB model. Changes are reported in terms of total number of cells *per* each of the twenty TL size classes, increase or decrease from the baseline (temperature scenario in grey) are highlighted respectively in green (increase) and red (decrease) with no change in orange (stable condition).



Temperature increase crossed to food decrease (-10% oligotrofic condition) and food increase (+10% eutrophic condition)

TRO (eggs per life span) classes	**0 - 5000** **RF**	5001 - 100000 **1**	100001 - 200000 **2**	200001 - 300000 **3**	300001 - 400000 **4**	400001 - 500000 **5**	500001 - 600000 **6**	600001 - 700000 **7**	700001 - 800000 **8**	800001 - 900000 **9**	00001 - 100000 **10**	1000001 - 110000 **11**	1100001 - 1200000 **12**
Current -10%NPP	66	45	61	49	67	55	20	16	7	1	0	0	0
Current	40	24	71	37	47	73	41	32	10	7	4	1	0
Current +10%NPP	114	42	57	97	40	25	9	3	0	0	0	0	0
Current +0.5°C -10%NPP	56	44	68	47	76	50	25	13	6	2	0	0	0
Current +0.5°C	35	25	71	35	46	86	36	32	11	7	3	0	0
Current +0.5°C +10%NPP	113	44	56	97	40	25	9	3	0	0	0	0	0
Current +1.0°C -10%NPP	48	47	66	44	67	54	40	10	10	1	0	0	0
Current +1.0°C	34	26	68	39	37	69	54	27	19	8	5	1	0
Current +1.0°C +10%NPP	92	58	59	74	54	26	21	2	1	0	0	0	0
Current +1.5°C -10%NPP	45	49	60	49	53	69	24	24	11	3	0	0	0
Current +1.5°C	33	18	63	41	39	72	59	27	21	12	1	1	0
Current +1.5°C +10%NPP	87	58	57	78	41	41	15	9	1	0	0	0	0
Current +2.0°C -10%NPP	43	44	61	41	53	75	34	22	7	5	2	0	0
Current +2.0°C	30	11	62	42	37	63	67	28	20	13	7	6	1
Current +2.0°C +10%NPP	82	56	53	64	63	37	22	7	3	0	0	0	0

Temperature increase only *per* current trophic condition

TRO (eggs per life span) classes	**0 - 5000** **RF**	5001 - 100000 **1**	100001 - 200000 **2**	200001 - 300000 **3**	300001 - 400000 **4**	400001 - 500000 **5**	500001 - 600000 **6**	600001 - 700000 **7**	700001 - 800000 **8**	800001 - 900000 **9**	00001 - 100000 **10**	1000001 - 110000 **11**	1100001 - 1200000 **12**
Current	40	24	71	37	47	73	41	32	10	7	4	1	0
Current +0.5°C	35	25	71	35	46	86	36	32	11	7	3	0	0
Current +1.0°C	34	26	68	39	37	69	54	27	19	8	5	1	0
Current +1.5°C	33	18	63	41	39	72	59	27	21	12	1	1	0
Current +2.0°C	30	11	62	42	37	63	67	28	20	13	7	6	1

🟩	Increase total number of pixel per class size
🟨	No change total number of pixel per class size
🟥	Decrease total number of pixel per class size
⬜	Baseline temperature increase only
⬛	RF = Reproductive failure

Table 2. Changes of number of cells per Total Reproductive Output (TRO) classes across the study area for each scenario as predicted by the DEB model. Changes are reported in terms of total number of cells *per* each of the thirteen TRO classes, increase and decrease from the baseline (temperature scenario in grey) are highlighted respectively in green (increase) and red (decrease) with no change in orange (stable condition). Reproductive failure (RF) is reported in black.

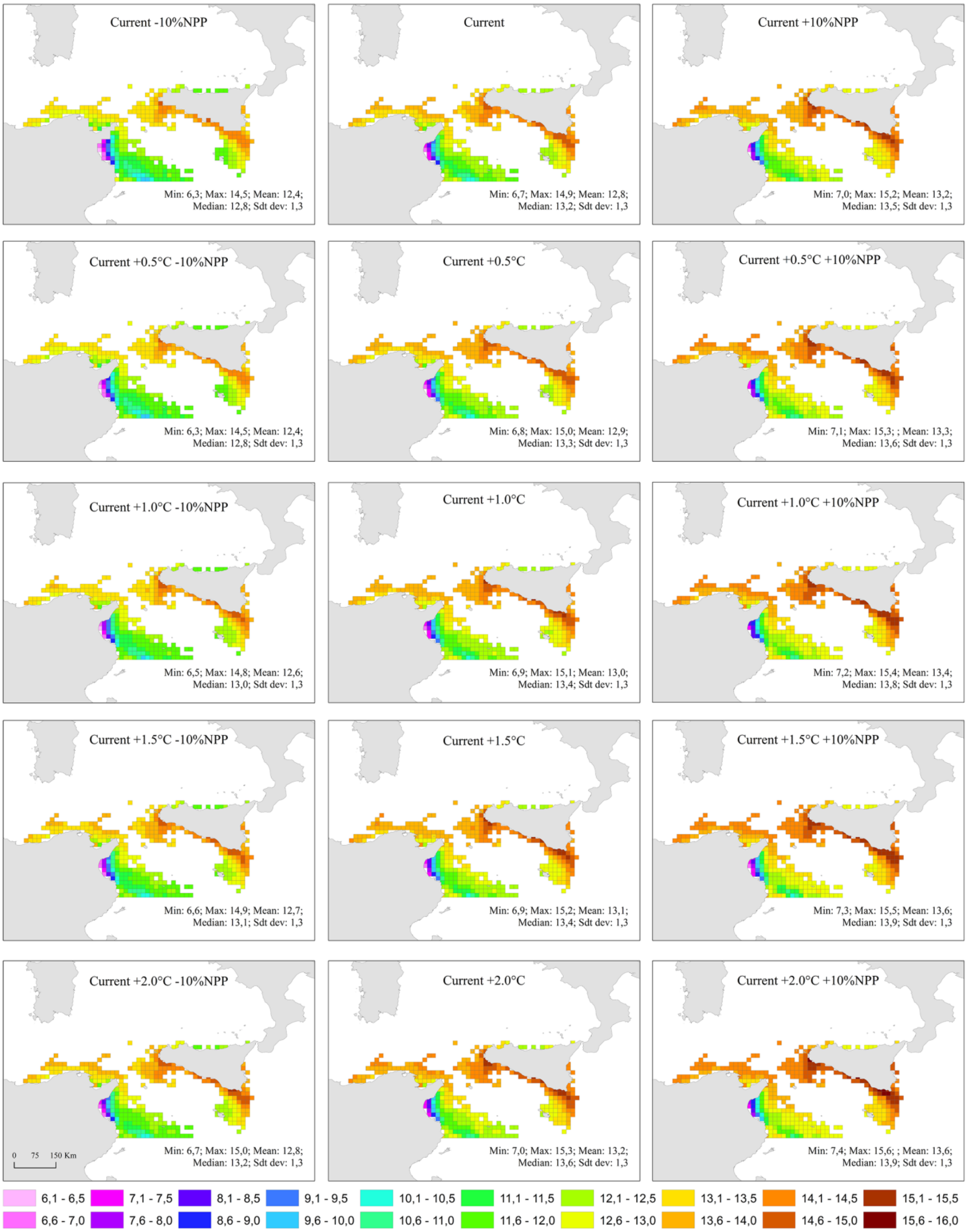


Figure 3. Scenario-based quantitative maps of Total Length (TL, cm) under current conditions of both temperature and food (C, Current; central panel) and under four increasing temperature scenarios (+ 0.5; + 1.0; + 1.5; + 2.0 °C) coupled with decreasing (- 10 oligotrophic, left panel) and increasing trophic conditions (+ 10 eutrophic right panel). Twenty TL size classes are represented ranging from 6 to 16 cm TL. Minimum, maximum, mean and median values for each scenario have been also reported inside each map. Maps were created using ArcGIS software by ESRI (Environmental Systems Resource Institute, ArcMap 10.1, (www.esri.com).

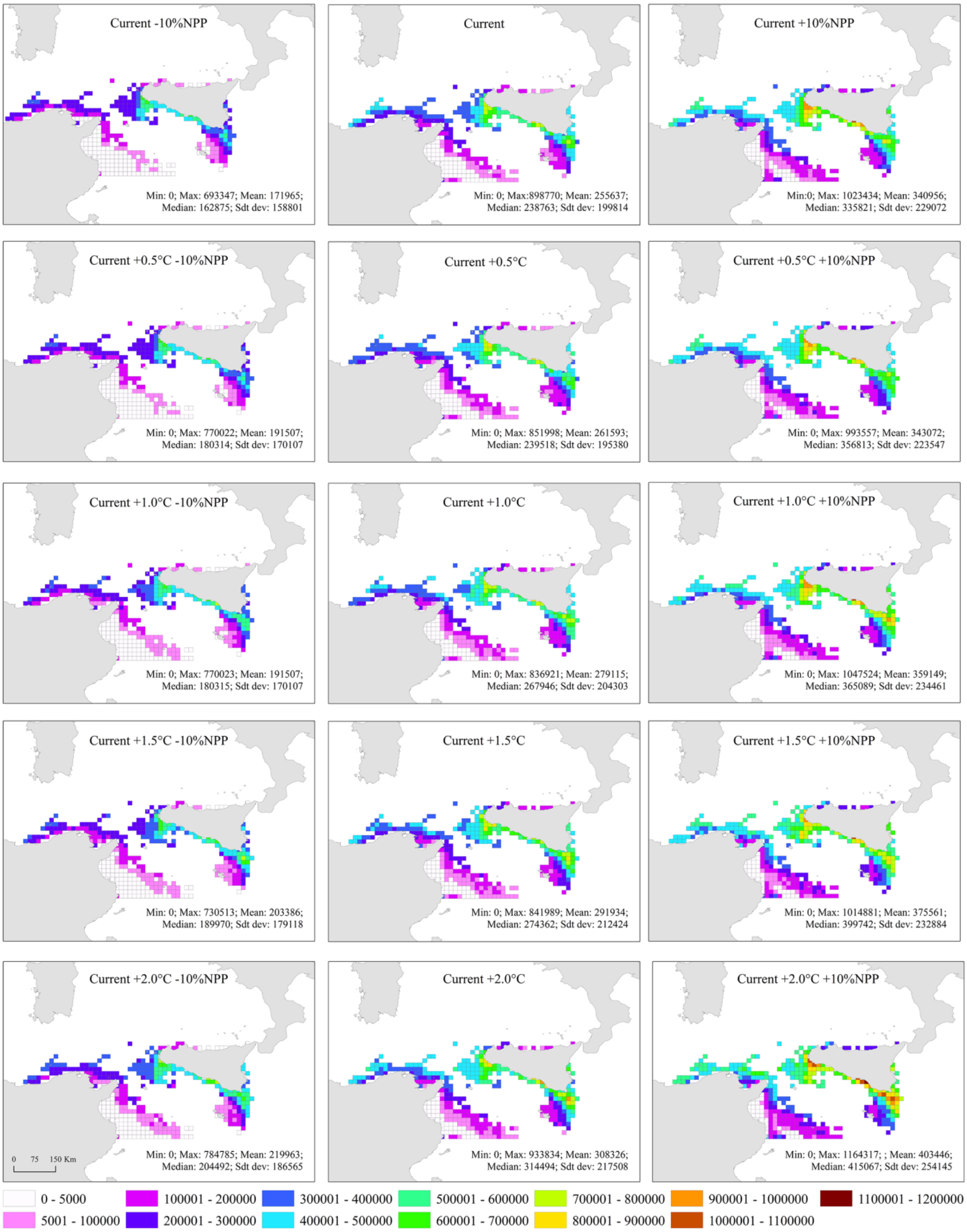


Figure 4. Scenario-based quantitative maps of the Total Reproductive Output (TRO). under current conditions of both temperature and food (C, Current; central panel) and under four increasing temperature scenarios (+ 0.5; + 1.0; + 1.5; + 2.0 °C) each one coupled with decreasing (- 10 oligotrophic, left panel) and increasing trophic conditions (+ 10 eutrophic right panel). Twenty TRO classes are represented ranging from 0 to 1,200,000 eggs per life span. Minimum, maximum, mean and median values per each scenario have been also reported inside each map. (Maps were created using ArcGIS software by ESRI (Environmental Systems Resource Institute, ArcMap 10.1, (www.esri.com).

3.1 Growth patterns (TL size classes)

Generally, increasing temperatures promoted the spatial extension of the larger size classes (Table 1; from 13.6 to 15 cm). Responses of the largest size class to the various scenarios (Figure 3, dark brown class 15.5 – 16 cm) represent perhaps the most crucial information needed to set tailored, local scale management plans. Temperature increases under oligotrophic conditions led to a decrease in the spatial extent of the largest class size, whereas eutrophic conditions facilitated an increase. Anchovies were predicted to never achieve the largest size classes under a temperature increase of +1.0°C coupled with oligotrophic conditions. Under the highest temperature scenario, +2°C, coupled with oligotrophic conditions, the nine largest size classes were forecast to experience a spatial contraction; under these conditions the anchovy will stop growing at a length of 15.5 cm. Under eutrophic conditions, however, the species will achieve the largest class size recorded by the model (16 cm), with a general spatial increase in the largest nine size classes (Table 1).

3.2 Fecundity patterns (TRO size classes)

Similar spatial heterogeneity characterised the TRO simulated responses. The model identified areas predicted to be more productive (*i.e.* areas with the highest TRO values; Figure 4; dark brown class >1,100,000 eggs *per* life span). Moreover, a loss of productivity in half of the analysed size classes was predicted under temperature increases and under any scenario of food availability (Table 2). Under all the considered scenarios of increasing temperature, the model predicted a spatial increase of reproductive failure, *i.e.* areas with no egg production (*i.e.* “zero value”, reproductive failure – RF in black; Table 2). The first TRO class (5001-100000 eggs *per* life span) showed a constant increase in spatial distribution under all the considered scenarios of temperature and food. However, for sites with the highest TRO values under current conditions, no spatial changes were predicted, suggesting the presence of refugia despite overall negative impacts of temperature increase on reproduction.

3.3 LH traits spatial analysis

The spatial analysis of patchiness among the TL size classes indicated a low spatial fragmentation as testified by the mean high clustering of class sizes across the study area (positive z-score values were recorded for TL). A decreasing trend was recorded with increasing temperature under both oligo- and eutrophic conditions (Figure 5A). The highest z-scores were reported under eutrophic conditions. Negative z-score values were recorded for TRO indicating a mean low clustering, high fragmentation and low patchiness, with a general increase of values along the increasing gradient of temperature. The highest values of patchiness were reported under oligotrophic conditions followed by eutrophic and current conditions (Figure 5B).

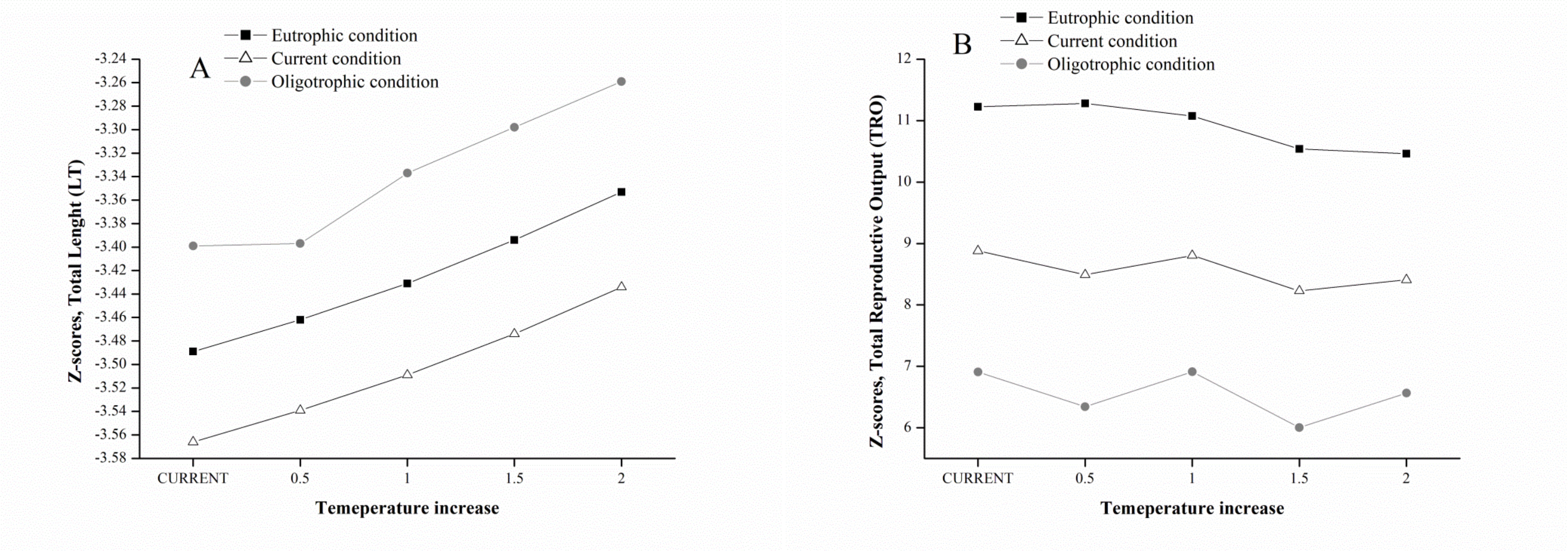


Figure 5. Z-score values (positive = high clustering; zero no apparent clustering; negative = low clustering) estimated through the Spatial Analyst tool of ArcGIS and reported for each scenario and LH trait respectively TL and TRO.

4 DISCUSSIONS

The mechanistic approach applied using DEB theory, allowed for a comparison of current baseline conditions of European anchovy LH traits distribution against those that can be expected under future climate scenarios. The present model is capable to provide highly reliable, quantitative, spatially-explicit predictions of how changes in climate-related environmental conditions will potentially affect LH traits such as growth (TL) and reproduction (TRO). These traits were selected as they drive population dynamics and are essential information for managing commercially important species both currently and in the future (Queirós et al. 2018). Our approach generates spatial and temporal forecast data with a previously unachievable fine-scale (~12.5 km) resolution, allowing the identification of threats and opportunities for the long-term sustainability of the commercially important anchovy, with implications for the European anchovy fisheries sector (three stocks over eight in Mediterranean Sea are “currently lying outside safe biological boundaries” *litt*. Vasilakopoulos et al., 2014). The presented approach has a potential wide range of applications to fisheries stocks globally, assisting in the implementation of existing management evaluation strategies and helping to develop more resilient, trans-boundary resource management planning options.

4.1 Fecundity as an early warning proxy of species vulnerability: a baseline tool to formulate control measures

To date, some examples of European anchovy management based on long-term monitoring of fish stocks coupled with environmental indices and simulation, have been proven unsuccessful (Punt et al. 2013). Management measures of this species have mainly consisted of technical measures such as: the establishment of Minimum Conservation Reference Size, catch regulation, limitation of fishing areas, closed seasons and mandated changes in gear size. The harvest control rule drives the ICES advice on setting the Total Allowable Catch (TAC quota; *e.g.* Subarea 8, Bay of Biscay). Therefore, bio-economic simulations performed to assess the biological and economic consequences of a fixed TAC to manage an anchovy stock in Spain (purse seine fleet, Gulf of Cádiz), described this strategy as causing biological and economic vulnerabilities with a consequent fragmentation of sustainability (Ruiz et al. 2017). The same authors explored an adaptive TAC, such an alternative strategy to account for the inherent stock variability and resulting in balancing profits and sustainability. The limits of the classical use of a TAC levels have been underlined by Lehuta et al. (2010) which suggested a more adaptive TAC setting, proportional to recruitment.

Interestingly, a sensitivity analysis confirmed the importance of fecundity for population growth, supporting proposals to protect spawning. As highlighted from Pecquerie et al. (2009) in the Bay of Biscay, “the model can be used to realistically predict spawning in spatially and temporally varying environments” (Litt.). Therefore, such measures cannot yet be applied in annual management cycle due to the difficulty of predicting recruitment that, instead, would require a seasonal-based management (Fréon et al., 2005). Fecundity was recently suggested as reference point to estimate the Stock Reproductive Potential, improving the understanding of stock dynamics and the promotion of fishery-independent data collection (*sensu* Kell et al. 2015). As the first input-driver of a species’ population dynamics, fecundity can be coupled with Lagrangian physical-biological models to predict species local persistence over time, source areas, and dispersal over time, sink areas, at fine spatial resolution (Falcini et al. 2015; Politikos et al. 2015). Persistence is an essential component of predictive forecasts of future status of commercial stocks and one of the most important population traits for the efficient creation of dynamic ecosystem-based management plans (Munroe et al. 2012).

In this context the scenario-based forecasts of phenological shifts for target species, such as the European anchovy, can be used to address the development of seasonal (or even higher such as monthly) TAC. Our TRO outcomes may help in improving the degree of accuracy when performing modeling exercise to evaluate strategies and the robustness of management options. Interestingly, our approach, although applied in a limited geographic region, demonstrates new capabilities for predicting areas of future species vulnerability in terms of changes in spatial connectivity (fragmentation/patchiness among TRO classes) and increase/decrease of reproductive failure (Montalto et al. 2016). Quantitative information on fragmentation of spawning areas, recognized as Essential Fish Habitat (EFH, *sensu* Benaka 1999), fills a critical knowledge gap regarding the capacity to implement spatially-explicit management strategies. It also facilitates more accurate spatial mitigation of fisheries pressures (*i.e.* control provision measures such as special rules concerning fishing permits, vessel monitoring systems, effort and catch cross-checks), which can be used to increase stock resilience, within or between reproductive patches. As a proxy for recruitment variability, predicted fecundity can represent an effective metric for defining sustainable exploitation strategies (Shelton et al. 2014).

4.2 Spatial-temporally explicit identification of source areas: a baseline tool to address protection and adaptation measures

The need to increase knowledge of population shifts of this species is also crucial because anchovy is the most common forage fish eaten by large predators in the Mediterranean Sea, including Atlantic Bluefin tuna and European hake (Olson et al., 2016). Detecting shifts in the anchovy population can provide a means of foreseeing and disentangling interconnected responses within the multiple hierarchical levels of the food web that this species sustains. Our model results clearly identified source areas (Lewin 1989) where anchovy will still be capable of reproducing under oligotrophic conditions, and therefore serve as “rescue sites” (Assis et al. 2017). The number, distribution and extent of source areas could provide the most reliable baseline information for identifying and prioritizing areas for protection (*e.g.* no-take areas; Giannoulaki et al. 2013). Other sites may in contrast serve as sinks where fish are able to rapidly grow, but may fail to reproduce. Recent models of the impacts of Marine Protected Areas on anchovy stocks in the Bay of Biscay, Lehuta et al. (2010) have emphasized high uncertainty in the values of mortality of larvae and juveniles, growth, and reproduction and this invalidates the effectiveness of the simulated MPA designs. The localization of areas of highest productivity coupled with other factors, including local and regional oceanography (Falcini et al. 2015; Politikos et al. 2015), can allow identification of sink areas forecast under future climatic scenarios and can be useful to redirect research and management strategies. This knowledge is essential for an effective and successful adaptive management of exploitation by fishing activities and for the maintenance and enhancement of resilience in the context of ecosystem-based management (Pikitch et al. 2004; Berkes 2012).

Our scenario-based quantitative maps are expected to improve our ability to cope with expected changes in fishery practices at sea (*e.g.* fleet behaviour shifts) and to better manage the relocation of human activities (*e.g.* fish farms, wind farms) and the enactment of an efficient Maritime Spatial Planning (Domínguez-Tejo et al. 2016). Fishery-dependent communities along cross-border coastal areas could be offered the opportunity to maximize their adaptive capacity and minimize their socio-economic vulnerability (*i.e.* climate-proofing for development) with a general improvement of social-ecological system resilience (Folke 2006).

Instead of long-term and fixed solutions, more flexible, tailored and adaptive tools and strategies would facilitate the implementation of fisheries management plans that incorporate the recovery of populations overfished or threatened by stressors with both local (*e.g.* pollution) and global origins (Folke 2006; Halpern et al. 2008; King et al. 2015; Queirós et al. 2018). Mechanistically-based forecasts can help to promote more flexible management plans based on a system of year-by-year assessment of marine resources (*i.e.* based on seasonal assessments and revision of benchmarks and protection, adaptation, mitigation management options) and facilitate more appropriate and specifically tailored monitoring plans.

5 CONCLUSION

One of the most critical issues in the Ecosystem Based Fisheries Management approach is represented by trade-offs among fisheries or between fisheries and other management objectives, such as conservation. This can be exemplified by the European anchovy case study. Management objectives need to account not only for the stock availability for fisheries (*i.e.* the economic role), but also the biomass available to sustain natural predators and species persistence through time (*i.e.* the ecological role). Clearly there is an important contribution offered by mechanistic approaches to increase predictive capability with respect to where and when fish stocks will become more vulnerable to collapse, serving as a sensitive, geographically-explicit, early-warning system (Teal et al. 2018; Sarà et al. 2018). For policy-makers it would be exceptionally difficult, if not impossible, to accurately generate climate-proof economies, dependent upon exploitable marine resources, without accounting for changes in the environment in which a stock/natural population occurs. These cannot be based on global trends such as increases in global temperature and even regional models may be insufficient unless they consider the coincidence of multiple drivers interacting on local scales (*e.g.* Kroeker et al. 2016). Often these drivers are manifest as mosaics rather than as geographic gradients (*e.g.* Helmuth et al. 2006), making the application of spatially-explicit models increasingly important. The future coupling with other analytical tools (*e.g.* physical and topographic barriers; Bacha et al., 2014; or food web models) could provide a promising approach towards the implementation of Ecosystem Based Management within the context of global change.

Waiting for the next policy window (*sensu* Rose et al. 2017), this study reinforces the growing chorus of scientific literature calling for a more ecologically sound reframing of management areas established based on political and statistical considerations by the Scientific Advisory Committee of the GFCM (GFCM, 2012), which otherwise risk being invalidated, threatening the effectiveness of the enormous efforts which now proliferate in current management policies.

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Supplementary Data: Chapter 7

Model description. In order to avoid considerable repetitions of the considerable amount of literature already published on this topic, this section comprises a short preface, as an excellent comprehensive descriptions of the Standard DEB model and its fundamentals is widely available (van der Meer 2006; Kooijman, 2010; Sousa et al., 2010). Dynamic Energy Budget theory incorporates whole-organism bioenergetics, connecting individual behaviours to population growth and rely on the assumption that flows of energy and matter (and time) through habitats and organisms are subjected to conservation laws (Charnov and Krebs, 1974) and, consequently, are traceable (and budgetable) processes. The standard DEB model (Kearney, 2012) deals with one type of food, one reserve and one structure compartment, for an isomorph (i.e. an individual that does not change in shape during growth). The mechanistic nature of the model made it an extremely powerful tool to link individual bioenergetics to environmental forcing variables (*i.e.* temperatures and food), so that the functioning of each species and thereby the magnitude and variability of LH traits (Loreau, 2010; Kearney, 2012; Pethybridge, 2013) can be reliably predicted. This is only feasible if the organismal body temperature (BT) and food densities are available for the target species, here the European anchovy, and above all if the full set of DEB parameters has been previously estimated (Pethybridge, 2013) (Table S1, Figure S1).

Table S1 List of DEB parameters used in this study for European anchovy model, central Mediterranean Sea.

			Engraulis encrasicolus	
Primary DEB parameters	Symbol	Units	Value	Reference
Structural volume at birth	V_b	cm^3	0.0002	Pethybridge et al., 2013
Structural volume at puberty	V_p	cm^3	1.73	Pethybridge et al., 2013
Shape coefficient	δ_m	-	0.17	Pethybridge et al., 2013
Max. surface area-specific ingestion rate	$\{\dot{p}_{Xm}\}$	$\mathrm{J\ cm^{-2}\ h^{-1}}$	13.50	Pethybridge et al., 2013
Assimilation rate	ae	-	0.71	Pethybridge et al., 2013
Saturation coefficient	X_k	$\mathrm{mg\ m^{-3}}$	33	Pethybridge et al., 2013
Volume-specific cost of growth	$[E_G]$	$\mathrm{J\ cm^{3}}$	4,000	Pethybridge et al., 2013
Maximum storage density	$[E_m]$	$\mathrm{J\ cm^{3}}$	2,700	Pethybridge et al., 2013
Volume-specific maintenance cost	$[\dot{p}_M]$	$\mathrm{J\ cm^{-3}\ h^{-1}}$	2.00	Pethybridge et al., 2013
Fraction of utilized energy spent on maintenance plus growth	κ	-	0.7	Pethybridge et al., 2013
Fraction reproductive energy fixed	κ_r	-	0.95	Pethybridge et al., 2013
Arrhenius temperature	T_A	°K	9,800	Pethybridge et al., 2013
Lower boundary of tolerance range	T_L	°K	278	Teal et al., 2012
Upper boundary of tolerance range	T_H	°K	305	Teal et al., 2012
Rate of decrease at lower boundary	T_{AL}	°K	50,000	Teal et al., 2012
Rate of decrease at upper boundary	T_{AH}	°K	100,000	Teal et al., 2012

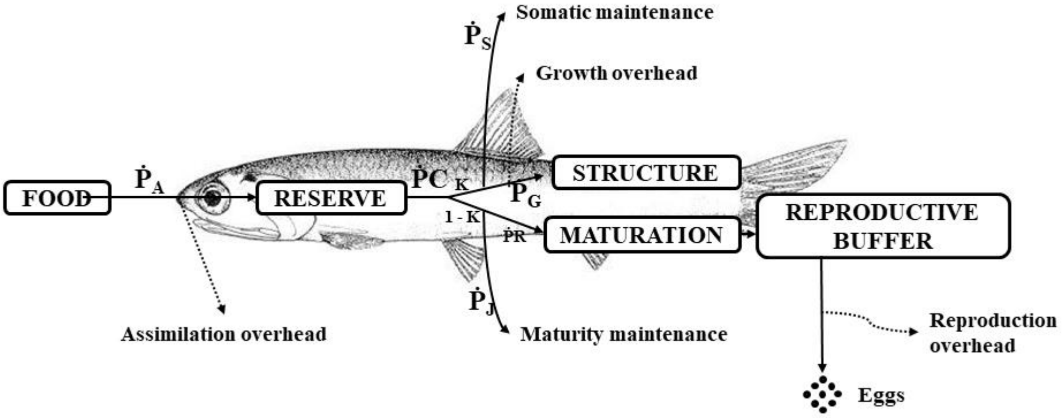

Figure S1 Schematic representation of the standard Dynamic Energy Budget model (Kooijman 2010) and the flux of energy through an organism coming from the environment; ṖA = assimilation flux, ṖC = mobilisation flux, ṖG = growth flux, ṖR = reproduction flux, ṖS = somatic maintenance flux; and 1b) flow diagram of study approach (modified from Sarà et al., 2012).

Forcing variables: food density.

To obtain a spatially continuous dataset on the distribution of food throughout the study area and across time we followed the recent approach proposed by Strömberg et al. (2009) and applied by Mangano et al. (2018). This approach involves transforming weekly Net Primary Productivity (NPP) into wet mass of zooplankton (mg m-3) starting from NPP values of Carbon per Unit Volume expressed as grams of Carbon per cubic meter per day. The NPP dataset was obtained from Oregon State University (2017), values were extracted for each cell over 4 years (2011 - 2014). The NPP values for each cell, obtained from current trophic conditions, were first transformed into zooplankton carbon mass (mg C/m^3) (Strömberg et al., 2009) and then converted into zooplankton wet mass, using the conversion coefficient provided by the ICES Committee on Terms and Equivalents (Cushing, 1958).

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CHAPTER 8 FINAL CONSIDERATIONS

This thesis represents a contribution to the understanding of the effect of Multiple Stressor on marine organisms, with the aim to understand the ecological consequences and quantify multiple impacts for management purposes. In this context we have already highlighted how the study of a single anthropogenic disturbance or Climate Change-derived alteration on multi-level ecological responses is very often misleading and generates unrealistic conclusions. Ecological research has begun to document the individual effects of these various stressors on species and ecosystems, but research into the cumulative and interactive impacts of multiple stressors is less frequent. We know that every single stressor is able to exert clear and quantifiable negative effects on marine biota, but at the same time they are also likely to have interactive effects on biodiversity and ecosystem functioning that are difficult to predict. For this reason, the study of a "single stressor *per* time" has been actually recognized as the main limitation of the current ecosystem management approach. Through **Chapter 1** has been already described the ecosystem based management's (EBM) focus on ecosystem *equilibria*, and the special need for quantities in order to provide realistic management measures for important activities at sea such as conservation, fisheries and aquaculture. Actually one way to deal with acting and interacting stressors generating quantities is to follow a mechanistic approach based on physiological processes, underlying climate change effects on organisms. One such bio-energetic model, which has been successfully applied for modelling species distributions, is the Dynamic Energy Budget (DEB) model, which is able to deal with multiple stressors and other environmental parameters that are expected to affect individual performance, such as growth and reproduction. The overall goal of this study was to introduce a comprehensive approach that integrates quantities generated by a mechanistic DEB based application, and in order to complete the framework, to include a spatially-explicit module that allow to spatially visualize potential management issues and predicted future scenarios of climate effects on target species.

This thesis opens with a **Chapter 1** that introduce the general topic, starting from the concept of disturbance, up to Ecological responses, the way in which stressors interact, the concept of ecomechanics, and the way DEB models are used as a tool to disentangle the effect of multiple stressor and generate quantities on Life History traits such as MHIS, MT, RE and TRO (refer to section 1.5.2). The estimation of DEB parameters has been further introduced in section 1.5.3 and the core parameters of the standard DEB models have been summarized in Table 2.

In **Chapter 2** we experimentally investigated the effects of a novel prey and a chronic increase in temperatures on functional traits and global fitness of the whelk *Stramonita haemastoma*. This first work reported as even though the consumption rates of two different bivalves were affected by increased temperature, on the contrary the ~2°C higher temperature condition based on a projected future climate change scenario (IPCC, 2014; Paris COP21) was not sufficient (or not maintained for sufficient time) to significantly affect the growth rates of our whelks. Anyway, this result may be consistent with the theory that assumes ectotherms follow the Von Bertalanffy growth function (VBGF), where the asymptotic length in VBGF does not strictly depend on body temperature (Kooijman, 2010). This supports the concepts that ectotherms such as gastropods and bivalves, living under different body temperature conditions (as our experimental animals did), should not record significant deviations from natural common patterns (as those at ambient temperature did) in reaching the maximum/asymptotic size. In indeterminate growing ectotherms, maximum length is directly linked to the absolute reproductive output (i.e. Darwinian fitness), and then food quality and quantity may become main determinants of fitness of our investigated species. That growth rate can be directly linked with food value and density has been shown in many studies (Walne, 1963; Verity and Villareal, 1986; Kawamura et al., 1998), in particular when food is supplied ad libitum under experimental conditions. The diet relied on the Lessepsian prey promoted the most rapid growth and achievement of egg production, while growth of whelks relying on the indigenous *M. minimus* was slower and the energy ingested was not sufficient to reach reproduction; temperature did not influence these processes. Although a previous study showed a similar energetic value of flesh for g^{-1} for the two prey species (Giacoletti et al., 2016), the differences in the amount of edible tissue per individual were, on average, of such a magnitude to promote the Lessepsian mussel as the most profitable prey with respect the native bivalve. Such observations should further raise awareness on the possible role of multiple stressors (here alien vs. increasing temperature; Vye et al., 2015) in reinforcing the effect of increasing temperature on life history traits of marine invertebrates.

In **Chapter 3,** we applied a new approach using DEB models to investigate the effects of an anthropogenic pollutant on Life-History (LH) traits of marine organisms, providing stakeholders and policy makers an effective tool to evaluate the best environmental recovery strategy. Integrated modelling and experimental studies based on mechanistic simulations, to predict the effects of an oil spill on *M. galloprovincialis* populations, have never been performed before in a Mediterranean context, suggesting the importance of this tool in helping the near future remediation technique move a step forward to become the most developed integrated-monitoring-studies already successfully applied (Gorbi et al., 2008; Gomiero et al., 2011a, b). Our mesocosm experiment reported a better performance, in terms of reducing the availability of the contaminant to the organisms, for the dispersant respect to the nano-bubbles. However probably, over a longer observation period, the efficiency of the two selected approaches should be more comparable due to the increased contribution of microbial activity. Our simulations led also to predict the timing effect of the disturbance that demonstrated a differing pattern between intertidal and subtidal populations. From the comparison of model outputs, it has been demonstrated that the disturbance strongly affected the growth of the intertidal populations from the second to the fourth year, and maturation mostly in the first and second year. The disturbance instead acted on subtidal population with a lower intensity on growth across the period from the second to the fourth year, but with a higher intensity on maturation during the first year, affecting the reproduction mostly in the second and third year. There are actually no data in the current literature regarding the timing of disturbance to refer to, or compare with, so this actually represent the first exercise to assess and predict the differing effects of a contaminant across the life-span of a model species through mechanistic models. This chapter showed as our pilot experiment, coupled with bioenergetics models predicting the growth and the potential fecundity of a model species, may represent a new approach in testing, on a broad spatial and time scale, the effects of any anthropogenic pollutant, and as a potential tool that will lead stakeholders and policy makers to evaluate current, and propose future remediation strategies to achieve the Good Environmental Status.

In **Chapter 4** we used DEB models to determine the effect of changing environmental conditions and pollution on the Indo-Pacific *Perna viridis* aquaculture. Here, we first parameterised the mussel *Perna viridis* and then used the mechanistic, predictive power of the DEB model to investigate the effects of changing somatic maintenance costs on the main LH end-points (ultimate size, growth as expressed in time to reach commercial size, reproductive potential etc.) using different scenarios. Such an approach allowed us to predict *P. viridis* performance under different aquaculture conditions. Specifically, we investigated how environmental pollution may affect the mussel's energy allocation strategies (by varying the somatic maintenance DEB parameter), and this approach can be used to provide quantitative baseline information when assessing issues dealing with aquaculture management for *P. viridis* by scientists, policy makers and stakeholders. Indeed, the main objective of modern management strategies such as ecosystem based management is to provide tailored management measures based on ecological functioning principles at a sufficient scale to reduce impacts on economic yield. The DEB model has proven to be able to quantify species' LH traits as function of potential temporal fluctuations in local environmental variables (Sarà et al., 2012). Such an approach, therefore, makes the model extremely useful for prediction of time for the mussel to reach commercial size and subsequently estimating the operational cost for a selected site (Sarà et al., 2018b). With such flexibility, the DEB model becomes increasingly important for predicting species performance under varying environments, providing important information for the management and the increasing competitiveness of aquaculture to meet future demands.

In **Chapter 5** we proposed a DEB application to study the link between future COP21 predicted temperature scenarios and varying food availability on LH-traits of some Mediterranean fishery and aquaculture target species, exploring the efficiency of Integrated Multitrophic Aquaculture as a potential management solution. In so doing, we introduced a comprehensive approach that integrates quantities generated by a mechanistic DEB based application, with the aim to operationalize, inform and support a more adaptive management of marine resources and the related development (e.g. fisheries ad aquaculture). The Dynamic Energy Budget (DEB) theory (Kooijman 2010) feeding the functional-based approach (Schoener, 1986; Kearney and Portner 2009; Kooijman 2010; Sarà et al. 2014; 2018c) was here tested and exploited as a tool able to provide those kinds of quantities to inform effective and flexible fisheries and aquaculture management plans. A sensitivity analysis was applied to simulate the effects of future environmental change on the time needed to reach the commercial size and the length at first maturity. We also explored the efficiency of Integrated Multitrophic Aquaculture (IMTA) as a potential management solution in a context of an adaptive EBM. This framework involved a spatially-explicit module that, by mapping our outcome, allowed disentangling potential conflicts among human activities at sea. Our integrated experimental and modelling approach shows that the increasing temperature within the current thermal species-specific boundaries of present target organisms will cause a general shortening of both cultivation time and length at first maturity (TIME) with an effect which was variable among present target species. Up to date, DEB has not been applied to assess the potential role of phenotypic plasticity in pushing adaptation of species under climate change; this will be a fruitful research ground for DEB scientists, evolutionary biologists and ecologists in the near future. When the DEB functional-traits outcome is translated at spatial level, by a spatially-contextualized and mapped analysis, it generates easy-to-read maps which are useful to engage with the stakeholders. They can easily identify and proactively implement adaptive site-specific management strategies tailored to target species. By adopting a spatial resolution of 0.11°, which corresponds to about 13 km pixels, we are able to underline some spatial bottlenecks where both fisheries and aquaculture will be not supported by local conditions or where they can be a win-win solution and become successful drivers for local economies. In conclusion, our final maps can be read as a quantitative informational baseline indicator of climate risk that can be shared and discussed by scientists, policy makers and stakeholders when producing management plans at local level under pressures of climate change. The quantitative mapping of changes in species' thermal habitats and growth performance are an easy-to-communicate-tool that allows to enlarge the common people's understanding while narrowing the science-policy communication gap and ensuring a more interactive science-policy interface (Kearney & Porter, 2009; Hickey *et al.*, 2013; Shelton, 2014; Pacifici *et al.*, 2015; Payne *et al.*, 2015; Gluckman, 2016; Mangano and Sarà 2017). Our DEB functional-based approach and the provided scenario-based quantitative maps, showing different simulation outcomes, can represent a tool to analyze and help narrow the field of action to be taken in place (e.g. Decision Support Tools; *sensu* Punt et al. 2016) and to discuss possible future activities on which to build local socio-economies (Mullon et al., 2016; Fernandes et al., 2017). An adaptive management based on multiple species mechanistic quantities - according to the innovative Ecosystem-Based Fisheries Management (EBFM) - are what European coastal countries need today.

Through **Chapter 6** we investigated the site-specific effects of environmental changes represented by Ocean Acidification (OA) and hypoxia on the functional and behavioural traits of the mussel *Mytilus galloprovincialis* by simulating the potential effects on growth and reproduction trough a Dynamic Energy Budget (DEB) model under a multiple stressor scenario. While much research showed that low pH may impair most functional traits (e.g. respiration, ingestion, absorption and excretion; Pörtner et al., 2004), no studies are yet available to assess the potential effects of OA on the magnitude of Life History (LH) traits, such as maximum habitat body size, fecundity, time to reach maturation and the number of spawning events under future conditions of environmental change (*sensu* Kearney and Porter, 2009; Sarà et al., 2011; 2013b). Thus, apart from long term experiments carried out in few field sites where lowered pH seawater is naturally available, the recent introduction of mechanistic functional trait-based (FT) models based on the Dynamic Energy Budget theory (DEB; Kooijman, 2010) can offer a reliable opportunity for disentangling the effect of OA on LH traits. FT-DEB can provide information about the effect of OA on the fecundity (as expressed by the number of gametes per life span, the so-called Darwinian fitness; Bozinovic et al., 2011) and the degree of reproductive failure of species providing theoretical predictions about. LH traits having implications on population dynamics and community structure throughout the species range (Sarà et al., 2013a). Here, through this chapter we specifically exploited the FT-DEB model spatially and explicitly contextualised along the Italian coasts under subtidal conditions, using four-year thermal series and satellite chlorophyll-a (CHL-a) concentrations, to test the multiple effect due to the combination of pH and hypoxia on the physiological and behavioural traits of our target species, the bivalve *Mytilus galloprovincialis* (Lamarck, 1819). We further documented the effects of those stressors on *M.*

galloprovincialis' shells through the use of a scanning electron microscope (SEM) and compared the maximum shell breaking load of treated vs. control specimens. A behavioural analysis completed the frame concerning the individual's response to both single and combined stressors. This study showed the major threat represented by the hypercapnia and hypoxia phenomena under the current climate change context, and that a mechanistic approach can illustrate complex and site-specific effects of environmental change, producing that kind of information useful for management purposes, at larger temporal and spatial scales. Moreover, carried out in a context of OA, this exercise comprises a first step in linking the fields of ecomechanics and climate change ecology, which should yield a more mechanistic understanding of how biodiversity will respond to environmental change (*sensu* Buckley et al., 2012). In addition, we believe that an appropriate knowledge of species' biological traits and a mechanistic understanding of the effect of each stressor, reached through an FT-based approach, will allow the translation of the effects of environmental change into realistic management measures taking into account the optimisation of the species' biological traits (Sarà et al., 2018a, b).

Finally, in **Chapter 7** presented a proof-of-concept study using the European anchovy as a model species to show how a trait-based, mechanistic species distribution model can be used to explore the vulnerability of marine species to environmental changes. Scenarios of temperature and food were crossed to generate quantitative maps of selected mechanistic model outcomes. By translating environmental change into biological effects through a downscaled approach, we compared the current status of this population in a core area of the Mediterranean distributional range (i.e. the Strait of Sicily, Southern Mediterranean, recognized hotspot for this species; Basilone et al., 2006) with its future responses to predicted temperature increases. We developed scenario-specific quantitative maps to show the different simulation outcomes, which allowed: (1) the identification of current source and sink areas and the detection of future temporal and spatial shifts and (2) the predictions of reproductive failure and size-structure shifts due to climate change of the anchovy. This study at the end demonstrates that model predictions based on functional traits can reduce the degree of uncertainty when forecasting future trends of fish stocks. Such a sensitive and spatially-explicit predictive approach may be used to inform more effective adaptive management strategies of resources in novel climatic conditions. Our approach generates spatial and temporal forecast data with a previously unachievable fine-scale (~12.5 km) resolution, allowing the identification of threats and opportunities for the long-term sustainability of the commercially important anchovy, with implications for the European anchovy fisheries sector (three stocks over eight in Mediterranean Sea are "currently lying outside safe biological boundaries" litt. Vasilakopoulos et al., 2014). The presented approach has a potential wide range of applications to fisheries stocks globally, assisting in the implementation of existing management evaluation strategies and helping to develop more resilient, trans-boundary resource management planning options. Our scenario-based quantitative maps are expected to improve our ability to cope with expected changes in fishery practices at sea (e.g. fleet behaviour shifts) and to better manage the relocation of human activities (e.g. fish farms, wind farms) and the enactment of an efficient Maritime Spatial Planning (Domínguez-Tejo et al. 2016). Fishery-dependent communities along cross-border coastal areas could be offered the opportunity to maximize their adaptive capacity and minimize their socio-economic vulnerability (i.e. climate-proofing for development) with a general improvement of social-ecological system resilience (Folke 2006). Instead of long-term and fixed solutions, more flexible, tailored and adaptive tools and strategies would facilitate the implementation of fisheries management plans that incorporate the recovery of populations overfished or threatened by stressors with both local (*e.g.* pollution) and global origins (Folke 2006; Halpern et al., 2008; King et al. 2015; Queirós et al., 2018). Mechanistically-based forecasts can help to promote more flexible management plans based on a system of year-by-year assessment of marine resources (*i.e.* based on seasonal assessments and revision of benchmarks and protection, adaptation, mitigation management options) and facilitate more appropriate and specifically tailored monitoring plans. Clearly there is an important contribution offered by mechanistic approaches to increase predictive capability with respect to where and when fish stocks will become more vulnerable to collapse, serving as a sensitive, geographically-explicit, early-warning system (Teal et al. 2018; Sarà et al. 2018c).

Here I made a little attempt in giving my contribution to fill some unclear knowledges, trying to contribute to the understanding of the effect of Multiple Stressor, proposing an approach meant to overcome the main limitation of the current ecosystem management approach exploiting the extremely powerful tool represented by a mechanistic DEB based application in order to disentangle the single and cumulative effects of environmental or anthropogenic stressor on marine organism. In this context, this thesis might also identify the need to expand the number of mechanistic studies dealing with individual responses to environmental and anthropogenic stressors in other sectors and environments.

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