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A macroecological projection for the future of the Mediterranean marine space management in a Ecosystemic Community Strategy context

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Abstract

Over the past few decades we have witnessed rapid ecological changes that have occurred in the world's oceans and that have mainly affected the resilience and resistance of ecosystems and the vulnerability of communities living in these ecosystems. The real challenge will be to identify and select techniques and approaches based on a macroscopic vision in order to reduce the effect of global warming, the impact of human activities and their consequences on the marine environment. These interactions can generate effects that influence the functioning of oceanic and coastal ecosystems and, consequently, goods and services, such as fishing and aquaculture production. In fact, these activities must be managed and administered responsibly to avoid competition for space which cause numerous conflicts and increase the human impact on ecosystems. In Europe, the Marine Strategy Framework Directive (MSFD) was established in 2008. It represents an important legal instrument for Member States to protect and preserve the marine environment, prevent deterioration and, where possible, restore marine ecosystems in areas where they have been damaged. The directive obliges Member States to determine the characteristics of "Good Enviromental Status" (GES) defined by descriptors, criteria and indicators. This assessment should be carried out in a complementary manner by all Member States, however, in most countries the precise means of implementation of the MSFD are not yet clear. To meet the necessity to increase MSFD implementation, we need to gather organic information in order to better address the decision making process. Here, the **chapter 1** proposes an "analysis of the evidence", by which I defined a complete picture of the EU Marine Strategy showing important gaps of the type of approaches and the means to be used for a correct analysis of the state of the environment. Based on this analysis, I selected two MSFD descriptors (D. 2 “Non-indigenous species” and D. 3 ”Commercial species”) which were the main subject of the following chapters**. Chapter 2** aims to study the effect that climate change, specifically the increase of temperature, had on the presence of non-indigenous species (NIS) in the entire Mediterranean basin. In this basin, complex and fundamental alterations which have consequences on the structure and functioning of the sea and the consequent supply of goods and services, are still underway. Furthermore, threats due to temperature increase may also alter the spread of shellfish aquaculture, hitherto recognized as the best candidate for mitigating the effects of overfishing. This topic was developed in **chapter 3**. In fact, by using a study carried out along the Italian peninsula, we highlighted the effects of the increase in temperature on the model species, *Mytilus galloprovincialis*. This was done by measuring the characteristics of the thickness and the condition index. This information will be invaluable for the examination of any possible deviation from natural models due to the increase in temperature and could represent an important context in which to face the future understanding of

the feasibility and reliability of the economic activities of shellfish aquaculture. Lastly, **in chapter 4**, the main objective was to understand and predict the possible impacts of one among the most important human economic activities: aquaculture in coastal habitats. The main question was about the possibility of combining the experimental procedures with the mechanistic bioenergetic models based on functional traits, in order to effectively predict the life history traits of the cultivated species. The need to adopt an ecosystem approach to site selection and the need to define the allocation of areas dedicated to aquaculture activities in the wider context of marine spatial planning, requires the use of modeling tools to support decision-making in aquaculture.

Therefore, the main objective of my thesis was that to determine cumulative pressures caused both by the increase in temperature and by human activities, so as to provide useful information for the destination and sustainability of use of Marine Spatial Planning (MSP), in the evaluation of the GES, using an ecosystem-like approach.

List of paper

- Montalto, V., **Martinez, M.**, Rinaldi, A., Sarà, G., & Mirto, S. (2017). The effect of the quality of diet on the functional response of *Mytilus galloprovincialis* (Lamarck, 1819): Implications for integrated multitrophic aquaculture (IMTA) and marine spatial planning. Aquaculture, 468, 371-377.

- **Martinez, M.**, Mangano, M. C., Maricchiolo, G., Genovese, L., Mazzola, A., & Sarà, G. (2018). Measuring the effects of temperature rise on Mediterranean shellfish aquaculture. Ecological Indicators, 88, 71-78.

In Preparation

- **Martinez, M.,** Mangano, M.C., Sarà, G. (2018). Up to the North: invasiveness of species in the Mediterranean Basin.

Chapter 1

General Introduction

1. Introduction

1.1 Global changes and temperature increase.

Although marine ecosystems are fundamental to the ecology of the planet, we still do not have a complete understanding of the effects of climate change on them (Hoegh-Guldberg et al., 2010). Recent studies (e.g. Hansen et al., 2006) and the IPCC Special Report "Global Warming of 1.5 °C" (Seneviratne et al., 2018) indicate that the rapid growth of greenhouse gas concentrations produced by human activities will lead to an increase of global average temperatures of ~ 1.5 ° C in only next few years (expected for 2030). This phenomenon is driving ocean systems towards conditions that have not been seen for millions of years (*sensu* Walther et al., 2002). The impacts of climate change have so far led to a decrease in ocean productivity, changes in food chain dynamics, reduced abundance of habitat-forming species, shifting species distributions and a higher incidence of disease (Hansen et al., 2010). Furthermore, the complex behavior of these drivers in ecological systems can increase the possibility of triggering feedback with domino effects (Hansen et al., 2008). The impacts of climate change have an impact on the functioning of oceanic and coastal ecosystems and consequently on the goods and services they provide to humans such as fishing and aquaculture production (Borja et al., 2013; Sarà et al., 2018a; Sarà et al., 2018b) . Specifically, little is known about the direct consequences of rising temperatures on marine ecosystems (Doney et al., 2011). Negative effects on survival, physiological responses and thermal tolerance of organisms have been found (Gilman et al., 2006; Pörtner et al., 2007; Harley et al., 2017), which have been important investigative outbreaks for decades (*sensu* Helmuth et al., 2006).

This is due to the fact that temperature plays a fundamental role in biological processes, such as biochemical reactions, metabolic and growth rates, but also interactions between species and their distribution (*sensu* Kroeker et al., 2014). In many cases, however, our understanding of how the physical environment, particularly climate change, can change the distribution of organisms is limited by our rather poor understanding of how environmental factors vary in space and time (Hallett et al., 2004). These processes determine an alteration of the structure and diversity of communities, including the possible emergence of new ecosystems (Doney et al., 2011).

In this context, the rapid ecological changes occurring in the world's oceans have challenged the leaders and the political leaders, who, in order to face and contain these changes, have adopted community marine strategies. Their challenge will be to identify and select the most effective techniques and approaches to reduce the effect of global warming, the impact of human activities and their consequences on marine ecosystems (Hansen et al., 2010).

Although we underline the urgency with which the international community must act to reduce the serious risks of climate change, these are not explicitly considered in European Community strategies (European Commission, 2014). Global warming will continue to have an increasing influence on habitats and marine species as temperature is an environmental factor affecting most marine organisms (*sensu* Walther et al., 2002). Therefore, it will become an important factor in the assessment of marine biodiversity and coastal marine space planning (European Commission, 2014).

1.2 Macroecology approach.

The effects of global changes and human activities pose a threat to ecosystem species and services (May et al., 1995). The effective solutions to this multifaceted crisis require scientific answers that embrace different disciplines and scales (Kerr et al., 2007). Macroecology fully responds to this request. In fact, this discipline deals with understanding of mechanisms and processes driving the abundance and distribution of diversity at large spatial and temporal scales (Maurer et al., 1999). It includes various fields of biology, including ecology, biogeography and macroevolution (Gaston et al 2008). The macroecological findings are based in particular on the correlative methods, which have proved effective in predicting the impacts on ecosystems (Brown et al., 1995).

The philosophy underlying the macroecological approach, discussed by Brown (1995), Gaston and Blackburn (1999) and Maurer (1999), seeks to understand ecological systems through the study and analysis of the properties of the entire ecosystem, preferring a top-down approach. This may be in contrast to a more traditional approach, based on the study of ecosystem components (bottom-up) (Maurer et al., 1999). However, the two approaches are clearly complementary. For example, an examination of the properties of an ecological community could suggest the characteristics of the species that compose it and allow us to define the structure of the populations, while the study of the behavior of the species could provide information on the characteristics of the community (Gaston and Blackburn 2008). In fact, by following both the "bottom-up" and "top-down" paths, a better understanding is achieved than the one deriving from the use of the single approaches. (Kent, et al 2005).

This philosophy is not peculiar to macroecology (Gaston and Blackburn 2008). A complete understanding of most, if not all, of scientific disciplines can only come about by integrating observations made from a series of points of view, or on a variety of spatial and temporal scales (Gaston and Blackburn 1999). Obviously, large- and small-scale approaches are both tools for examining and trying to understand the complexity of ecological systems (Gaston and Blackburn, 2008). The macroecological approach can be developed through the use of three rigorous phases (Gaston and Blackburn 1999). The first phase answers the question "What?" and document the models to be used. The second phase answers the question "How?" and check the structure of the models. Finally, the last phase answers the question "Why?" and it concerns the mechanisms that explain the observed models (*sensu* Gaston and Blackburn 2008).

In addition to describing and explaining large-scale species distributions, macroecology deals with the search for laws, theories and general principles related to the processes that underlie large-scale ecological spatial and temporal models. In this context, various researchers have tried to use the theory to link together different models that could be explained by a limited set of processes (Kent et al., 2005). The result is usually defined with a "unified theory" (McGill and Collins 2003). At present, evidence for a clear unified theory has yet to emerge, but, as Kent (2005) shows, there is no doubt that significant progress has been made in recent years and that the future has interesting prospects for both biogeographers that for the ecologists. These macroecological perspectives will however help to address the biotic consequences of global change and human activities. The policy responses needed to overcome these enormous challenges must draw on many scientific disciplines and must be applied to the full range of spatial scales on which global changes and human action exert their effects (*sensu* Kerr et al., 2007).

1.3 Policy Contest: Marine Strategy Framework Directive and Maritime Spatial Planning.

Marine waters have traditionally been used by society for various activities (e.g. fishing, aquaculture, navigation, tourism, agricultural discharges and urban areas (Borja et al., 2011). New activities are currently being developed or increased (e.g. renewable energy, etc.), which can compete for space compared to traditional activities, causing numerous spatial conflicts and increasing the human impact on marine ecosystems (Ban and Alder 2008; Halpern et al., 2008). While the legal framework for 'marine spatial planning' (MSP) is relatively new (Ehler and Douvere 2009; European Commission, 2013), most of the legislation to protect, conserve or enhance marine ecosystems is based on the United Nations Convention on the Law of the Sea (UNCLOS, 1982).

Starting in 2008, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) came into force, defining a framework for community action in the field of marine environmental policy. The MSFD is the environmental pillar of the integrated maritime policy (IMP). It aims to achieve or maintain the Good Environmental Status (GES) in the marine environment by 2020, using an ecosystem approach. To achieve this objective may include spatial measures, spatial and temporal distribution controls and management coordination measures. MSP can therefore be an important tool to enable Member States to protect and preserve the marine environment, prevent its deterioration or, where practicable, restore marine ecosystems in areas where they have been adversely affected. Furthermore, inputs into the marine environment are to be prevented and reduced, with a view to phasing out pollution, so as to ensure that there are no significant impacts on, or risks to, marine biodiversity, marine ecosystems, human health or legitimate uses of the sea (Article 1, paragraph 2, MSFD).

According to the MSFD, the environmental status is defined by 11 descriptors, and forms a proposed set of 29 associated criteria and 56 indicators that include biological, physico-chemical indicators as well as pressure indicators—including hazardous substances, hydrological alterations, litter and noise, and biological disturbance such as introduction of non-indigenous species (Cardoso et al., 2010; European Commission, 2010) (table 1, supplementary material).

The Directive requires Member States to determine the characteristics of GES, that is, "what does GES look like", and to develop environmental targets and associated indicators. These environmental targets and associated indicators should help guide progress towards achieving or maintaining GES. 'Good Environmental Status' shall be determined at the level of marine regions or subregions (Article 3, paragraph 5, MSFD) (figure 1).

Determining GES and setting environmental targets and associated indicators are to be coordinated with other Member States in their marine region or subregion and should reflect closely the EU Commission Decision 2010/477/EU of 1 September 2010 on Criteria and Methodological Standards of Good Environmental Status (COM Decision 2010/477/EU). This includes the preparation, by 2012, of an initial assessment of the marine environment, a characterization/ determination of GES and a suite of appropriate environmental targets and associated indicators. by 2014, Member States shall have established fit-for-purpose monitoring programmers and developed (by 2015) and implemented (by 2016) programmers of measures designed to achieve or maintain GES by 2020 (Article 11 and 13, MSFD) (figure 2).

This assessment should be carried out in an integrative way, including measurement of many ecosystem components together with physicochemical parameters and elements of pollution (Borja et al., 2009). However, in most countries, the precise means of implementing the MSFD are yet unclear. In most cases, MSFD are focusing on individual descriptors and then criteria and indicators within the descriptors, with apparently little or no attention being paid to the means of combining the indicators, criteria and descriptors into a holistic assessment of the environmental status (http:// ec.europa.eu/environment/marine/public-consultation/index_en. htm).

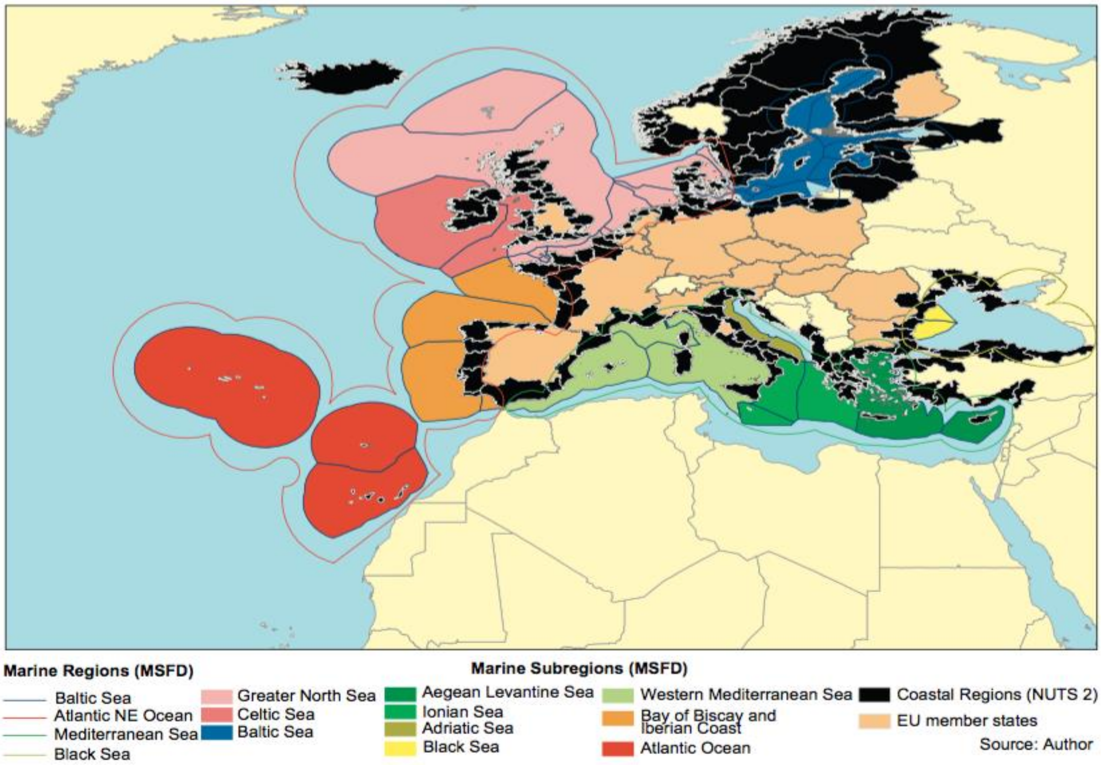

Fig. 1. European marine regions and subregions.

Fig. 2. Steps of the MSDF.

1.4 Literature search: State of the Art of Marine Strategies

In response to numerous attempts to assess the good state of the environment for the management of human activities in European regional seas. Here we present a complete picture on the types of descriptors studied up to now and on the approaches hitherto used to reach the GES. Once highlighted any gaps in marine strategies, these will represent the basis on which this doctoral thesis was developed.

1.4.1 Materials and methods

An extensive analysis of the literature has been completed at 16/04/2016 a systematic review, designed to investigate, deepen the knowledge on the basis of the main objectives, applications highlighting any gaps in marine strategies, focus of my thesis.

The search was carried out using prominent or substantial keywords forming a simple search string ((*"Good Environmental Status" OR "GES") OR ("Marine strategy Framework" OR "MSFD") AND "Marine Spatial Planning" AND "Mediterranean Sea"*). The research was carried out by selecting articles starting from 2008, the year in which the directive was published until 15 April 2016, the day on which the research was completed. The search string was entered into scientific computerized databases including: ISI Web of Sciences, Scopus. Additional general search engines were used (Google and Google Scholar) limiting the search for appropriate data to the Word, PDF and/or Excel documents and to the first 50 hits (Mangano & Sarà, 2017a, 2017b). A hand search was performed on reference list of relevant review articles to identify any additional references. Hits generated from the search were collated in a database, examined for relevance and critically appraised (table 2a and 2b, supplementary material). Data and evidence extraction from peer review and grey literature were organized and synthesized according to specific criteria, e.g. geographic area, habitat preferences, associated species, with a complete list of the collated studies for each Mediterranean sector (table 2a and 2b, supplementary material). All quantitative information of each sheet was extracted from a complete picture on marine strategies used in the Mediterranean Sea.

1.4.2 Results

The results of the research generated a baseline of about 200 scientific articles. Only 91 were selected and evaluated as relevant to the research carried out until April 2016. In Figure 1, the selected articles are grouped by year of publication. It can be noted that with increasing time starting from the year 2008 the publication number tends to increase with a maximum of 31 articles in 2015, and a minimum of 1 in the years 2010 and 2009.

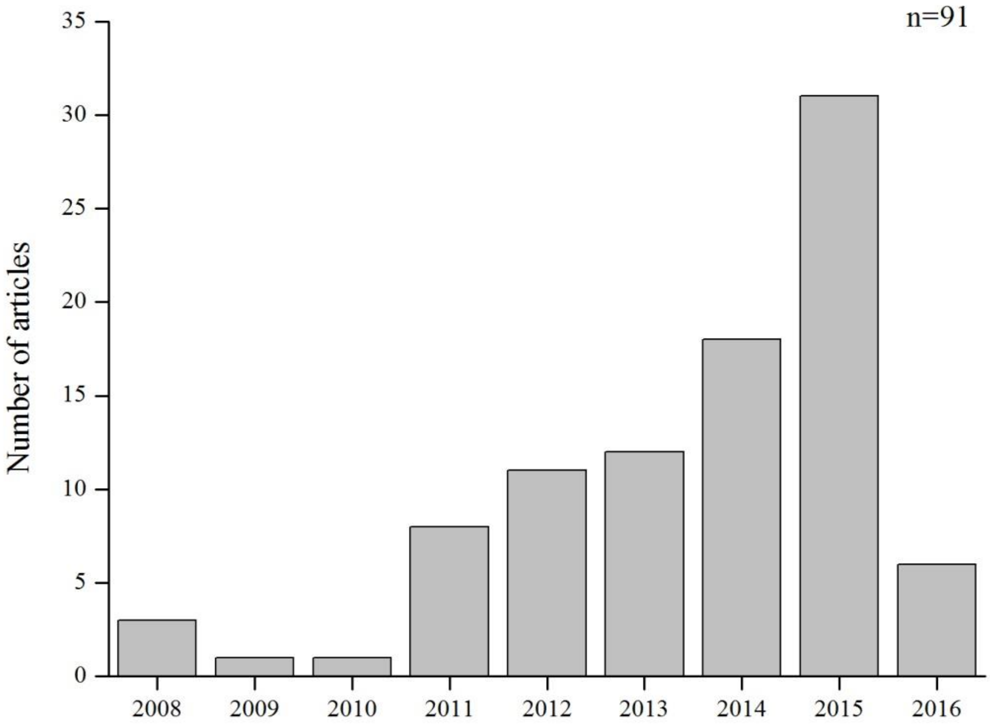

Fig. 1. Year of publication of the selected articles.

Figure 2 shows the number of sources for each type of source analyzed. As we can see the scientific articles are the most consulted sources (54), to follow we find Conference Paper (16), Review (6), Book chapter and Viewpoint (4), Short Communication (3) and finally Report of project, Editorial, Preface and Perspective (1).

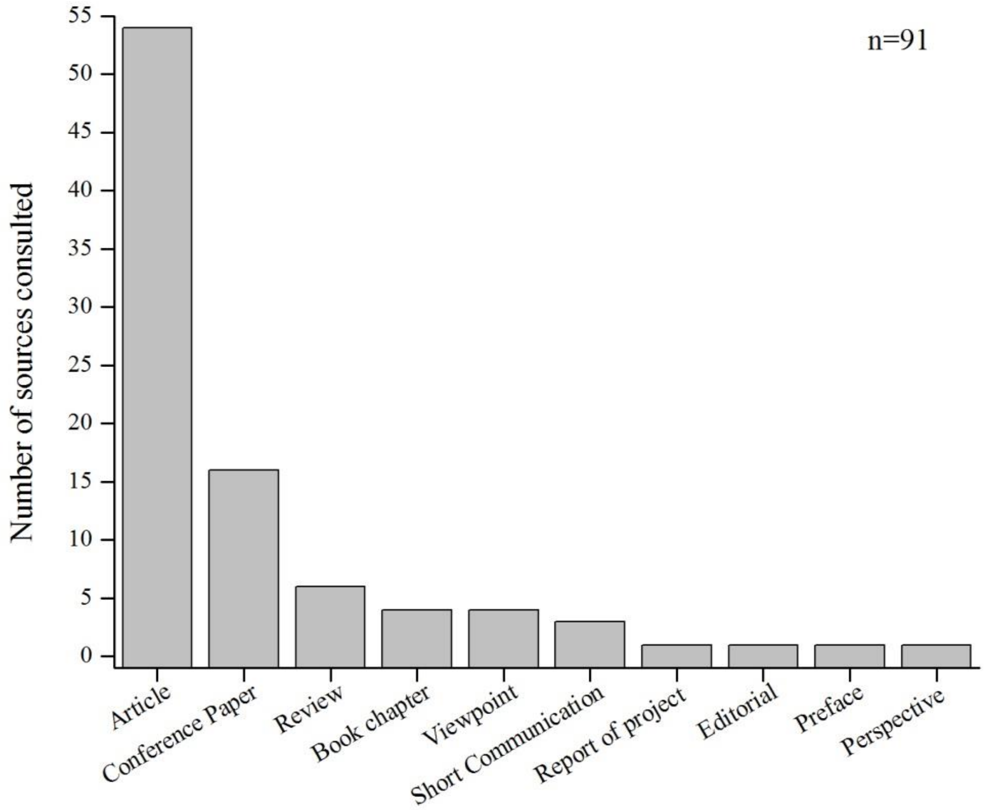

Fig. 2. Number and type of source consulted.

Starting from the analysis of the previous results shown, Figure 3 shows the number of sources consulted in each geographical area under study. This was indicated only in 77 out of 91 sources consulted. The highest number of sources can be highlighted in Northern European regions including the North Sea with a value of 16, followed by the Baltic Sea with a value of 15 sources consulted, followed by 14 in Europe, 12 in Atlantic Ocean, 11 in Mediterranean sea, 3 in Black sea, 1 source consulted in the geographical regions of the Czech Republic, Pacific Ocean, Indian Ocean and Irish sea, Slovakia, Weddell Sea.

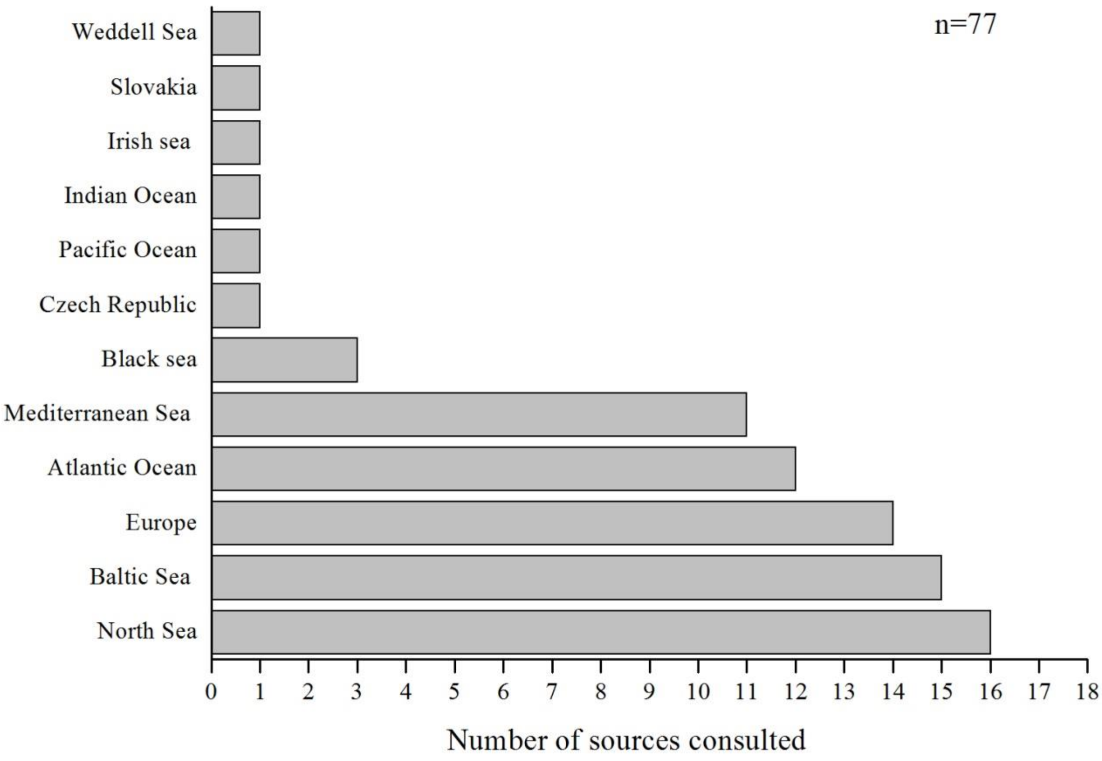

Fig. 3. Geographical distribution of the number of source consulted.

Figure 4 shows the number of sources consulted in each country under study. Out of 91 consulting sources, only 42 reported information on the country under study. The highest value we find in Germany with 9 sources, followed by 4 sources in Spain, 3 in the UK, 2 in Romania, Sweden, Bulgaria, Denmark Lithuania, Portugal, Greece, Norway and France. One source consulted for Ireland, Czech Republic, Turkey, Finland, Netherlands, Adriatic, Slovakia and Belgium.

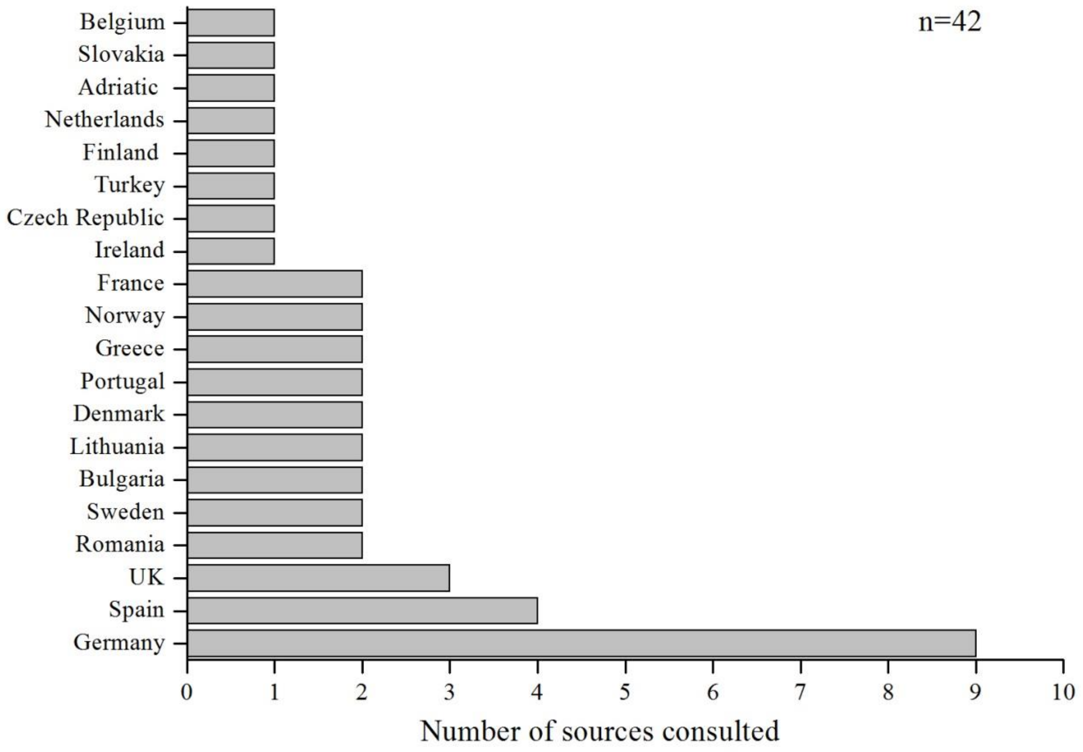

Fig. 4. Distribution of the number of sources consulted in European countries.

Figure 5 shows the number of sources consulted for each type of approach used. Out of 91 sources, the approach that presents the highest values is the ecosystem approach with a value of 54. Below we find the Integrated approach with a value of 12 sources consulted and the Risk Analysis with only 2 sources. Furthermore, no approach is defined in 14 sources consulted.

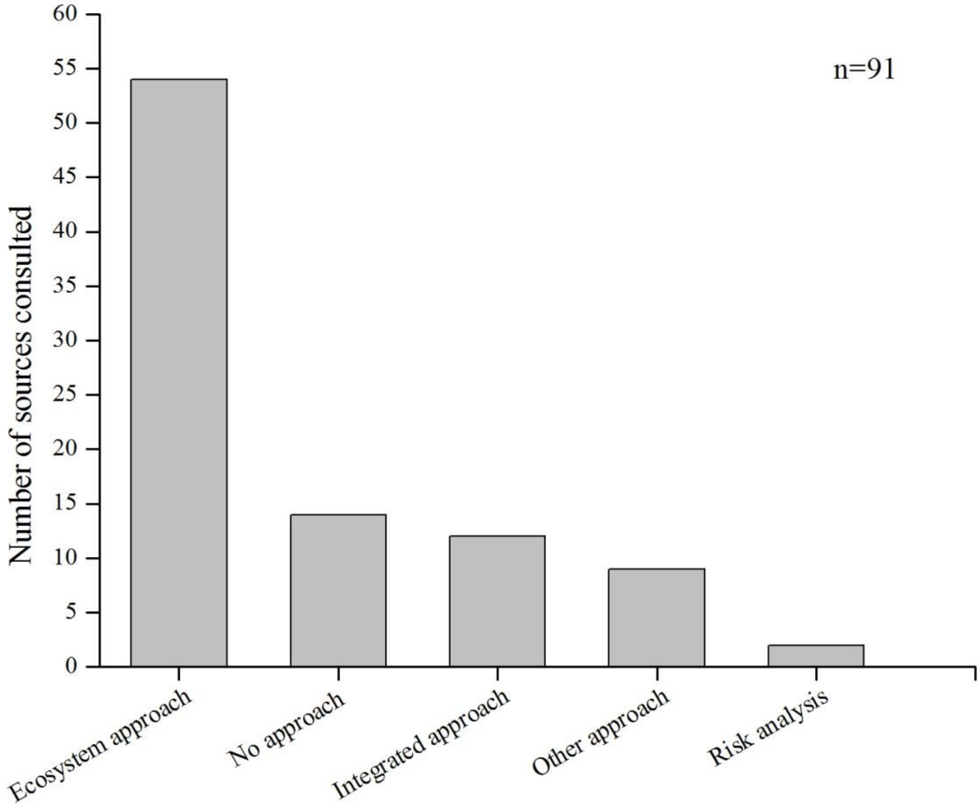

Fig. 5. Number of sources consulted and types of approach used.

At this point it is possible to select the articles and to define information about the presence of MSDF descriptors. This is important for understanding the state of the art and defining which and how many descriptors until now are more or less studied. Figure 6 indicates the presence / absence of the descriptors defined in the MSDF in the sources consulted. Out of 91 articles, 58 have the descriptors as their research object, while 33 speak of MSFD but do not take into account the descriptors of the legislation.

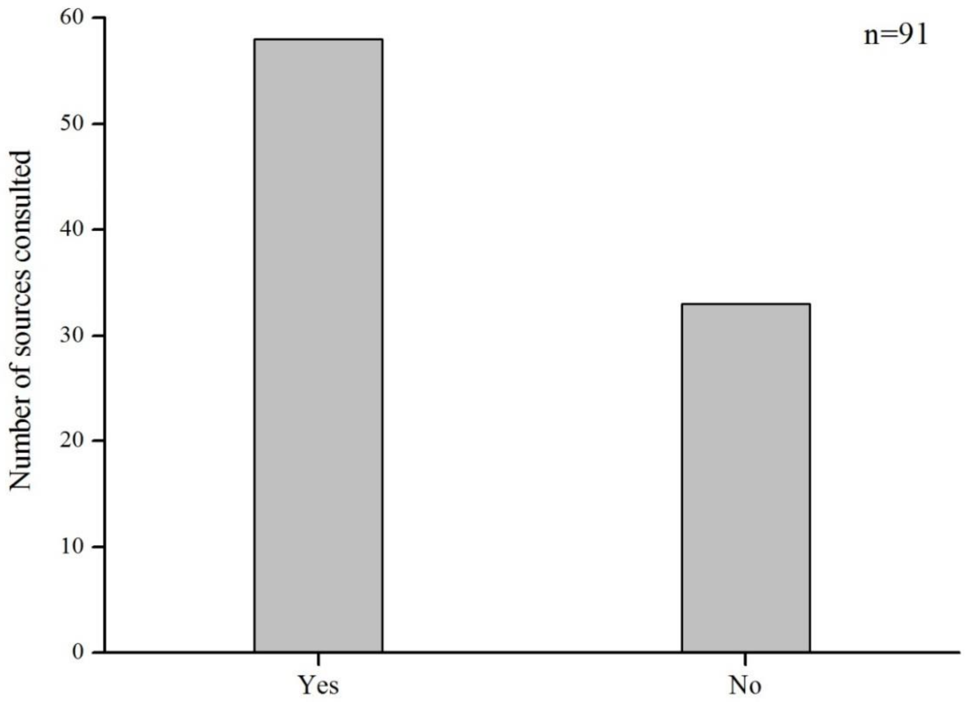

Fig. 6. Presence/absence of the descriptors defined in the MSDF in the number of sources consulted.

Starting from the analysis of the previous results shown, in figure 7 we show the number of sources consulted for each descriptor of the MSDF object of the sources themselves. On a number of 58 sources consulted, we obtained 146 observations. The highest values correspond to the descriptor 1 (D. 1) with 21 articles, followed by the descriptor 3 (D. 3) with 19 articles and the descriptor 4 (D. 4) with 16 articles, followed by 14 for both descriptor 11 (D. 11) and the descriptor 6 (D. 6) and the descriptor 8 (D. 8), 13 for the descriptor 2 (D. 2), 10 for the descriptor 10 (D. 1), 7 for the descriptor 9 (D. 9) and 5 for the descriptor 7 (D. 7).

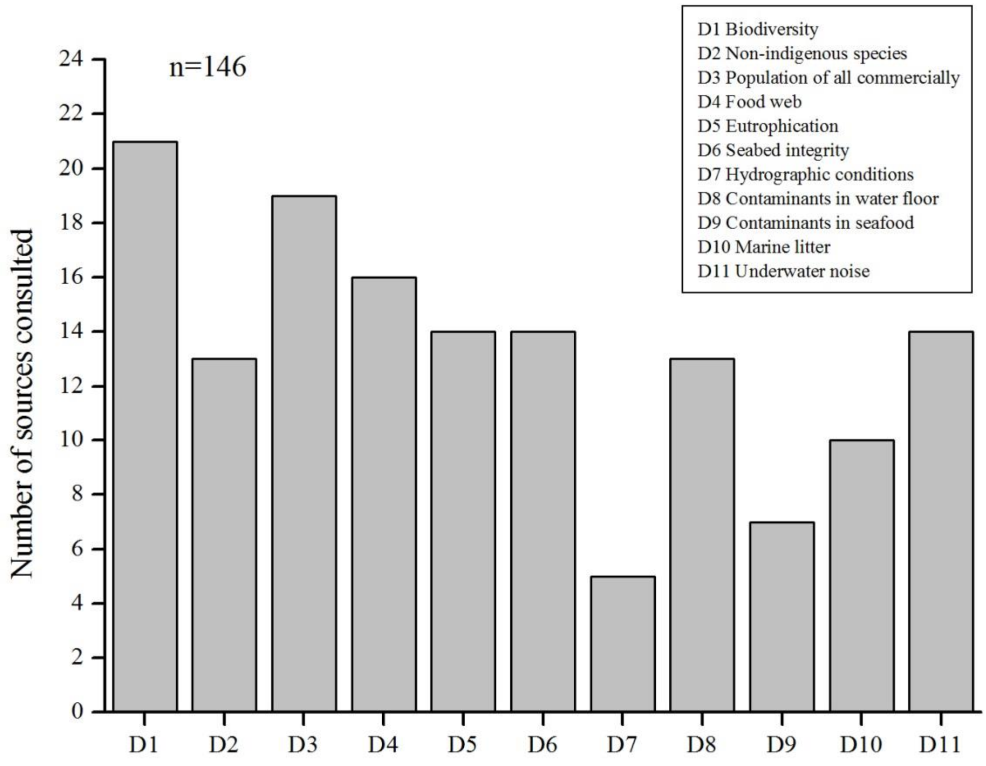

Fig. 7. Number of source consulted and descriptor of MSFD.

Figure 8 focuses on the descriptor 3 showing the number of sources consulted and for each type of fishing of this descriptor. Of the 19 sources, the type of fishing with the highest values is Fish Management with a value of 15. Below we find Shell management with a value of 4 sources consulted.

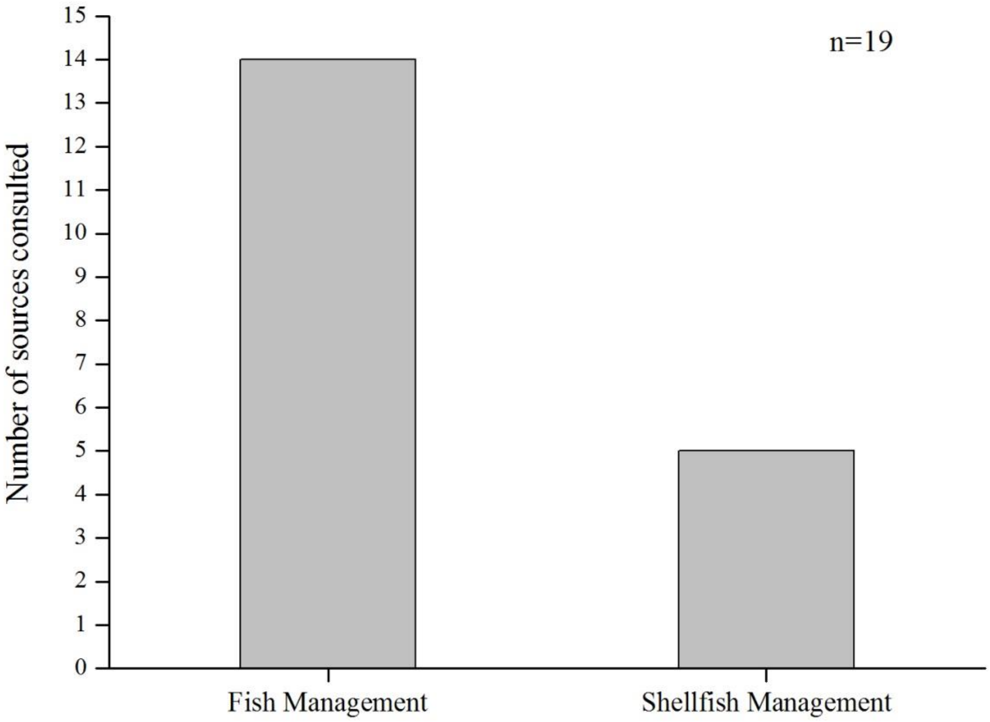

Fig. 8. Number of source consulted and the types of fishing of the descriptor 3 (D. 3).

1.4.3 Discussions and conclusions

Although several attempts have been made to assess the environmental status of marine waters, based on the notions of the ecosystem, significant gaps still remain concerning: (i) the understanding of marine ecosystems and their responses to human activities; (ii) basic knowledge of basic knowledge of MSFD; (iii) understand the meaning of GES, and (iv) define a complete picture of the different descriptors used up to now, (v) identify the types of approaches hitherto used to reach the GES.

The results showed a temporal trend starting from the year 2008, when the MSFD directive was established (2008/56 / EC, European Commission, 2008) until the year 2016, when the following search has been completed. As we note, the results show that the number of sources consulted increases with the increase in the time trend, and two peaks can also be identified. The first peak corresponds to the year 2012, which coincides with the initial assessment of the current ecological status of marine waters and the environmental impact of human activities on these waters, as defined in accordance with Article 8 of 2008/56 / EC. The second peak is present in 2015 where we find a higher number of sources consulted, which coincides with the preparation of a program of measures aimed at achieving or maintaining a good ecological status, in accordance with Article 13, paragraphs 1, 2 and 3 of 2008/56/EC.

The research phase of the sources consulted was carried out through 2 methods. The first was performed using the computerized scientific databases where it emerged that most of the types of sources were mainly scientific articles, we also find conferences, papers, reviews, book charters, report of projects following viewpoint and short communication, editorial, preface, perspectives. These sources address topics such as the harmonization, uniformity and clarity of the MSFD and GES concepts between the countries of the union and between the different research groups, as for example it was realized in the document of the Common Understanding (Claussen et al., 2011). The second modality was carried out through web research and suggests that the sources consulted above all present material of projects already completed or in progress. In fact, the types mainly include pdf, articles, web site and project.

As stated in article 1, paragraph 3, MSFD and in Berg et al. (2015), also in this research it has been shown that the approaches used are mostly of an ecosystemic type, but there are also case studies of integrated approaches (Borja et al., 2016) and Risk analysis techniques (Tallins et al., 2010). This means that it is necessary to consolidate the knowledge of ecosystem functions and services, in particular based on a common European strategy, which defines a shared vision of ecological terminologies and concepts (Borja et al., 2016).

As shown by the results, only a part of the sources consulted presented explicit references on the types of descriptors examined. This is important for understanding the state of the art and defining which and how many descriptors so far are more studied than others.

In this doctoral thesis, I decided to select and analyze only the D. 2 descriptor "Non-indigenus species", which in this study showed lower values compared to other descriptors. This descriptor was chosen because in the Mediterranean Sea the increase in the number of non-indigenous species (NIS) is causing major alterations affecting the structure and functioning of the sea and the consequent supply of goods and services (Galil et al., 2016).

The D. 3 "Commercial Fish and Shellfish" descriptor was also selected, although this showed high values in our study. However, the results say that most of the sources consulted for the D. 3 descriptor had the main objective of Fishing Management and not Shellfish Management. This was one of the reasons that led me to select the D. 3 descriptor and specifically selected bivalve species (such as *Mytilus galloprovincialis*) which represent an important segment of the world aquaculture (Martinez et al., 2018).

Screening and analysis of the evidence have therefore defined a complete picture of the marine strategy, but above all have revealed important gaps on the determination of a correct analysis of the state of the environment through the use of an ecosystem approach. In particular as claimed by De Jonge et al. (2012) it is necessary to consider the evaluation of ecological, economic and social aspects of fundamental importance. This is to allow the sustainable use of goods and services, while maintaining a good ecological status and preventing marine deterioration (Borja and Elliott 2013). However, in my opinion, the effective implementation of this paradigm faces many challenges, including: (i) The need to involve stakeholders (van Hoof et al., 2014), (ii) the aggregation of multiple indicators, descriptors and spatial scales effectively (Borja et al., 2014), (iii) the overlapping of the various directives, the legislative and government instruments (Boyes and Elliott 2014). Therefore, I recommend consolidating scientific knowledge, in particular based on an alignment of vision of ecological terminologies and concepts, to promote the sustainable use of the oceans and seas. These results have allowed me to acquire a deeper knowledge about the main topic of the thesis. This step was necessary to outline the future steps for the selection of the descriptors used as a proxy, to define the times and methods of implementation of the doctoral thesis.

1.5 Summary of the project and select descriptor

The impacts of climate change are perceived on a global scale and are likely to intensify considerably in the future, affecting, above all, phenomena such as (i) resilience and endurance of ecosystems around the world, (ii) the vulnerability of communities living in these ecosystems.
In this context, the true challenge of the common marine strategies adopted in each country, will be to identify the most effective techniques and approaches to reduce system vulnerability.
The main objective of the Marine Strategy framework directive (MSFD) is to achieve or maintain a good environment status (GES) in the EU's marine by 2020, as defined by the Directive:

"... The environmental state that preserves the ecological diversity, vitality and productivity of the seas and oceans, which are clean, healthy and productive, thus safeguarding the potential for uses and activities for present and future generations …" (Article 3).

The MSFD requires Member States to apply an ecosystem approach to the management of human activities of the European regional, seas by providing for each region and subregion of marine Member States, a set of requirements to determine GES based on declarers, criteria and qualitative indicators. To achieve good ecological status, all relevant human activities must be carried out in compliance with the requirement to protect and preserve the marine environment.
In fact, the aim proposed in this thesis is to offer useful tools for the determination of the GES, through case studies using a macroscopic approach in order to evaluate all those processes that influence the responses on the state of the environment. Indeed, this study could be seen as an opportunity to raise awareness in the public and the scientific community to take actions and define strategies to mitigate the impact that humans have on marine ecosystems, based on the MSFD. Specifically, the knowledge gathered from the systematic literature review performed in **chapter 1** allowed me to define the strategies to reduce human impact on marine ecosystems, based on MSFD. This step was necessary to outline the future steps for the selection of the descriptors used as a proxy, to define the times and methods of implementation of the doctoral thesis.
In fact, the strategy proposed here the case studies and the questions I have elaborated will be articulated in 3 main chapters. The chapters contain two among descriptors selected in the MSFD:

- **Case of study of Descriptors D. 2 (**Non-indigenous species) developed in **chapter 2**
- **Case of study of Descriptor D. 3** (Commercial Fish and shellfish) developed in **chapters 3-4**.

Chapter 2 aims to study the effect that climate change, specifically the increase in temperature and the concentration of chlorophyll has had on the presence of non-indigenous species (NIS) on the entire Mediterranean basin. Where alterations are still ongoing complex and fundamental affecting the structure and functioning of the sea and the consequent supply of goods and services (Galil et al., 2016). A recent synthesis on marine biodiversity of the Mediterranean (Coll et al., 2010) described the Mediterranean as a hot spot of biodiversity. It hosts about 17.000 marine species, and more than 600 (3.3%) are not indigenous and they are defined as Non-indigenous species (NIS) (Zenetos et., al 2010). In fact, the number of introductions recorded in the Mediterranean Sea is much higher than in other European seas, caused by the warming of water and the impact generated by man (Galil et al., 2014). These motivations are cause to a progressive migrations of the alien species tropical and subtropical species in the Mediterranean Sea, changing the ecosystem stability in the basin (Zenetos et al., 2010). The continuum flow of NIS and they growing presence and stability in the Mediterranean basin implies a profound alteration and continues in the distribution patterns of the species that appear to be significantly increased in recent years (Galil et al., 2014). In fact, there is an increasing need to take action to control biological invasions and thus mitigate their impacts on biodiversity, ecosystem services and human activities thereby (Katsanevakis et al., 2013a). Regulate the flow of alien species is of particular importance for future community actions and political strategies covering major maritime strategic objectives, such as the Marine Strategy Framework Directive (MSFD) (2008/56/EC), which specifically targets the introduction of marine alien species as a major threat to European biodiversity and ecosystem health, requiring Member States to include alien species in the definition of GES and to set environmental targets to reach it (Katsanevakis et al., 2013b). The goal of this chapter was to try to quantify the geographic distribution of invasive species in the Mediterranean Sea. We present an analytical structure that can be useful to the scientific community for the possibility of using information regarding temperature and the number of occurrence to define the invasion status of alien species on the entire Mediterranean basin.

Chapter 3 focuses on the possible unforeseen and still unforeseen, poorly-known effects of climate change can inflict on shellfish aquaculture, which extends along the Mediterranean coasts. Threats due to temperature rise can alter the deployment and development of this sector, up until now recognised as the best candidate to mitigate the effects of fishery overexploitation.

By adopting a study along the Italian peninsula, we investigate the effects of temperature increase on the model species, *Mytilus galloprovincialis*, measuring the characteristics of the valve fragility (thickness) and the condition index. Such information is valuable when investigate any possible deviation from natural patterns in response to increasing temperature and may represent important background in which to address the future understanding of feasibility and reliability of shellfish aquaculture economic activities. This study may further provide the opportunity to raise awareness in public and scientific communities (*sensu* Mangano et al., 2015; Mangano and Sarà, 2018) to inform on the importance of building upon common actions and strategies to mitigate the impact of climate change on several aspects of the food chain production based on marine sectors. The thickness (and fragility) of the valves in shellfish could also be considered as a new reliable indicator when informing the Marine Strategy Framework Directive (MSFD) (European Commission, 2008), which sets the overall objective of achieving or maintaining the "Good Ecological Status" (GES) in European Marine Waters by 2020 under a context of environmental and climate change, as for example within the specific Descriptor 3 "Commercial Fish and shellfish".

In chapter 4 I defined as a central objective the understanding and prediction of probable impacts of one among the most important human economic activity like the aquaculture in coastal habitats. The main question deals with the possibility to combine experimental procedures with the new mechanistic functional trait based bioenergetic models in order to effectively predict life history traits of cultivated species. This practice very often involves filter feeders, such as bivalves, by the use of which bioenergetics budgets are strongly influenced by the quality and quantity of different foods. However, to date, scant information is available, to really understand the rebounds of food availability on the growth performances of these harvested biomasses in the natural environment.
By choosing the mussel *Mytilus galloprovincialis* as a model, this study aims to (1) characterize the functional response of the species to define all parameters related to food intake strategies and (2) to investigate how responses change as a function of varying food sources. The need for adopting an ecosystem approach to site selection and framing the allocation of areas dedicated to aquaculture activities within the broader context of the Marine Spatial Planning requires the use of modeling tools as a support for decision-making in aquaculture. The integrated model described in this study can provide a useful means to design responsible aquaculture production systems for tomorrow. The mechanistic nature of such models combined with broad applications to other species, allows the consideration of the effects of different environmental drivers such as water temperature and food availability inexplicitly calculating the metabolism of the cultivated species, increasing our ability to prevent impacts and to assist with site selection, moving toward the sustainability of integrated multi-trophic aquaculture.

The chapters of the present thesis have been written as stand-alone studies to allow autonomy in the reading of each chapter. For this reason, some repetition of introductory information may occur throughout the thesis.

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1.7 Supplementary material

Table 1. Descriptors, Criterion and Indicator of the MSDF, 2008/56/EU, European Commission.

Descriptor	Criterion	Indicator
D1 Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions.	1.1 Species distribution	1.1.1 Distributional range
		1.1.2 Distributional pattern within the latter, where appropriate
		1.1.3 Area covered by the species (for sessile/benthic species)
	1.2 Population size	1.2.1 Population abundance and/or biomass, as appropriate
	1.3 Population condition	1.3.1 Population demographic characteristics (e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates)
		1.3.2 Population genetic structure, where appropriate
	1.4 Habitat distribution	1.4.1 Habitat distributional range
		1.4.2 Habitat distributional pattern
	1.5 Habitat extent	1.5.1 Habitat area
		1.5.2 Habitat volume, where relevant
	1.6 Habitat condition	1.6.1 Condition of the typical species and communities
		1.6.2 Relative abundance and/or biomass, as appropriate
		1.6.3 Physical, hydrological and chemical conditions
	1.7 Ecosystem structure	1.7.1 Composition and relative proportions of ecosystem components (habitats and species)
D2 Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems.	2.1 Abundance and state characterisation of non- indigenous species, in particular invasive species	2.1.1 Trends in abundance, temporal occurrence and spatial distribution in the wild of non-indigenous species, particularly invasive non-indigenous species, notably in risk areas, in relation to the main vectors and pathways of spreading of such species
	2.2 Environmental impact of invasive non-indigenous species	2.2.1 Ratio between invasive non- indigenous species and native species in some well studied taxonomic groups (e.g. fish, macroalgae, molluscs) that may provide a measure of change in species composition (e.g. further to the displacement of native species)
		2.2.2 Impacts of non-indigenous invasive species at the level of species, habitats and ecosystems, where feasible
D3 Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock.	3.1 Level of pressure of the fishing activity	3.1.1 Fishing mortality (F)
		3.1.2 Ratio between catch and biomass index ('catch/biomass ratio')

	3.2 Reproductive capacity of the stock	3.2.1 Spawning Stock Biomass (SSB)
		3.2.2 Biomass indices
	3.3 Population age and size distribution	3.3.1 Proportion of fish larger than the mean size of first sexual maturation
		3.3.2 Mean maximum length across all species found in research vessel surveys
		3.3.3 95% percentile of the fish length distribution observed in research vessel surveys
		3.3.4 Size at first sexual maturation, which may reflect the extent of undesirable genetic effects of exploitation
D4 All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.	4.1 Productivity (production per unit biomass) of key species or trophic groups	4.1.1 Performance of key predator species using their production per unit biomass (productivity)
	4.2 Proportion of selected species at the top of food webs	4.2.1 Large fish (by weight)
	4.3 Abundance/distribution of key trophic groups/species	4.3.1 Abundance trends of functionally important selected groups/species
D5 Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters.	5.1 Nutrients level	5.1.1 Nutrients concentration in the water column
		5.1.2 Nutrient ratios (silica, nitrogen and phosphorus), where appropriate
	5.2 Direct effects of nutrient enrichment	5.2.1 Chlorophyll concentration in the water column
		5.2.2 Water transparency related to increase in suspended algae, where relevant
		5.2.3 Abundance of opportunistic macroalgae
		5.2.4 Species shift in floristic composition such as diatom to flagellate ratio, benthic to pelagic shifts, as well as bloom events of nuisance/toxic algal blooms (e.g. cyanobacteria) caused by human activities
	5.3 Indirect effects of nutrient enrichment	5.3.1 Abundance of perennial seaweeds and seagrasses (e.g. fucoids, eelgrass and Neptune grass) adversely impacted by decrease in water transparency
		5.3.2 Dissolved oxygen, i.e. changes due to increased organic matter decomposition and size of the area concerned
D6 Sea-floor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected.	6.1 Physical damage, having regard to substrate characteristics	6.1.1 Type, abundance, biomass and areal extent of relevant biogenic substrate
		6.1.2 Extent of the seabed significantly affected by human activities for the different substrate types
	6.2 Condition of benthic community	6.2.1 Presence of particularly sensitive and/or tolerant species

		6.2.2 Multi-metric indexes assessing benthic community condition and functionality, such as species diversity and richness, proportion of opportunistic to sensitive species
		6.2.3 Proportion of biomass or numbers of individuals in the macrobenthos above some specified length/size
		6.2.4 Parameters describing the characteristics (shape, slope and intercept) of the size spectrum of the benthic community
D7 Permanent alteration of hydrographical conditions does not adversely affect marine ecosystems.	7.1 Spatial characterisation of permanent alterations	7.1.1 Extent of area affected by permanent alterations
	7.2 Impact of permanent hydrographical changes	7.2.1 Spatial extent of habitats affected by the permanent alteration
		7.2.2 Change in habitats, in particular the functions provided (e.g. spawning, breeding and feeding areas and migration routes of fish, birds and mammals), due to altered hydrographical conditions
D8 Concentrations of contaminants are at levels not giving rise to pollution effects.	8.1 Concentration of contaminants	8.1.1 Concentration of the contaminants mentioned above, measured in the relevant matrix (such as biota, sediment and water) in a way that ensures comparability with assessments under Directive 2000/60/EC
	8.2 Effects of contaminants	8.2.1 Levels of pollution effects on the ecosystem components concerned, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship has been established and needs to be monitored
		8.2.2 Occurrence, origin (where possible), extent of significant acute pollution events (e.g. slicks from oil and oil products) and their impact on biota physically affected by this pollution
D9 Contaminants in fish and other seafood for human consumption do not exceed levels established by EU legislation or other relevant standards.	9.1 Levels, number and frequency of contaminants	9.1.1 Actual levels of contaminants that have been detected and number of contaminants which have exceeded maximum regulatory levels
		9.1.2 Frequency of regulatory levels being exceeded
D10 Properties and quantities of marine litter do not cause harm to the coastal and marine environment.	10.1 Characteristics of litter in the marine and coastal environment	10.1.1 Trends in the amount of litter washed ashore and/or deposited on coastlines, including analysis of its composition, spatial distribution and, where possible, source

		10.1.2 Trends in the amount of litter in the water column (including floating at the surface) and deposited on the sea- floor, including analysis of its composition, spatial distribution and, where possible, source
		10.1.3 Trends in the amount, distribution and, where possible, composition of micro-particles (in particular micro-plastics)
	10.2 Impacts of marine litter on marine life	10.2.1 Trends in the amount and composition of litter ingested by marine animals (e.g. stomach analysis)
D11 Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment.	11.1 Distribution in time and place of loud, low and mid frequency impulsive sounds	11.1.1 Proportion of days and their distribution within a calendar year over areas of a determined surface, as well as their spatial distribution, in which anthropogenic sound sources exceed levels that are likely to entail significant impact on marine animals measured as Sound Exposure Level (in dB re 1µPa2.s) or as peak sound pressure level (in dB re 1µPapeak) at one metre, measured over the frequency band 10 Hz to 10 kHz
	11.2 Continuous low frequency sound	11.2.1 Trends in the ambient noise level within the 1/3 octave bands 63 and 125 Hz (centre frequency) (re 1µPa RMS: average noise level in these octave bands over a year) measured by observation stations and/or with the use of models if appropriate

Table 2a. Literature search outcomes dataset (search ended at 16/04/2016).

Ref. ID	Sources	Year	Area	Region	Descriptors	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	Ecosystem approach	Integrated approach	Risk analysis	Other approach
MSP_1	Serial	2016			Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes			
MSP_2	Short Commun ication	2016			Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	Yes		
MSP_3	Serial	2016	North Sea	Germany	Yes	No	No	No	No	No	No	No	No	No	No	Yes	No			Yes
MSP_4	Serial	2016	Europe		Yes	No	No	No	No	No	No	No	No	No	No	Yes	No			Yes
MSP_5	Article	2016	Baltic Sea	Lithuanian	Yes	No	No	No	No	Yes	No	No	No	No	No	No	No			Yes
MSP_6	Article	2015	Atlantic	Ireland	Yes	No	No	No	No	No	No	No	Yes	No	No	No	Yes			

MSP_7	Article	2015	Danube Delta	Romania	No	No	No	No	No	No	No	No	No	No	No	No	No			Yes
MSP_8	Review	2015	North Sea	Germany	Yes	No	No	Yes	No	No	Yes	No	No	No	No	No	No		Yes	
MSP_9	Article	2015			Yes	Yes	No	Yes	Yes	No	Yes	No	No	No	No	No	No			
MSP_10	Article	2015	North Sea	Germany	Yes	No	No	Yes	No	No	No	No	No	No	No	No	Yes			
MSP_11	Review	2015	Europe		Yes	Yes	Yes	No	Yes	No	Yes	No	No	No	No	No	No	Yes		
MSP_12	Report	2015	Baltic Sea	Sweden	Yes	No	No	No	No	No	No	No	Yes	No	No	No	No	Yes		
MSP_13	Preface	2015			Yes	No	No	No	No	Yes	No	No	No	No	No	No	No	Yes		
MSP_14	Editorial	2015	Europe		Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	No	No	No	No	Yes		

MSP_15	Article	2015	Atlantic		Yes	Yes	Yes	No	Yes	Yes	No	No	No	No	No	No	Yes		
MSP_16	Article	2015	North Sea	UK	Yes	No	No	No	No	No	No	No	Yes	No	No	No	No	Yes	
MSP_17	Article	2015	North Sea	Germany	Yes	Yes	No	Yes	Yes	No	No	No	No	No	No	No	Yes		
MSP_18	Conferen ce Paper	2015	Czech Republic	Czech Republic	No	No	No	No	No	No	No	No	No	No	No	No	No		
MSP_19	Article	2015	Mediterran ean Sea	Greece	Yes	No	No	Yes	No	No	No	Yes	No	Yes	Yes	No	No	Yes	
MSP_20	Review	2015			Yes	No	No	No	No	No	No	No	Yes	No	No	No	Yes		
MSP_21	Article	2015	Baltic Sea/Atlanti c		Yes	No	No	Yes	Yes	No	No	No	No	No	No	No	No	Yes	
MSP_22	Perspecti ve	2015	North Sea		Yes	No	No	Yes	No	Yes	No	No	No	No	No	No	No		Yes

MSP_23	Article	2015	Baltic Sea	Germany	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
MSP_24	Article	2015	Atlantic	Spain	Yes	Yes	Yes	No	No	No	Yes	No	No	No	No	No	Yes		
MSP_25	Article	2015			No	No	No	No	No	No	No	No	No	No	No	No	No		Yes
MSP_26	Article	2015	North Sea	Germany	Yes	No	No	Yes	No	No	No	No	No	No	No	No	Yes		
MSP_27	Article	2015	North Sea	Germany	Yes	No	No	Yes	No	Yes	No	No	No	No	No	No	Yes		
MSP_28	Viewpoint	2015	Europe		Yes	Yes	Yes	No	Yes	No	Yes	No	No	No	No	No	No		Yes
MSP_29	Article	2015	Atlantic	Portugal	Yes	No	No	No	No	Yes	No	No	No	No	No	No	Yes		
MSP_30	Viewpoint	2015	Europe		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	

MSP_31	Article	2015	Atlantic		Yes	No	Yes	No	No	No	No	No	No	No	No	No	Yes	
MSP_32	Review	2015			Yes	Yes	No	No	Yes	Yes	No	No	Yes	Yes	No	No	No	Yes
MSP_33	Viewpoint	2015			Yes	Yes	Yes	No	Yes	No	Yes	No	No	No	No	No	Yes	
MSP_34	Article	2014	Pacific/Atlantic/Indian oceans		Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes	
MSP_35	Article	2014	Europe	Spain	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes
MSP_36	Conference Paper	2014	Europe		Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes	
MSP_37	Conference Paper	2014	Black Sea	Romania/Bulgaria	No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_38	Article	2014	Black Sea	Bulgaria/Turkey	Yes	Yes	Yes	No	Yes	No	Yes	No	No	No	No	No	Yes	

MSP_39	Article	2014	Black Sea		Yes	No	No	Yes	No	No	No	No	No	No	No	No	No	Yes
MSP_40	Article	2014	Baltic sea/Atlantic	UK/Spain	Yes	No	No	Yes	No	No	No	No	No	No	No	No	Yes	
MSP_41	Conference Paper	2014			Yes	Yes	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_42	Conference Paper	2014			No	No	No	No	No	No	No	No	No	No	No	No	No	
MSP_43	Conference Paper	2014			No	No	No	No	No	No	No	No	No	No	No	No	No	
MSP_44	Article	2014	Baltic Sea		Yes	Yes	No	No	No	Yes	No	No	Yes	No	No	No	Yes	
MSP_45	Article	2014	Baltic Sea	Lithuanian	Yes	No	No	No	No	No	No	No	No	No	Yes	No	Yes	
MSP_46	Article	2014	Mediterranean sea	Adriatic Sea	Yes	No	No	No	Yes	No	No	No	No	No	No	No	Yes	

MSP_47	Short communication	2013			Yes	No	No	No	Yes	No	No	No	No	No	No	No	Yes
MSP_48	Baseline	2013	North Sea	Germany	Yes	No	No	No	No	No	No	No	No	No	Yes	No	Yes
MSP_49	Article	2013	Europe		No	No	No	No	No	No	No	No	No	No	No	No	Yes
MSP_50	Article	2013	North Sea		Yes	No	No	Yes	No	No	No	No	No	No	No	No	Yes
MSP_51	Conference Paper	2013	Europe		Yes	No	No	No	No	No	No	No	No	No	No	Yes	No
MSP_52	Article	2013	Baltic Sea	Denmark	Yes	No	No	No	No	No	No	No	Yes	No	No	No	Yes
MSP_53	Article	2013	IRISH SEA	Uk	Yes	No	No	Yes	No	No	No	No	No	No	No	No	Yes
MSP_54	Article	2013	Baltic Sea/North Sea		Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes

MSP_55	Conference Paper	2012			Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes	
MSP_56	Article	2012			Yes	No	No	No	No	No	Yes	No	No	No	No	No	Yes	
MSP_57	Article	2012	Atlantic /Baltic Sea	Portugal/Polonia	No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_58	Article	2012	Baltic Sea		No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_59	Article	2012			No	No	No	No	No	No	No	No	No	No	No	No	No	
MSP_60	Conference Paper	2012			No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_61	Short communication	2012			Yes	No	No	No	No	No	No	No	Yes	No	Yes	No	Yes	
MSP_62	Article	2012	Europe		No	No	No	No	No	No	No	No	No	No	No	No	No	Yes

MSP_63	Article	2011	Baltic Sea	Denmark/Finland	No	No	No	No	No	No	No	No	No	No	No	No	Yes
MSP_64	Article	2011	North Sea	Belgium/Denmark /France Germany/Netherlands/Norway UK	No	No	No	No	No	No	No	No	No	No	No	No	Yes
MSP_65	Article	2011	Europe		No	No	No	No	No	No	No	No	No	No	No	No	Yes
MSP_66	Review	2011	Baltic Sea/North Sea		No	No	No	No	No	No	No	No	No	No	No	No	Yes
MSP_67	Article	2011	Mediterranean Sea	Spain	No	No	No	No	No	No	No	No	No	No	No	No	Yes
MSP_68	Conference Paper	2011	North Sea		No	No	No	No	No	No	No	No	No	No	No	No	No
MSP_69	Conference Paper	2011	Atlantic	Norway	No	No	No	No	No	No	No	No	No	No	No	No	Yes

MSP_70	Conference Paper	2011			No	No	No	No	No	No	No	No	No	No	No	No	No	
MSP_71	Article	2010	Baltic Sea		No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_72	Conference Paper	2009	Slovakia	Slovakia	No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_73	Article	2008	North Sea	Germany	No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_74	Book	2008	North Sea		No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_75	Article	2008	Baltic Sea	Sweden	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes
MSP_76	Viewpoint	2013	Europe		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
MSP_77	Article	2015	Atlantic		Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	

MSP_78	Article	2012	Europe		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	
MSP_79	Review	2015	Atlantic	Spain	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	Yes
MSP_80	Article	2013	Europe		No	No	No	No	No	No	No	No	No	No	No	No	No	
MSP_81	Article	2015	Baltic Sea		No	No	No	No	No	No	No	No	No	No	No	No	No	
MSP_82	Conferen ce paper	2013	Weddell Sea	Artic	Yes	No	No	No	No	No	No	No	No	No	No	Yes	No	
MSP_83	Article	2014	Mediterran ean Sea		No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_84	Conferen ce paper	2013	Mediterran ean Sea		No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_85	Conferen ce paper	2014	Mediterran ean Sea		Yes	Yes	No	No	No	No	No	No	No	No	No	No	Yes	

MSP_86	Article	2012	Mediterranean Sea		Yes	No	No	No	No	No	Yes	No	No	No	No	No	Yes	
MSP_87	Article	2014	Mediterranean Sea		Yes	No	No	No	No	No	No	No	No	No	Yes	No	No	
MSP_88	Article	2012	Mediterranean Sea		No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes
MSP_89	Article	2014	Mediterranean Sea	Greece	No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_90	Article	2016	Mediterranean Sea		No	No	No	No	No	No	No	No	No	No	No	No	Yes	
MSP_91	Article	2014	Rhône River (France) and Valencia City (Spain)	France/Spain	Yes	No	No	No	No	No	No	No	No	No	No	Yes	No	

Table 2b. Summary of the 91 scientific articles of the bibliographic research (search ended at 16/04/2016).

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MSP_2	Probst, W. Nikolaus, and Christopher P. Lynam. "Integrated assessment results depend on aggregation method and framework structure–A case study within the European Marine Strategy Framework Directive." Ecological indicators 61 (2016): 871-881.
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MSP_4	Dekeling, René, et al. "The European Marine Strategy: Noise Monitoring in European Marine Waters from 2014." The Effects of Noise on Aquatic Life II. Springer, New York, NY, 2016. 205-215.
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MSP_18	Kozel, R., et al. "Legal aspects of environmental issues in the Czech republic."(2015).
MSP_19	Kontogianni, A., et al. "Modeling expert judgment to assess cost-effectiveness of EU Marine Strategy Framework Directive programs of measures." Marine Policy 62 (2015): 203-212.
MSP_20	Justino, Celine IL, et al. "Sensors and biosensors for monitoring marine contaminants." Trends in Environmental Analytical Chemistry 6 (2015): 21-30.
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Chapter 2

Up to the North: invasiveness of the species in Mediterranean basin

Abstract

The continuous arrival of the non-indigenous species (NIS) and their growing presence and stabilization in the Mediterranean basin implies a profound and continuous alteration in the models of distribution of the species that seem to have increased significantly in recent years. In fact, there is a growing need to try to define preventive measures to control biological invasions and therefore mitigate the related impacts on biodiversity, ecosystem services and human activities. The number of introductions recorded in the Mediterranean Sea is much higher than in other European seas, caused by the warming of water and the impact generated by man. These motivations have led to a progressive migration of tropical and subtropical NIS species in the Mediterranean, modifying the stability of the basin ecosystem. In fact, the results of this study show a high correlation between the number of occurrences and the difference in temperature (Td) between the points of presence in the Mediterranean (Arrival) and the points of origin (Departure) of the alien species. This work aims to provide a contribution that can be used to highlight the distribution of those invasive systematic groups that represent the greatest threat to the Mediterranean Sea.

Keywords: distribution, non-indigenous species, temperature, ecosystem services, Mediterranean Sea

2.1 Introduction

In the Mediterranean Sea complex and fundamental alterations are under way, including the increase in the number of non-indigenous species (NIS), which affected the structure and functioning of the sea and the consequent supply of goods and services (Galil et al., 2016). A recent summary on marine biodiversity (Coll et al., 2010) described the Mediterranean as a biodiversity hotspot housing about 17.000 marine species, of which more than 600 (3.3%) are foreign (Zenetos et al., 2010). In fact, the number of introductions recorded in the Mediterranean Sea is much higher than in other European seas, caused by the warming of water and the impact generated by man (Galil et al., 2014). These motivations have led to a progressive migration of tropical and subtropical NIS species in the Mediterranean, modifying the stability of the basin ecosystem (Zenetos et al., 2010). The continuous arrival of the NIS and their growing presence and stabilization in the Mediterranean basin implies a profound and continuous alteration in the models of distribution of the species that seem to have increased significantly in recent years (Galil et al., 2014). Only for the Mediterranean, the rate of new introductions has been estimated to be one every 1.5 weeks and the total number of reported NIS species has approached about 1000 species (Katsanevakis et al., 2013a). In fact, there is a growing need to try to define preventive measures to control biological invasions and therefore mitigate the related impacts on biodiversity, ecosystem services and human activities. (Katsanevakis et al., 2013b). Pro-active actions are generally far more convenient and cost-effective than post-active measures, such as elimination, or long-term mitigation measures of alien species (Galil et al., 2018). However, there is no way to predict the invasion of ecosystems (Zenetos et al., 2010).

This is because there are countless variables that can influence the invasion and the way in which the invader can colonize new habitats, think for example to the theory of "Biotic Resistance" of Elton (1958), which defines how the communities with high levels of specific wealth, are more resistant to the invasion of the NIS species. This depends on the fact that high levels of species richness communities are able to use resources more effectively than communities with low levels of species richness (Elton et al., 1958). These capacities should correspond to a set of biological traits that allow them to become successful colonizers, by comparing the performances of these species in their native ecosystem and in the newly invaded ecosystem (*sensu* Cardeccia et al., 2016).

Regulating the flow of alien species is of particular importance for future Community policy actions and strategies covering key maritime strategic objectives, such as the Marine Strategy Framework Directive (MSFD) (2008/56/EC). MSFD specifically highlights the introduction of marine alien species as a major threat to European biodiversity and ecosystem health, which requires Member

States to include NIS in the definition of GES and to set environmental targets for achieving it. (Katsanevakis et al., 2013b).

The present study shows an analytical structure that can be useful to the scientific community for the possibility to use the information regarding the species, starting from their native ecosystem and the one just invaded by comparing information regarding temperature and the number of occurrence to define the invasion status of alien species on the entire Mediterranean basin. This is aimed at extracting useful information to be able to understand the future geographical distribution of alien species in a context of anthropogenic global change.

2.2 Materials and Methods

2.2.1 Selection of species

Following the scientific criteria of the works listed (table 1, supplementary material), we have created a list with about 587 non-indigenus species (NIS) updated until November 2016 throughout the Mediterranean basin. The checklist is divided into the following systematic groups: *Macrophytes*, *Polychaeta*, *Crustacea*, *Mollusca*, *Bryozoa*, *Chordata / Ascidiacea*, *Cnidaria*, *Porifera* and *Pisces*.

2.2.2 Database construction

Starting from the previous check list, we have selected 69 species (figure 1). The criteria that have put the exclusion of other species into consideration depends on that information concerning the place of origin. In fact, all those species that did not have an origin or had an area so extensive that they could not define its origin were excluded (for example Circumtropical, Circumboreal Circumglobal, Pantropical, Tropical and subtropical). This information was extracted from the Aquanis database (http://www.corpi.ku.lt/databases/index.php/aquanis). The database has been enriched with other data, such as the number of occurrences of every single species downloaded from two different sources. The first refers to the points of origin, also referred to as the starting point, selected from the Worms database(http://www.marinespecies.org/). The second type concerns the arrival points in the Mediterranean basin, which have been extrapolated from a mix of sources downloaded from the Obis database (http://iobis.org), project reports, scientific articles.

These scientific articles have been selected thanks to one of a bibliographic research carried out with the use of search engines such as Google Scholar and Scopus (table 2, supplementary material). Specifically, the bibliographic research was performed using and creating strings that contained keywords: for example "... name of the NIS species, the synonym and our range of action, Mediterranean Sea or Mediterranean ...".

As the following: TITLE-ABS-KEY(("*Amphicorina pectinata*" OR "*Oriopsis alata pectinata*") AND ("*Mediterranean Sea*" OR "*Mediterranean*")).

Furthermore, on both types of occurrence points we have determined satellite temperature information for the entire year 2016 and 2010 by downloading the information from the Emis website (http://mcc.jrc.ec.europa.eu/emis/) choosing the Modis satellite with a resolution of 9km. Specifically we have selected boxes within a depth of 100 meters from the coast at each single point of departure and arrival that has been previously defined. On the basis of the previous phase, we estimated the temperature difference (Td) between the arrival time minus the departure difference for both the year 2010 (Td1) and for the year 2016 (Td2). This led us to group all the species into 2 groups, defining them as a species "From Cold to Hot" and "From Hot to Cold" (table 3, supplementary material).

2.2.3 Map processing in GIS

Maps were created in GIS, inserting the points of occurrence obtained as previously described. The maps were created through the use of the geographic information system (GIS). This system analyzes the spatial position and organizes layers of information in views using maps. The program used to map was ArcMap ver 10.5.1 and the geolocation system chosen was the World Geodetic System 1984 (WGS84).

2.3 Statistic Analysis

A simple first-order exponential decay approach was applied to test the relationship between the number of occurrence and the temperature difference (Td). The species were divided in two categories "From Hot to Cold" and "From Cold to Hot" and we used an independent sample test such as the Welch's test to define the independence of the data in relation to the temperature differences and the two categories for both the year 2016 and 2010. We chose the Whelch's test because the sampled averages did not have the same standard deviation. The Welch's test was conducted with the R studio software, applying the lsr package. Furthermore, to assess the existence of significant differences in the number of occurrence between the 2 categories we performed a univariate permutational analysis of variance (PERMANOVA; Anderson, 2001), using the temperature difference as a fixed factor (with 2 levels: From Cold to Hot and From Hot to Cold).

PERMANOVA was carried out using the PRIMER 6+ Package (Plymuth Marine Laboratory, Clarke, 1993). The program used to map was ArcMap ver 10.5.1 and the geolocation system chosen was the World Geodetic System 1984 (WGS84).

2.4 Results

The figures about the occurrence of the 69 selected a priori species showed a significant correlation with the number of occurrence and temperature, which is meant as the difference (Td) between the arrival temperature and the departure one for the year 2010 (Td1; $R2 = 0.46$, $p < 0.001$) and for the year 2016 (Td2; $R2 = 0.44$, $p < 0.001$) (figures 2 and 3).

The Welch's test showed the strong independence of the data the temperature difference, allowing to categorize the species in "From Cold to Hot" and "From Hot to Cold" both for the year 2010 (table 1a) and 2016 (table 1b). The number of occurrence of the species "From Hot to Cold" (68.32 ± 11.94) was significantly higher (PERMANOVA; $p < 0.001$) (table 2) compared to the number of occurrences of species "From Cold to Hot" (13.79 ± 2.10) in the Mediterranean (figure 4) both in the year 2010 and in the year 2016. The maps realized on both the "From Cold to Hot" and "From Hot to Cold" categories show the spatial distribution and current number of occurrences (until November 2016) of species in the Mediterranean basin (figures 5 and 6).

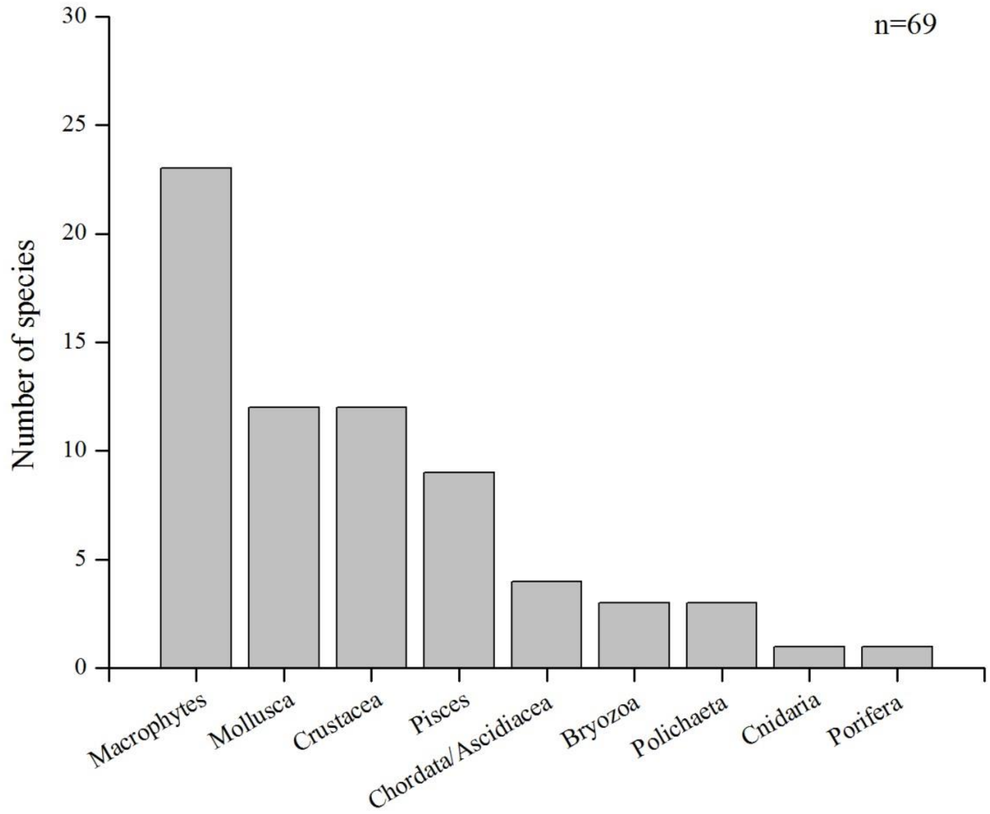

Fig. 1. Number and systematic group of species.

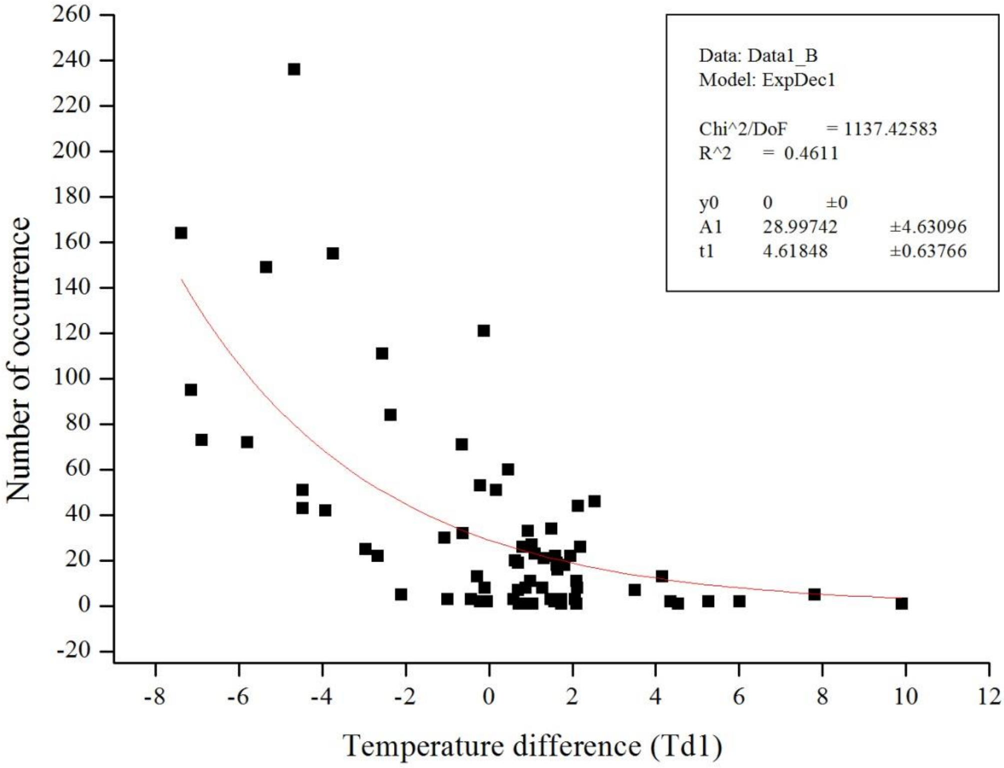

Fig. 2. Temperature difference (Td1) and number of occurrence for the year 2010.

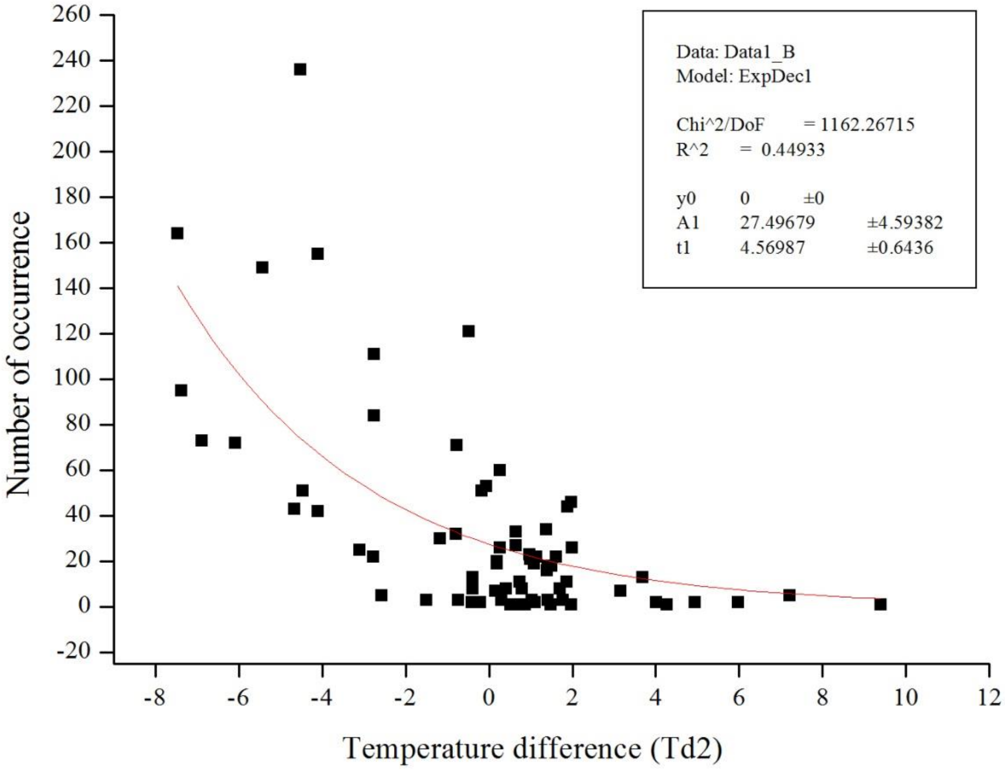

Fig. 3. Temperature difference (Td2) and number of occurrence for the year 2016.

Table 1a. Temperature difference (Td1) and categories "From Cold to Hot" and" From Hot to Cold" in 2010.

Welch Two Sample t-test							
Source	**t**	**df**	**p-value**	**Ho**	**Confidence interval**	**Sample estimates: mean in group "From Cold to Hot"**	**Sample estimates: mean in group "From Hot to Cold"**
	8.136	40.089	$5.081e^{-10}$	True	3.5599 to 5.9127	2.0274	-2.7088
Total	69						

Table 1b. Temperature difference (Td2) and categories "From Cold to Hot" and "From Hot to Cold" in 2016.

Welch Two Sample t-test							
Source	**t**	**df**	**p-value**	**Ho**	**Confidence interval**	**Sample estimates:mean in group "From Cold to Hot"**	**Sample estimates:mean in group "From Hot to Cold"**
	7.834	39.904	$1.35e^{-09}$	True	3.3686 to 5.7112	2.0274	-2.7088
Total	69						

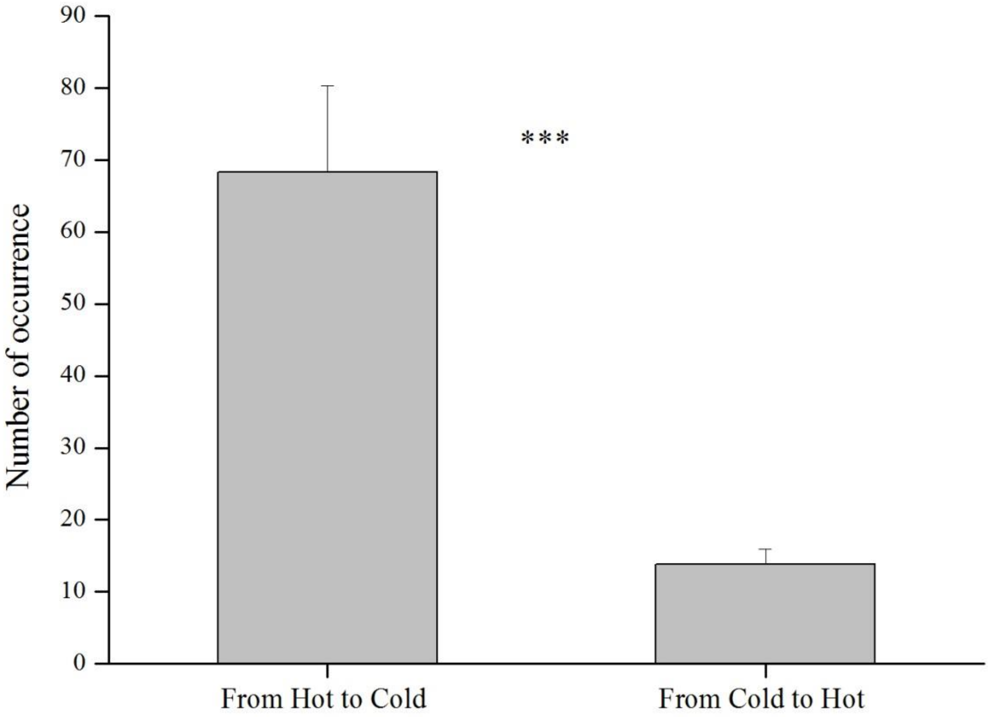

Fig. 4. Number of occurrence in each "From Cold to Hot" and "From Hot to Cold" categories for the year 2010 and 2016.

Table 2. Results of PERMANOVA analysis performed to test for differences between "From Hot to Cold" and "From Cold to Hot" in number of occurrence for the year 2010 and 2016 (df = degree of freedom, MS = mean s quare, Pseudo-F = F statistic, P(MC) = probability level; *** = P < 0.001; ** = P < 0.01; * = P < 0.05, ns = not significant).

PERMANOVA table of results						
Source	df	SS	MS	Pseudo-F	P(perm)	Uniqueperms
Td	1	47394	47394	33,774	0,0001	943
Res	67	94019	1403,3			
Total	68	1,4141E5				

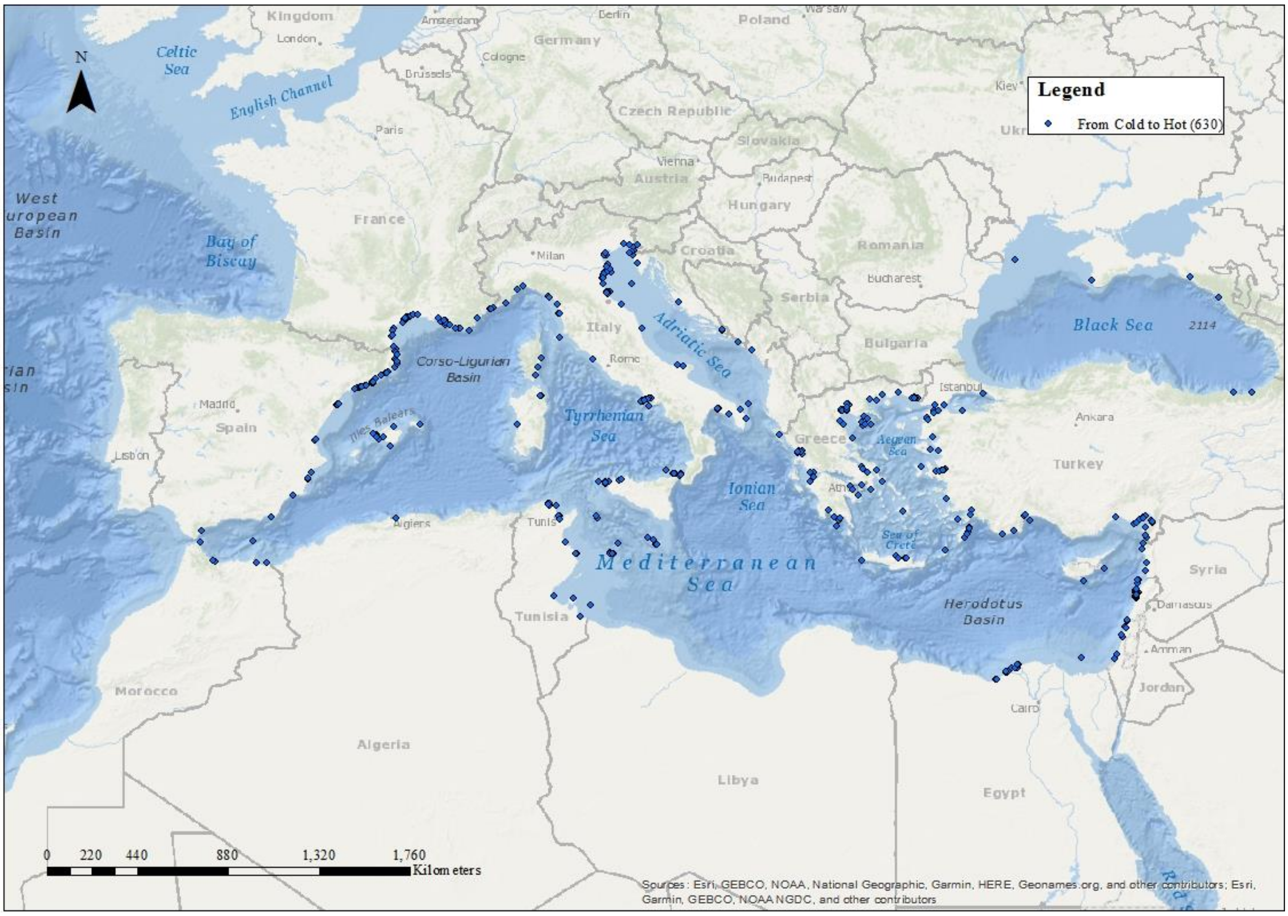

Fig. 5. Number of occurrence of the "From Cold to Hot" category.

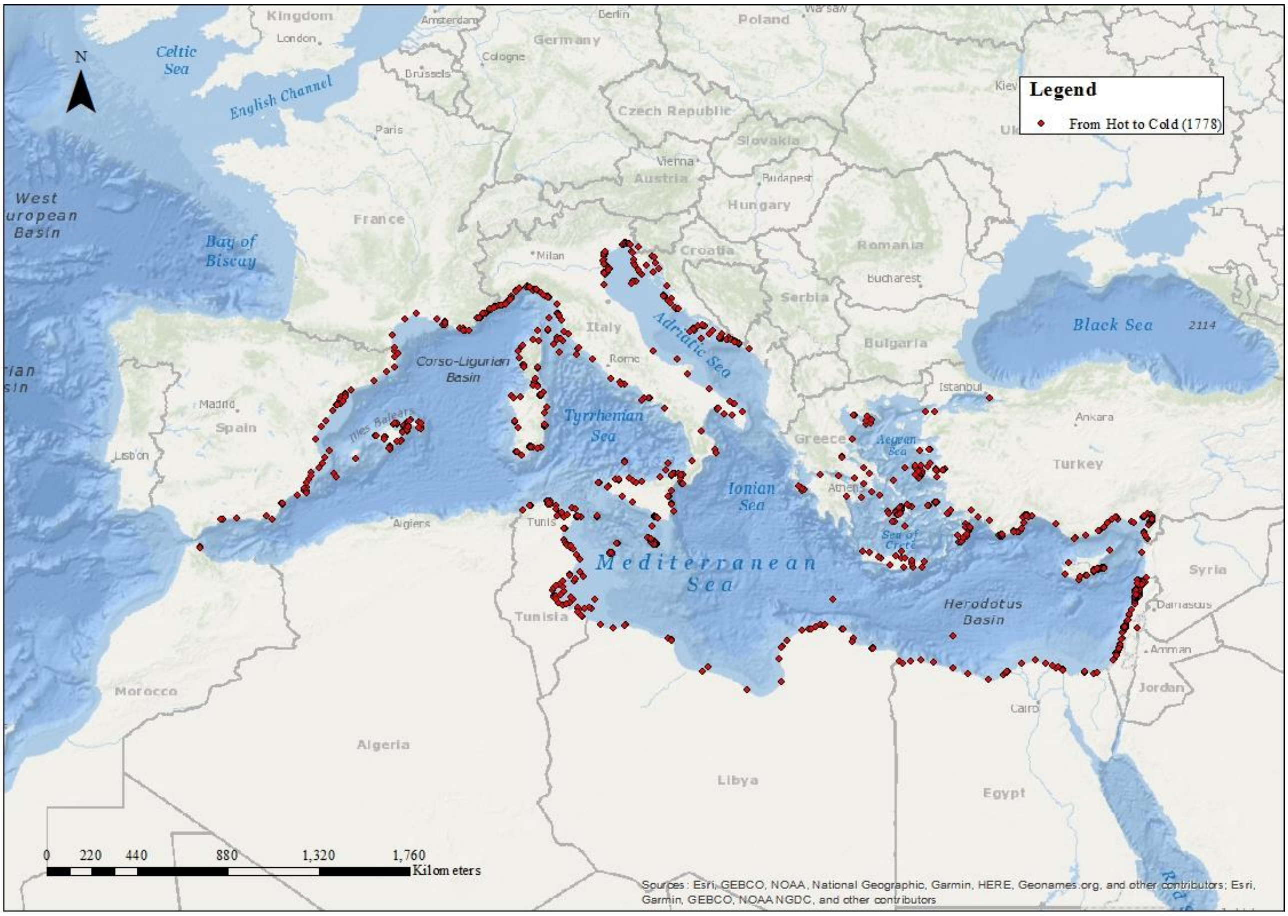

Fig. 6. Number of occurrence of the "From Hot to Cold" category.

2.5 Discussions and Conclusions

The continued arrival of non-indigenous species (NIS) and their increasing presence and stabilization in the Mediterranean basin trigger a deep and continuous alteration of the models of distribution of the species, which seem to have significantly increased in recent years (Galil et al., 2014). In fact, there is a growing need to try to define preventive measures to control biological invasions and therefore mitigate impacts on biodiversity and ecosystem services (Katsanevakis et al., 2013b). The number of introductions recorded in the Mediterranean Sea is much higher than in other European seas, and it is (partially) caused by water heating and the impact of human activities (Galil et al., 2014). As it is remarked in Occhipinti-Ambrogi et al. (2007), the temperature increase in the Mediterranean has led over time to a continuous stabilization of the Nis species, triggering competitive interactions between NIS and native species. Since studies on the influence of temperature rise on the stabilization of NIS in the Mediterranean have never been conducted on a small or large scale, this has been the purpose of the present study. First of all, 9 systematic groups of the 69 previously selected species were highlighted. Specifically, a predominance of Macrophyte, Mollusca, Crustacea and Pisces was highlighted in accordance with Zenetos et al. (2010), and in Galil et al. (2017). The taxa were influenced by the introduction vectors and by the receiving environment, which also affects its distribution and role in the ecosystem. (*sensu* Galil et al., 2018). In fact, as it is shown in our study, the number of species occurrence, a priori selected, presented a significant correlation with the temperature difference (Td) between the arrival point and the starting points for both the year 2010 and 2016. The year 2016 corresponds to the year in which our check list was created on the basis of a bibliographic research, instead the year 2010 was selected randomly to evaluate the existence of the same correlation in different years. Furthermore, in this study, based on the difference in temperature (Td) the species were divided into two different categories, defined as "From Hot to Cold" and "From Cold to Hot". This allowed us to infer that the number of occurrence of the "From Hot to Cold" species is significantly higher than the number of occurrence of "From Cold to Hot" species in the Mediterranean, both in the year 2010 and 2016. This could be explained by the general temperature increase in the Mediterranean Sea, which means that species accustomed to higher temperatures can easily settle in the basin. As shown in Zenetos et al. (2010) there has been a progressive migration of tropical and subtropical NIS species in the Mediterranean, which modifies the stability of the basin ecosystem. In fact, the increase in temperature is one of the main drivers that can generate effects on the survival and physiological responses of organisms, as well as on thermal tolerance and distribution of species (*sensu* Helmuth et al., 2006). However, in many cases our understanding of how the physical environment, in particular climate modification, can change the distribution of organisms is limited by our rather poor knowledge of how physiologically environmental factors vary in space and time (Hallett et al., 2004).

To conclude, this paper aims to provide a contribution that can be used to try to understand the complex interactions between the increase in temperatures and the distribution of non-indigenous species (NIS) in the entire Mediterranean basin. Indeed, regulating the flow of alien species will be of particular importance for future Community policy actions and strategies covering the main maritime strategic objectives, as they are expressed in the Marine Strategy Framework Directive (MSFD) (2008/56 / EC). That identifies the introduction of alien marine species as a major threat to European biodiversity and ecosystem health, calling Member States to include NIS in the definition of GES and to set environmental targets for achieving it (Katsanevakis et al., 2013b).

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2.7 Supplementary material

Table 1. Scientific literature used to define the checklist of alien species (search ended at 16/11/2016).

Ref_	Article
Ref._1	Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Lasram, F. B. R., Aguzzi, J., ... & Danovaro, R. (2010). The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PloS one*, 5(8), e11842.
Ref._2	Crocetta, F., Agius, D., Balistreri, P., Bariche, M., Bayhan, Y. K., Çakir, M., ... & Ergüden, D. (2015). New Mediterranean Biodiversity Records (October 2015). *Mediterranean Marine Science*, 16(3), 682-702.
Ref._3	Galil, B. S. (2009). Taking stock: inventory of alien species in the Mediterranean Sea. *Biological Invasions*, 11(2), 359-372.
Ref._4	Galil, B. S., Marchini, A., Occhipinti-Ambrogi., A. (2016). East is East and west is west? Management of marine bioinvasions in the Mediterranean Sea. Estuarine*, Coastal and Shelf Science*, In Press.
Ref._5	Gollasch, S. (2007). International collaboration on marine bioinvasions–The ICES response. *Marine Pollution Bulletin*, 55(7), 353-359.
Ref._6	Karachle, P. K., Angelidis, A., Apostolopoulos, G., Ayas, D., Ballesteros, M., Bonnici, C., ... & Crocetta, F. (2015). New mediterranean biodiversity records (March 2016). *Mediterranean Marine Science*, 17(1), 230-252.
Ref._7	Mavruk, S., & Avsar, D. (2008). Non-native fishes in the Mediterranean from the Red Sea, by way of the Suez Canal. *Reviews in Fish Biology and Fisheries*, 18(3), 251-262.
Ref._8	Mytilineou, C., Akel, E. K., Babali, N., Balistreri, P., Bariche, M., Boyaci, Y. Ö., ... & Dereli, H. (2016). New mediterranean biodiversity records (November, 2016). *Mediterranean Marine Science*,17(3), 794-821.
Ref._9	Zenetos, A., Gofas, S., Verlaque, M., Çinar, M. E., Garcia Raso, J. E., Bianchi, C. N., Siokou, I. (2010). Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution.
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Ref._11	Zenetos, A., Akel, E. H. K., Apostolidis, C., Bılecenoglu, M., Bitar, G., Buchet, V., ... & Drakulić, M. (2015). New Mediterranean biodiversity records (April 2015). *Mediterranean Marine Science*, 16(1), 266-284.

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Table 2. Literature search outcomes dataset (search ended at 23/11/2016).

Ref_	Taxa	Specie	Article
Nis-1	Crustacea	Caprella scaura	Prato, Ermelinda, Isabella Parlapiano, and Francesca Biandolino. "Seasonal fluctuations of some biological traits of the invader Caprella scaura (Crustacea: Amphipoda: Caprellidae) in the Mar Piccolo of Taranto (Ionian Sea, southern Italy)." Scientia Marina77.1 (2013): 169-178.
Nis-2	Crustacea	Caprella scaura	Dailianis, T., et al. "New Mediterranean Biodiversity Records (July 2016)." (2016).
Nis-3	Crustacea	Caprella scaura	Fernandez-Gonzalez, Victoria, and Pablo Sanchez-Jerez. "First occurrence of Caprella scaura Templeton, 1836 (Crustacea: Amphipoda) on off-coast fish farm cages in the Mediterranean Sea." Helgoland marine research 68.1 (2014): 187.
Nis-4	Crustacea	Caprella scaura	Marchini, Agnese, Jasmine Ferrario, and Dan Minchin. "Marinas may act as hubs for the spread of the pseudo-indigenous bryozoan Amathia verticillata (Delle Chiaje, 1822) and its associates." Scientia Marina 79.3 (2015): 355-365.
Nis-5	Crustacea	Caprella scaura	Prato, Ermelinda, Isabella Parlapiano, and Francesca Biandolino. "Seasonal fluctuations of some biological traits of the invader Caprella scaura (Crustacea: Amphipoda: Caprellidae) in the Mar Piccolo of Taranto (Ionian Sea, southern Italy)." Scientia Marina77.1 (2013): 169-178.
Nis-6	Crustacea	Caprella scaura	Ros, Macarena, Maite Vázquez-Luis, and José M. Guerra-García. "The role of marinas and recreational boating in the occurrence and distribution of exotic caprellids (Crustacea: Amphipoda) in the Western Mediterranean: Mallorca Island as a case study." Journal of Sea Research 83 (2013): 94-103.
Nis-7	Crustacea	Caprella scaura	Clemente, Maria,. Ros. "The spreading of the non-native caprellid (Crustacea: Amphipoda) Caprella scaura Templeton, 1836 into southern Europe and northern Africa: a complicated taxonomic history." Mediterranean Marine Science 15.1 (2014): 145-155.
Nis-8	Crustacea	Grandidierella japonica	Marchini, Agnese, Jasmine Ferrario, and Emanuele Nasi. "Arrival of the invasive amphipod Grandidierella japonica to the Mediterranean Sea." Marine Biodiversity Records 9.1 (2016): 38.
Nis-9	Crustacea	Grandidierella japonica	Munari, Cristina, Nadia Bocchi, and Michele Mistri. "Grandidierella japonica (Amphipoda: Aoridae): a non-indigenous species in a Po delta lagoon of the northern Adriatic (Mediterranean Sea)." Marine Biodiversity Records 9.1 (2016): 12.
Nis-10	Crustacea	Balanus eburneus	Kocak, Ferah, Zeki Ergen, and Melih Ertan Çinar. "Fouling organisms and their developments in a polluted and an unpolluted marina in the Aegean Sea (Turkey)." Ophelia 50.1 (1999): 1-20.
Nis-11	Crustacea	Austrominius modestus	Petrocelli, Antonella, Ester Cecere, and Marc Verlaque. "Alien marine macrophytes in transitional water systems: new entries and reappearances in a Mediterranean coastal basin." BioInvasions Record2.3 (2013).
Nis-12	Crustacea	Balanus trigonus	Haber, Markus, et al. "Barnacle fouling in the Mediterranean sponges Axinella polypoides and Axinella verrucosa." Marine Ecology 34.4 (2013): 467-473.
Nis-13	Crustacea	Balanus trigonus	Siokou, I., et al. "New Mediterranean Marine biodiversity records (June 2013)." Mediterranean Marine Science 14.1 (2013): 238-249.

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Nis-534	Macrophytes	Womersleyella setacea	Rindi, Fabio, Michael D. Guiry, and Francesco Cinelli. "Morphology and reproduction of the adventive Mediterranean rhodophyte Polysiphonia setacea." Sixteenth International Seaweed Symposium. Springer, Dordrecht, 1999.
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Nis-536	Macrophytes	Womersleyella setacea	Piazzi, Luigi, David Balata, and Francesco Cinelli. "Epiphytic macroalgal assemblages of Posidonia oceanica rhizomes in the western Mediterranean." European Journal of Phycology 37.1 (2002): 69-76.
Nis-537	Macrophytes	Womersleyella setacea	Piazzi, L., and F. Cinelli. "Distribution and dominance of two introduced turf-forming macroalgae on the coast of Tuscany, Italy, northwestern Mediterranean Sea in relation to different habitats and sedimentation." Botanica marina 44.5 (2001): 509-520.
Nis-538	Macrophytes	Womersleyella setacea	Piazzi, L., et al. "Seasonal dynamics of a subtidal north-western Mediterranean macroalgal community in relation to depth and substrate inclination." Botanica Marina 45.3 (2002): 243-252.
Nis-539	Macrophytes	Womersleyella setacea	Piazzi, Luigi, and Francesco Cinelli. "Evaluation of benthic macroalgal invasion in a harbour area of the western Mediterranean Sea." European Journal of Phycology 38.3 (2003): 223-231.
Nis-540	Macrophytes	Womersleyella setacea	Piazzi, Luigi, and David Balata. "Invasion of alien macroalgae in different Mediterranean habitats." Biological invasions 11.2 (2009): 193-204.
Nis-541	Macrophytes	Womersleyella setacea	Tomás, Fiona, E. Cebrian, and E. Ballesteros. "Differential herbivory of invasive algae by native fish in the Mediterranean Sea." Estuarine, Coastal and Shelf Science 92.1 (2011): 27-34.

Table 3. Matrix of the 69 species. Td1= Temperature difference (Mean _Temp Arrive - Mean _Temp Departure 2010) ; Td2= Temperature difference (Mean _Temp Arrive - Mean _Temp Departure 2016). Reference paragraph 2.2.

Taxa	Species	Origin	Categories	**Departure (2010)**						**Departure (2016)**						**Arrive (2016)**						**Td1**	**Td2**
				Mean_temp	dev_St-Temp	s_e_Temp	max_temp	min_temp	n_occurrence	Mean_temp	dev_St-Temp	s_e_Temp	max_temp	min_temp	n_occurrence	Mean _Temp	dev_St-Temp	max_temp	min_temp	s_e_Temp	n_occurrence		
Polichaeta	Branchiomma bairdi	Atlantic	From Cold to Hot	19.42	0.995	0.445	21	18.31	5	19.96	0.966	0.432	21.5	19	5	20.12	0.705	21.00	19.00	0.267	7	0.697	0.159
Polichaeta	Ficopomatus enigmaticus	Pacific	From Cold to Hot	17.27	0.590	0.264	18	16.51	5	17.72	0.708	0.317	18.6	17	5	21.42	0.874	22.51	20.00	0.242	13	4.150	3.692
Polichaeta	Hydroides dianthus	NW Atlantic	From Cold to Hot	20.11	0.683	0.305	21.1	19.24	5	20.25	0.896	0.401	21.2	19	5	21.74	0.625	22.00	19.72	0.147	18	1.630	1.490
Polichaeta	Spirorbis marioni	Pacific	From Cold to Hot	19.89	0.749	0.335	20.6	18.75	5	20.02	0.806	0.361	20.8	19.12	5	20.99	0.771	22.00	20.02	0.161	23	1.098	0.972
Bryozoa	Amathia (Zoobotryon) verticillata	Caribbea n Sea	From Hot to Cold	20.56	0.513	0.229	21	20	5	20.4	0.397	0.177	21.1	20.1	5	20.34	1.939	23.62	15.85	0.266	53	-0.215	-0.061
Bryozoa	Celleporaria brunnea	NE Pacific	From Cold to Hot	19.23	0.578	0.259	20	18.52	5	19.64	0.590	0.264	20.2	19	5	21.04	1.020	23.00	0.20	0.240	18	1.813	1.401
Bryozoa	Electra tenella	Atlantic	From Hot to Cold	19.46	0.871	0.389	20.8	18.62	5	19.65	0.797	0.356	20.9	19	5	19.25	1.209	20.10	18.39	0.855	2	-0.219	-0.403
Chordata/Ascidiacea	Botrylloides violaceus	NW Pacific	From Cold to Hot	18.95	0.649	0.290	19.7	18.24	5	19.14	0.879	0.393	20	18.02	5	20.00					1	1.048	0.862
Chordata/Ascidiacea	Didemnum vexillum	Japan	From Hot to Cold	18.41	0.855	0.382	19	16.96	5	18.56	0.899	0.402	19.2	17	5	18.35	2.122	19.85	16.85	1.501	2	-0.057	-0.211
Chordata/Ascidiacea	Microcosmus squamiger	Pacific	From Cold to Hot	18.31	1.120	0.501	20	16.95	5	18.56	0.899	0.402	19.2	17	5	20.44	0.997	22.00	18.00	0.150	44	2.137	1.883
Chordata/Ascidiacea	Styela clava	NW Pacific	From Cold to Hot	18.11	0.572	0.256	18.8	17.52	5	18.62	0.687	0.307	19.6	18	5	19.40	0.370	20.00	19.20	0.131	8	1.288	0.780
Cnidaria	Rhopilema nomadica	Red Sea	From Cold to Hot	21.56	0.536	0.240	22	20.95	5	21.75	0.719	0.322	22.6	21	5	22.02	1.267	23.54	15.00	0.164	60	0.461	0.269
Porifera	Paraleucilla magna	SW Atlantic	From Hot to Cold	22.14	0.447	0.200	22.9	21.72	5	22.43	0.393	0.176	23	22	5	22.04	0.797	22.60	20.20	0.282	8	-0.094	-0.386

Macrophytes	Caulerpa racemosa var. cylindracea	Indo-Pacific	From Hot to Cold	22.36	0.890	0.398	24	21.85	5	22.71	0.833	0.373	24	22	5	18.62	1.272	22.00	15.00	0.102	155	-3.746	-4.096
Macrophytes	Caulerpa taxifolia	Indo-Pacific	From Hot to Cold	23.92	1.273	0.569	25.1	22	5	24	0.707	0.316	25	23	5	16.54	1.841	23.00	14.00	0.144	164	-7.384	-7.464
Macrophytes	Codium fragile subsp. Fragile	NW Pacific	From Cold to Hot	20.38	0.988	0.442	21.6	19	5	20.8	0.842	0.377	22	20	5	22.50	0.007	22.52	22.50	0.002	8	2.121	1.705
Macrophytes	Colpomenia peregrina	Pacific	From Cold to Hot	14.21	0.871	0.390	15.5	13.29	5	14.8	0.837	0.374	16	14	5	22.02	0.045	22.10	22.00	0.020	5	7.808	7.220
Macrophytes	Sargassum muticum	NW Pacific	From Cold to Hot	18.09	0.791	0.354	19	17.25	5	18.48	0.915	0.409	19.6	17.5	5	19.11	0.868	22.00	17.02	0.167	27	1.021	0.635
Macrophytes	Stypopodium schimperi	Indo-Pacific	From Hot to Cold	22.67	0.319	0.143	23	22.24	5	22.83	0.836	0.374	24	22	5	22.04	0.618	23.00	19.19	0.109	32	-0.631	-0.793
Macrophytes	Undaria pinnatifida	Pacific	From Cold to Hot	18.00	0.756	0.338	18.8	16.86	5	18.24	0.829	0.371	19	17	5	20.09	0.938	21.10	18.00	0.283	11	2.094	1.854
Macrophytes	Halophila stipulacea	Red Sea	From Hot to Cold	22.43	1.187	0.531	24	20.84	5	22.54	0.938	0.419	23.3	21	5	22.15	1.090	23.40	19.50	0.302	13	-0.279	-0.387
Macrophytes	Acrothamnion preissii	Indo-Pacific	From Hot to Cold	22.05	0.475	0.212	22.8	21.59	5	22.24	0.434	0.194	23	22	5	17.58	0.925	20.00	17.00	0.141	43	-4.467	-4.661
Macrophytes	Antithamnion amphigeneum	SW Pacific	From Cold to Hot	17.46	0.635	0.284	18.2	16.77	5	17.72	0.701	0.314	18.6	17	5	18.45	0.934	20.00	17.00	0.282	11	0.997	0.735
Macrophytes	Asparagopsis armata	SW Pacific	From Cold to Hot	17.34	0.772	0.345	18.6	16.63	5	17.91	0.791	0.354	19	17	5	19.88	0.995	22.60	18.00	0.147	46	2.540	1.970
Macrophytes	Asparagopsis taxiformis	Atlantic	From Cold to Hot	20.22	0.765	0.342	21.3	19.52	5	20.77	0.867	0.388	22.1	20.12	5	21.04	1.268	24.60	19.05	0.249	26	0.816	0.270
Macrophytes	Bonnemaisonia hamifera	Japan	From Cold to Hot	18.65	0.521	0.233	19.2	18	5	18.94	0.851	0.381	20	17.62	5	19.24	0.014	19.25	19.23	0.008	2	0.594	0.296
Macrophytes	Ceramium strobiliforme	N Atlantic	From Cold to Hot	13.98	0.526	0.235	14.8	13.49	5	14.31	0.470	0.210	15	13.95	5	18.41	0.015	18.42	18.39	0.097	3	4.427	4.097
Macrophytes	Dasya sessilis	Pacific	From Cold to Hot	18.84	0.884	0.395	19.8	17.69	5	19.14	0.901	0.403	20.1	18	5	19.23	0.031	19.26	19.20	0.306	3	0.389	0.083
Macrophytes	Heterosiphonia japonica	NW Pacific	From Cold to Hot	20.22	0.832	0.372	21	19.2	5	20.72	0.947	0.423	21.6	19.4	5	22.00					1	1.784	1.280
Macrophytes	Gracilaria vermiculophylla	NW Pacific	From Cold to Hot	17.46	0.621	0.278	18	16.62	5	17.74	0.499	0.223	18	16.86	5	18.29	0.039	18.34	18.25	0.090	5	0.833	0.551
Macrophytes	Grateloupia turuturu	NW Pacific	From Hot to Cold	20.73	0.889	0.397	22.1	19.88	5	20.76	0.910	0.407	22.2	20	5	20.71	0.857	21.26	19.72	0.495	3	-0.017	-0.049
Macrophytes	Lophocladia lallemandii	Indo-Pacific	From Hot to Cold	24.97	0.664	0.297	26	24.15	5	25.2	0.837	0.374	26	24	5	17.83	0.756	22.00	17.00	0.078	95	-7.143	-7.373

Macrophytes	Neosiphonia harveyi	NW Pacific	From Cold to Hot	14.22	0.758	0.339	15	13.32	5	14.57	0.597	0.267	15.3	13.95	5	18.58	0.273	18.78	18.39	1.932	2	4.364	4.014
Macrophytes	Polysiphonia morrowii	NW Pacific	From Cold to Hot	19.35	0.671	0.300	20.3	18.58	5	19.82	0.844	0.377	21	19	5	20.23	0.311	21.00	20.12	0.810	8	0.876	0.410
Macrophytes	Polysiphonia paniculata	E Pacific	From Cold to Hot	12.91	0.796	0.356	14	12.02	5	13.03	0.769	0.344	14	12.1	5	15.00					1	2.092	1.968
Macrophytes	Womersleyella setacea	Indo-Pacific	From Hot to Cold	24.11	0.809	0.362	25	23	5	24.4	0.894	0.400	25	23	5	18.32	1.071	22.00	16.00	0.126	72	-5.785	-6.079
Crustacea	Caprella scaura	Indian	From Hot to Cold	23.01	0.740	0.331	23.9	22	5	23.2	0.837	0.374	24	22	5	19.09	0.982	20.80	18.00	0.151	42	-3.919	-4.109
Crustacea	Grandidierella japonica	Japan	From Cold to Hot	15.83	0.550	0.246	16.3	15	5	16.37	0.871	0.390	17	15	5	17.46	0.763	19.00	16.89	0.175	19	1.629	1.085
Crustacea	Balanus eburneus	W Atlantic	From Cold to Hot	19.82	0.671	0.300	20.8	19.16	5	20.02	0.594	0.266	21	19.53	5	22.01	0.046	22.23	22.00	0.009	26	2.194	1.994
Crustacea	Austrominius modestus	Tropical Pacific	From Cold to Hot	18.93	0.819	0.366	20.1	18.1	5	19.16	0.940	0.420	20.6	18.3	5	19.72					1	0.792	0.564
Crustacea	Mytilicola orientalisp	Pacific	From Cold to Hot	13.40	0.257	0.115	13.6	13.1	5	13.72	0.438	0.196	14	13	5	15.13	0.058	15.20	15.10	0.033	3	1.737	1.413
Crustacea	Parvocalanus crassirostris	Atlantic	From Cold to Hot	20.10	0.618	0.276	20.8	19.25	5	20.39	0.849	0.380	21.1	19.03	5	21.03	0.115	21.54	21.00	0.020	33	0.935	0.641
Crustacea	Callinectes sapidus	W Atlantic	From Cold to Hot	19.50	0.917	0.410	20.2	18	5	19.95	0.881	0.394	20.8	18.62	5	20.13	1.639	22.00	17.53	0.366	20	0.632	0.182
Crustacea	Hemigrapsus sanguineus	Pacific	From Cold to Hot	22.82	0.566	0.253	23.3	22.16	5	23.02	0.526	0.235	23.6	22.5	5	23.54					1	0.724	0.520
Crustacea	Marsupenaeus japonicus	Indo-Pacific	From Hot to Cold	26.02	0.713	0.319	27	25.2	5	26.02	0.713	0.319	27	25.2	5	21.55	0.757	23.00	21.00	0.106	51	-4.467	-4.467
Crustacea	Palaemon macrodactylus	Pacific	From Cold to Hot	20.36	1.013	0.453	22	19.52	5	20.82	0.983	0.439	22.4	20.1	5	21.93	0.891	22.56	21.30	0.630	2	1.572	1.106
Crustacea	Paralithodes camtschaticus	NE Pacific	From Cold to Hot	17.08	0.615	0.275	17.5	16	5	17.33	0.848	0.379	18.3	16.54	5	18.81					1	1.730	1.482
Crustacea	Percnon gibbesi	W Atlantic	From Cold to Hot	19.00	0.865	0.387	20.2	18.1	5	19.26	0.979	0.438	20.4	18.2	5	20.64	0.597	22.00	20.00	0.149	16	1.639	1.383
Mollusca	Anadara transversa	W Atlantic	From Cold to Hot	19.15	0.849	0.380	20.2	18.09	5	19.62	0.895	0.400	20.5	18.26	5	20.75	1.429	20.75	17.12	0.305	22	1.596	1.128
Mollusca	Brachidontes pharaonis	Indian	From Hot to Cold	25.54	1.069	0.478	27.1	24.3	5	25.4	0.894	0.400	26	24	5	20.88	1.023	23.00	16.85	0.067	236	-4.657	-4.519
Mollusca	Crassostrea gigas	NW Pacific	From Cold to Hot	18.08	0.657	0.294	19	17.25	5	18.2	0.837	0.374	19	17	5	19.58	1.573	22.65	16.00	0.270	34	1.503	1.379
Mollusca	Mercenaria mercenaria	W Atlantic	From Cold to Hot	19.10	0.322	0.144	19.5	18.76	5	19.12	0.914	0.409	20	17.84	5	25.10	0.141	25.20	25.00	0.100	2	6.004	5.978

Mollusca	Musculista senhousia	Pacific	From Cold to Hot	17.63	0.987	0.441	18.7	16.25	5	17.96	0.919	0.411	19	17	5	18.95	1.206	22.00	17.01	0.263	21	1.318	0.988
Mollusca	Mya arenaria	N Atlantic	From Cold to Hot	16.10	0.867	0.388	17.2	15.26	5	16.45	0.940	0.420	17.8	15.63	5	19.61	1.998	21.84	17.12	0.755	7	3.507	3.157
Mollusca	Petricola pholadiformis	W Atlantic	From Cold to Hot	21.04	0.979	0.438	22.1	20.19	5	21.49	0.883	0.395	22.6	20.78	5	22.52	0.289	22.54	22.50	0.167	3	1.485	1.031
Mollusca	Pinctada radiata	Red Sea	From Hot to Cold	22.73	1.882	0.842	26	21.3	5	22.85	0.845	0.378	24	22	5	22.08	1.081	24.00	19.37	0.128	71	-0.653	-0.771
Mollusca	Ruditapes philippinarum	Indo Pacific	From Hot to Cold	24.52	0.919	0.411	25.6	23.19	5	24.51	0.635	0.284	25.3	24	5	17.63	1.529	22.00	14.13	0.179	73	-6.885	-6.881
Mollusca	Bursatella leachii	Indian	From Hot to Cold	21.93	1.388	0.621	23	19.52	5	22.05	0.909	0.406	22.9	20.53	5	20.87	0.900	22.00	18.00	0.164	30	-1.063	-1.175
Mollusca	Crepidula fornicata	NW Atlantic	From Cold to Hot	14.09	1.173	0.524	15	12.1	5	14.6	0.809	0.362	15.1	13.2	5	24.00					1	9.906	9.400
Mollusca	Rapana venosa	Pacific	From Cold to Hot	19.35	1.581	0.707	21.6	17.37	5	19.86	1.396	0.624	22	18.3	5	20.05	1.677	22.52	17.02	0.385	19	0.695	0.185
Pisces	Etrumeus teres	Atlantic	From Cold to Hot	19.64	1.096	0.490	20.8	18.36	5	19.97	0.955	0.427	21	19	5	21.59	1.101	23.00	20.00	0.235	22	1.944	1.612
Pisces	Fistularia commersonii	Indo-Pacific	From Hot to Cold	24.31	0.921	0.412	25.1	22.84	5	24.4	0.894	0.400	25	23	5	18.97	1.548	22.00	14.13	0.127	149	-5.346	-5.434
Pisces	Hemiramphus far	Indo-Pacific	From Hot to Cold	24.84	0.374	0.167	25.4	24.51	5	24.98	0.716	0.320	26	24	5	21.88	0.449	22.00	20.00	0.090	25	-2.962	-3.104
Pisces	Lagocephalus sceleratus	Indo-Pacific	From Hot to Cold	24.21	0.373	0.167	24.8	23.84	5	24.6	0.894	0.400	26	24	5	21.85	1.024	23.00	0.20	0.112	84	-2.365	-2.755
Pisces	Pterois miles	Indian	From Hot to Cold	24.68	0.355	0.159	25	24.29	5	24.8	0.447	0.200	25	24	5	22.02	0.057	22.23	0.20	0.012	22	-2.667	-2.783
Pisces	Siganus luridus	Indian	From Hot to Cold	24.24	0.833	0.373	25	23	5	24.44	0.820	0.367	25.3	23.15	5	21.69	1.116	23.00	20.00	0.106	111	-2.552	-2.754
Pisces	Siganus rivulatus	Red Sea	From Hot to Cold	21.50	0.524	0.234	22	20.84	5	21.86	0.669	0.299	22.8	21	5	21.37	1.133	23.00	15.00	0.103	121	-0.127	-0.491
Pisces	Stephanolepis diaspros	Red Sea	From Hot to Cold	22.30	1.031	0.461	23.8	21	5	22.46	1.230	0.550	24.3	21	5	22.28	1.147	25.00	17.00	0.161	51	-0.022	-0.178
Pisces	Tridentiger trigonocephalu s	Pacific	From Cold to Hot	22.73	0.799	0.357	23.8	21.54	5	23.02	0.709	0.317	24	22	5	23.32	0.631	23.79	22.60	0.364	3	0.587	0.297

Chapter 3

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Measuring the effects of temperature rise on Mediterranean shellfish aquaculture

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Abstract

Shellfish aquaculture represents a worldwide valuable segment of the aquaculture market, spreading along the Mediterranean coasts, and is sensitive to the still unforeseen, poorly-known effects of climate change. Threats due to temperature rise can threaten the deployment and development of this sector, up until now recognized as the best candidate to mitigate the effects of fishery overexploitation. Here, we investigate the effects of temperature increase on the model species, *Mytilus galloprovincialis*, measuring outcomes from valve fragility (thickness) and condition index. Evidence of a reduction in the thickness of valves and the modulation condition of the mussels along with temperature increase have been gathered from simulations of a natural temperature gradient changing along latitude (the Italian Peninsula) and temperature risen (mesocosm trial). The obtained results offer a baseline to help the next generation of managers and stakeholders when assessing the reliability and feasibility of shellfish culture in a changing sea that can generate undetected and underestimated impacts on the sector.

Keywords: Thickness; Body condition; Temperature increase; Climate Change; Shellfish; Aquaculture

3.1 Introduction

Environmental change, including increasing temperature due to global warming, has direct effects on quality and quantity of cultivated bivalves by affecting their morphometric characteristics, growth rates and condition index (Mackenzie et al., 2014). Since bivalves represent an important segment of the aquaculture market worldwide, environmental change will risk reducing the role of this sector as the recognized best candidate of mitigating the effects of fishery overexploitation (FAO, 2016). Thus, the need for an accurate and proactive mechanistic understanding of "how", "where" and "when" the effects of global warming will manifest is becoming both pressing and compelling in a context of multiple stressors (Helmuth et al., 2014; Connell et al., 2017; Sarà et al., 2017). Temperature can affect the metabolism of cultivated molluscs according to specific rules following mechanistic relationships (e.g. Arrhenius temperature, Kooijman, 2010), with tested effects on both shell calcium fixation processes and the energy allocation to somatic and gonadic structures (Hiebenthal et al., 2012, 2013). A potential expression of this effect could be a reduction of thickness with a consequent increase in shell fragility (Olson et al., 2012; Briones et al., 2014). Valves play several primary ecological roles, such as reducing successful predation by crushers, protection from intense wave action and providing mechanical support from the effects of density and aggregation in beds, ropes or matrices (Elner, 1978; Briones et al., 2014 and references therein). Thus, any possible reduction in thickness and mechanical strength could have a profound effect on survival, not only by reducing protection of the soft tissues from predators and anthropogenic activity, but also by influencing the ability of bivalves to respond to environmental change (MacKenzie et al., 2014). The relationship between environmental temperature (Sea Surface Temperature, SST) and thickness in bivalves has had a new and recent impetus due to the results obtained by studies focusing on the expected increasing temperature effects on organismal performances (*sensu* McBryan et al., 2013; Helmuth et al., 2014). Overall, the experimental outcomes obtained by testing the relationship between temperature, latitude and thickness have shown contrasting trends (*sensu* MacKenzie et al., 2014), highlighting differences among cold and warm waters (Vermeij, 1993) in several invertebrates (Trussell, 2000; Trussell and Smith, 2000; Trussell and Etter, 2001; Sepúlveda and Ibáñez, 2012; Watson et al., 2012). Therefore, a general trend seems to be most commonly observed in that under higher temperature and at lower latitude, valves should be thinner (Briones et al., 2014). Considering the assumption that thickness correlates with valve strength (fragility), here we hypothesise that the expected increasing temperature under climate change may generate direct consequences on the amount of lost bivalves due to shell breakage caused during aquaculture facility operations, with direct implications on the amount of saleable product. Nowadays, the amount of lost product in aquaculture, due to breakage, is not usually recorded or taken into account by farmers, and it is neglected by shellfish managers, although anecdotal data reveal that it could depress the whole annual production by about 5–15% (G. Sarà pers. com).Contextually, according to bioenergetics extrapolations (*sensu* Kooijman, 2010; Sarà et al., 2014), animals living under higher temperature regimes could have a larger amount of organic structures (i.e. somatic and gonadic tissues; Matzelle et al., 2014). This can be mirrored in a more positive condition index (i.e. individual length-weight; Matzelle et al., 2014) with direct consequences on the quality of saleable product. The term 'condition index' is usually loosely used to describe the general performance of cultivated animals (Filgueira et al., 2013; Briones et al., 2014). It should decrease under oligotrophic conditions (Raubenheimer and Cook, 1990) and reach higher values under a richer food environment (Mackenzie et al., 2014). The relationships determining the organismal response to temperature increase, in terms of both valves fragility and individual condition, are usually neglected by managers when assessing the reliability and feasibility of shellfish culture in a changing sea. The relationship between the thickness of valves (as a potential proxy of fragility affecting product lost rate; *sensu*; Branch et al., 2013) and the condition of organisms (as a potential proxy of product quality; Watanabe and Katayama, 2010) is implicitly accounted in the product value and market price. Nevertheless, this information should be accounted in reliable metrics to seek economic trade-offs in order to manage aquaculture activities. In fact, the relationships among increasing temperature, local trophic conditions, morphometric traits and condition index in calcified shelled cultivable animals (e.g. mussels, oysters, cockles and clams) play a crucial role in our understanding of how global environmental change will affect productive systems, thus impairing the sustainability of commercial activities at sea.

This is much more crucial in the Mediterranean Sea where shellfish aquaculture spreads along the coast and where sea surface temperature is forecasted to increase at the northern sites (Lejeusne et al., 2010; Shaltout and Omstedt, 2014) and, as a possible secondary effect, to generate a trophic impoverishment (i.e. oligotrophication; *sensu* Nixon, 2009; Briones et al., 2014). Thus, northern sites are expected to become potentially warmer and food-poorer, weakening their productive potential with unpredictable socio-economic repercussions (IPCC, 2014). Here, we used the Mediterranean Mussel (*Mytilus galloprovincialis*) (Lamark, 1819), one among the most cultivated bivalve worldwide (FAO, 2016), as a model species to study whether the relationships between temperature, thickness, fragility (expressed as breaking load) and condition index were tested in the wild on a large spatial scale, under a sea surface temperature and trophic Mediterranean latitudinal gradient (9° degree). The adoption of the latitudinal gradient of temperature – to test the potential future expected effects of increasing temperature on organismal responses generated by climate change – is a common approach well-accepted across the current literature and it allows to increase the realism of climate change predictions (*sensu* Watson et al., 2012).

However, conscious that in designing an uncontrolled survey on a large spatial scale, the interpretation of organismal response can be biased by other factors (e.g. the local amount of food, the density of mussels in a bed, rope or matrix; Briones et al., 2014), here, to increase the realism of our predictions and the generalisation ability, we validated the information gathered from the larger spatial scale observations with a short-term mesocosm experiment (7 months). This was designed to monitor the effect of two different temperature treatments (ambient vs ambient +3 °C; Mackenzie et al., 2014) on the fragility of the valves by keeping the amounts of food constant for both treatments and by measuring "free" mussels (not twisted in a rope).

This design allowed to disentangle the observed trends by the effect oflocal trophic conditions and the position of individuals in the rope (or space). The proposed relationships, deriving from theoretical extrapolations, have never been specifically verified in a context of shellfish aquaculture and never carried out through appropriate studies on a large spatial scale by exploiting the latitudinal gradient of temperature change (Briones et al., 2014). Such information will be valuable when investigating any possible deviation from natural patterns in response to increasing temperature and may represent important background in which to address the future understanding of feasibility and reliability of shellfish aquaculture economic activities.

3.2 Materials and methods

This study articulates two separate steps; the first (hereafter called"Latitudinal survey") was designed to test the relationships between temperature, thickness and fragility (breaking load) on a large spatial scale by collecting mussels, *Mytilus galloprovincialis*, from farms located in 10 open-sea sites along the Italian Peninsula (Fig. 1). The salinity of sites was marine, ranging from 36 to 38‰. Several thousand of mussels were then collected from commercial farms at every site, in the framework of PRIN TETRIS project during the "ECOTRIP" survey in summer 2013. Samples were analysed at the Ecology Laboratory (DISTEM, University of Palermo), morphometric and age measures were collected. To establish the relationship between morphometric measurements and local Sea Surface Temperature (SST), hourly SST data were downloaded from the database provided by the Higher Institute for the Protection and Environmental Research (ISPRA) oceanographic national buoys network. To link the condition index to the trophic status of any cultivation sites, data of chlorophyll-a (CHL-a) concentration from satellite imagery were downloaded from the Environmental Marine Information System (EMIS) maintained at the

European Joint Research Centre website (http://emis.jrc.ec.europa.eu/), as in several previous companion papers (Sarà et al., 2013a, 2014).

The second step (hereafter called "Mesocosm") was a mesocosm experiment carried out between May and November 2014 to validate the outcomes from the large latitudinal survey.

3.2.1 Latitudinal survey: laboratory analysis

Mussels were individually measured and the age was determined; only individuals of a fixed range of 2/3 years and a standardised size of 60–70mm were selected (for a total of 100 mussels per site). The total length (TL, mm) was measured by using a Vernier calliper (to the nearest 0.01 mm; Sarà et al., 2007) from the front (umbonal) to the back. The age was estimated by using the shell rings analysis proposed by Peharda et al. (2011) and routinely applied in other previous companion studies (e.g. Matzelle et al., 2014; Rinaldi et al., 2014). Valves were cut with a Dremel rotary (Series 4000; Robert Bosch Tool Corporation) and the number of rings was counted under a stereomicroscope lens (Leica Z4). Mussels selected to the standardised size weredissected; somatic tissues, gonads and valves were both fresh and dry weighted separately. Valves were measured for thickness by using a Vernier calliper. Here, we expressed the thickness as the width (mm) measured along the profile of the right valve, which was previously cut with a circular saw. Thickness was standardised by averaging the thickness values, measured at three valve-points, identified respectively at the umbonal region, the mean and outer region of the right valve (Fig. 2). Thickness measurements were replicated three times and in blind by two operators. Once measured, 50 mussels from every site were measured for the mechanical strength of the valves. A crushing test, to estimate the maximum breaking load of the shell (Newton, N), was carried out through a crusher device calibrated with an Instron 3367 Tensile Test Machine controlled through the Bluehill 2.0 software.

The breaking load was a proxy of potential breakage and was correlated with temperature and thickness. Valve, somatic and gonadic weight measurements were combined in a condition index according to Walne (1976) and recently applied in Filgueira et al. (2013).

3.2.2 Mesocosm experiment to validate the relationship between thickness and fragility at two different temperatures

To validate the effect of temperature on thickness and fragility (breaking load), an experiment under Mesocosm conditions was carried out in the premises of CNR Lab (Messina, Italy). Mussels from a local farm located in Eastern Sicily (200 individuals) were acclimated under two different temperature regimes and the growth and thickness were monitored for 7 months. Mussels were subdivided into two groups of 100 animals each. The first group was placed into two tanks (A and B) and maintained under ambient temperature (here after called Ambient), the second group was placed into two tanks (tanks C and D) under an ambient temperature of plus 3 °C (here after called HOT). All tanks were maintained independently of each other to avoid any pseudoreplication. To reproduce a slight increase of temperature, heaters (1500 W) were placed into the tanks and the power was adjusted daily to generate a constant increment of 3 °C compared to the Ambient but respecting the same variability of the ambient temperature. Such a small increment mirrored the increasing temperature predicted by IPCC (2014) in AR5 scenarios, which is considered the worst-case scenario by the Paris Agreement of the Conference of the Parties (COP21) of the United Nations Framework Convention on Climate Change, judging it as the maximum sustainable temperature in the future before the productive collapse of ecological systems (*sensu* Hulme, 2016). Temperatures were hourly recorded through temperature data loggers (model: iButton G1, prec. ± 1 °C, res. ± 0.5 °C, http://www.alphamach.com). The trend is reported in Fig. 3. Mussels were hung up in the tanks, separately, to minimize the effect of competition typical of placement in the rope (Sarà and Mazzola, 2004). They were individually measured every six weeks to determine the length and weight. At every sampling date, 10 mussels were frozen measured and weighed, before an age and breaking load estimation was performed (as described above). Throughout the whole experimental period, mussels were fed twice a day with unicellular algae belonging to the genus *Nannochloropsis sp*. provided ad libitum in each tank (Sarà et al., 2013b) to maintain a constant mesotrophic concentration of about 3–4 μg l^{-1} kept over time.

3.3 Elaboration and statistical analysis

A simple linear regression approach was applied to test the relationship among all the variables and measurements taken into analysis during the Latitudinal survey (SST, latitude, chlorophyll-a, thickness, fragility; Sarà and Mazzola, 2004). The difference in temperature values coming from thermo-loggers in the Mesocosm experiment was tested by ANOVA, with temperature (Ambient vs. HOT; 2 levels) as a fixed factor, Tank as a random factor. The relationship between thickness and temperature under the two treatments (Ambient vs. HOT) was tested through linear regressions, and ANCOVA was used to test the heterogeneity of slopes and differences between intercepts of regressions according to Sarà and Mazzola (2004) and Bracciali et al. (2016).

The GMAV (1997) statistical package (University of Sydney, Australia) was used to perform ANOVA, Microsoft Excel and PAST (Natural History Museum, University of Oslo) to calculate heterogeneity of slopes and differences between intercepts, while other statistics were assessed using the STATISTICA (Statsoft Inc., USA) statistical package.

3.4 Results

The thickness of mussels a priori selected for standardise size (60 and 70 mm) and age (2–3 years old) collected from 10 farms around the Italian peninsula showed a significant dependency with latitude (Fig. 4a): the lower the latitude, the thinner the valves ($p < 0.05$; i.e. going southward the thickness decreased). The strong correlation between latitude and local mean water mass temperature (Local Water Temperature=29.43 (±2.78)−0.273 (±0.065) ∗ Latitude; r=0.835; N=10; p=0.00327) drove the relationship between thickness and mean local water mass temperatures (Fig. 4b) that gave significant results only after the three-sampling site of Licata, Chioggia and Portonovo were not considered in the model. Thickness was significantly (positively) correlated with the fragility (breaking load; Fig. 4c) while the condition index was significantly correlated with temperature (Fig. 5). Temperature and chlorophyll-a were significantly and negatively correlated (CHL-a=18.94 (±1.57)−0.97 (±0.09) ∗ Temperature; r=-0.97; n=10; $p < 0.001$). To investigate the dependency of condition index on local trophic conditions of water masses, the condition index was correlated with CHL-a (Fig. 5b). Such relationships justified the Mecososm experiment to disentangle the latitudinal outcome and to validate it under controlled trophic conditions that were constantly kept over time at high level (∼3.5 μg l−1) for both treatments (Ambient vs HOT). Our experiment corroborated the relationship between temperature and thickness (Fig. 6 and 3) and showed that temperature could be the main factor driving the relationship, as showed by the significant difference between intercepts of two regressions (ANCOVA test; Ambient vs. HOT; p=0.028; see Fig. 6) and the consequent relationship between thickness and breaking load (AMBIENT: Breaking load=−49.67+288.35 ∗ Thickness; r=0.93; n=5; p=0.02; HOT: Breaking load=−36.38+277.47 ∗ Thickness; r=0.97; n=5; p=0.01). Comparison between intercepts were significant (ANCOVA test: Ambient vs. HOT; p=0.024) confirming an effect of temperature on thickness in terms of increasing fragility.

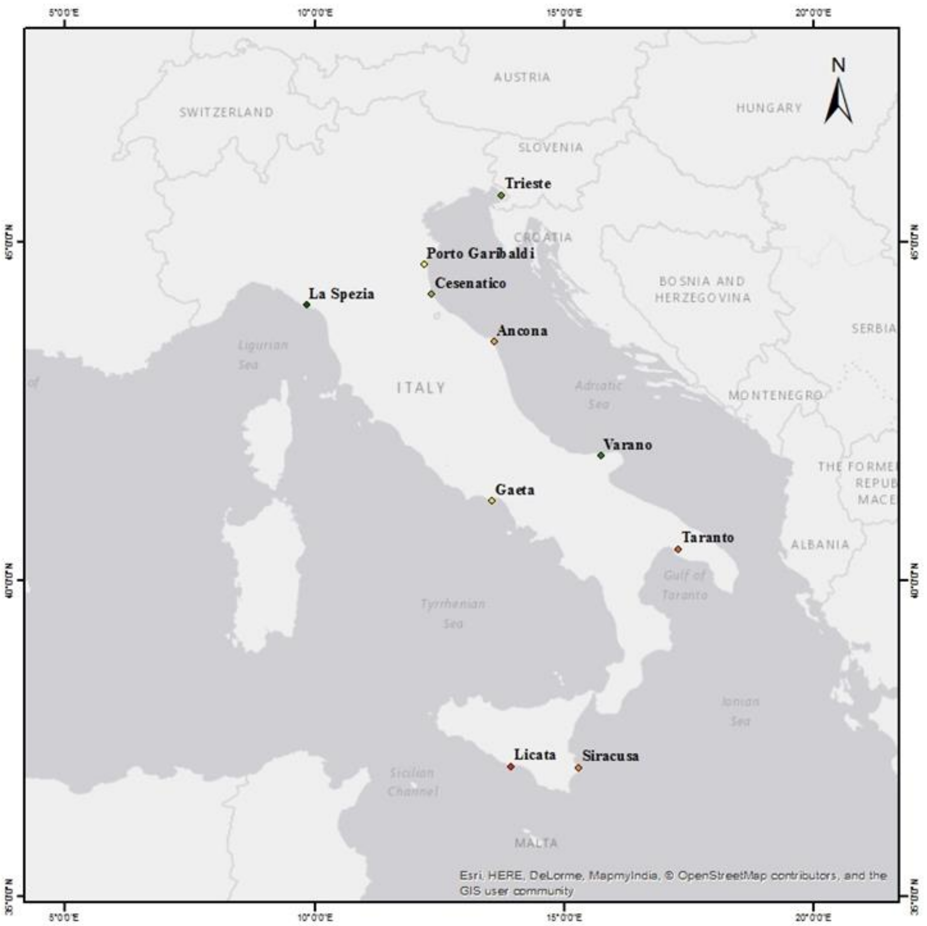

Fig. 1. Sampling sites along the Italian Peninsula. The redstar bullet indicates the locality (Messina) where the mussel were collected to carry out the mesocosm experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

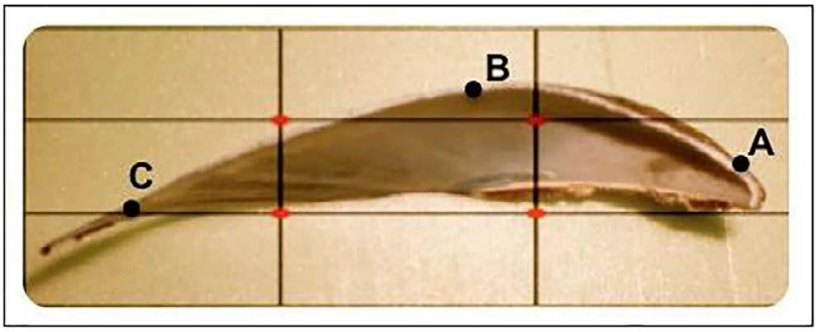

Fig. 2. Valve measurement points A, B and C on the profile of the right valve.

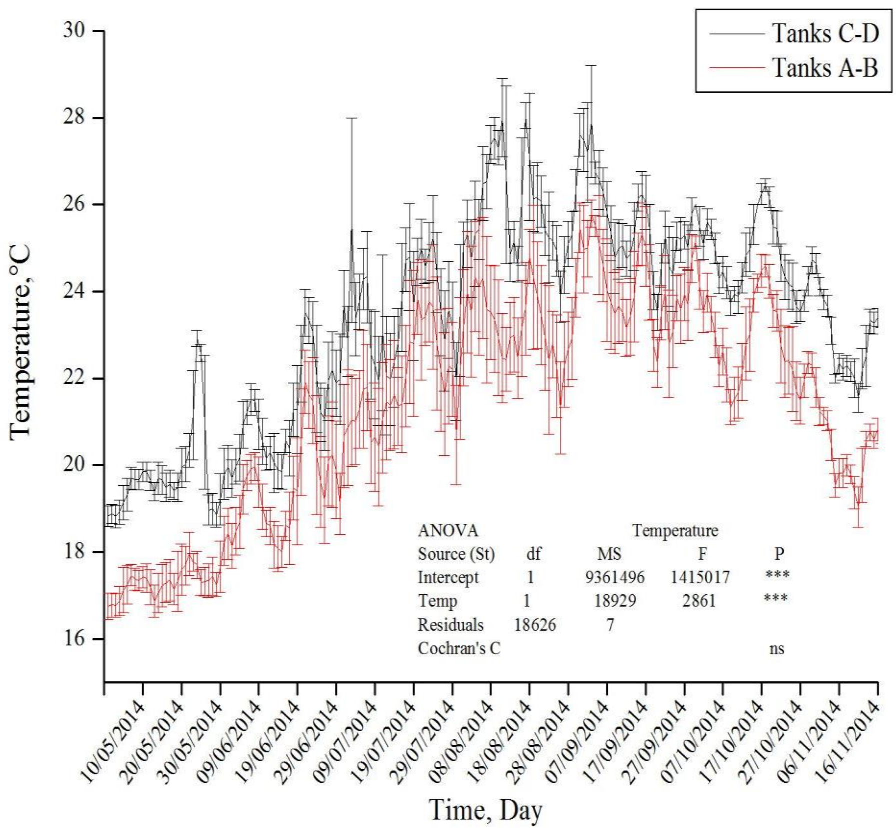

Fig. 3. Temperature of the tanks. The average ambient temperature was 21.31 ± 2.43, and the HOT was 23.35 ± 2.37.

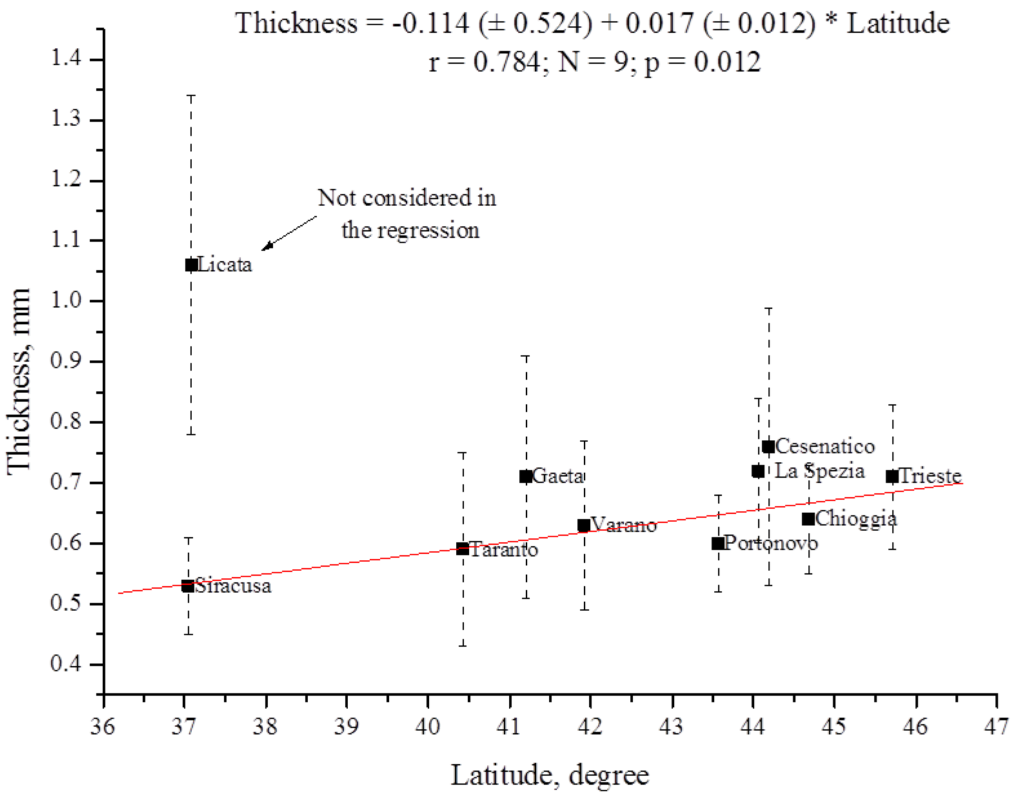

Fig. 4a. Relationship between thickness and latitude.

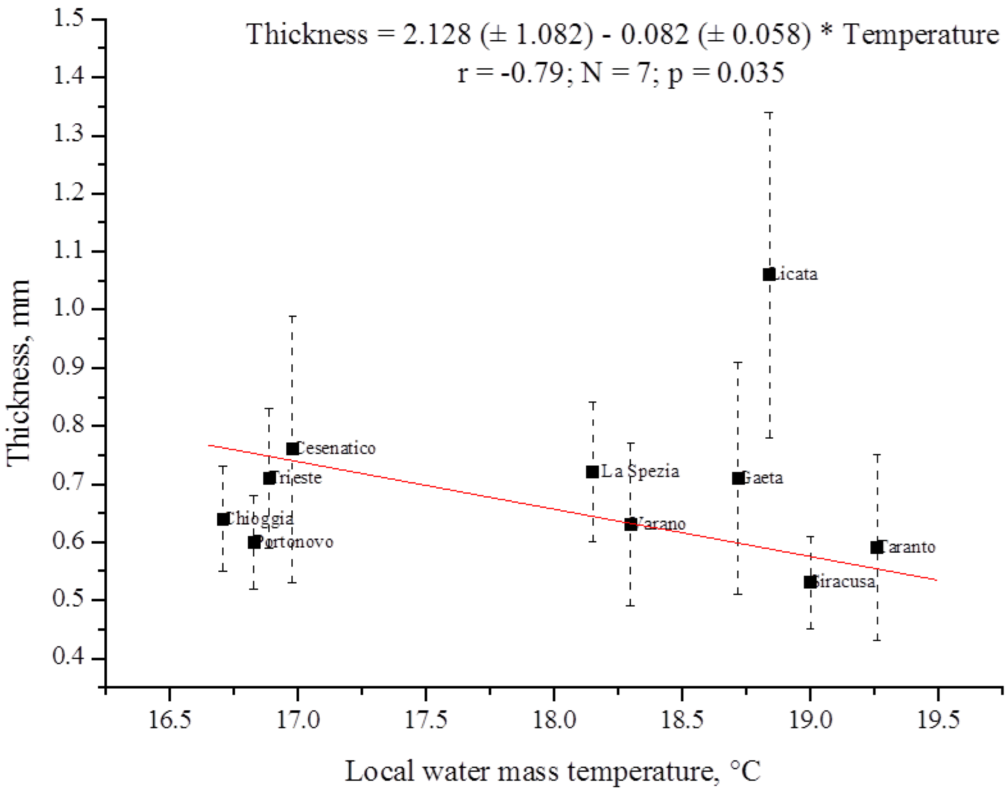

Fig. 4b. Relationship thickness and temperature.

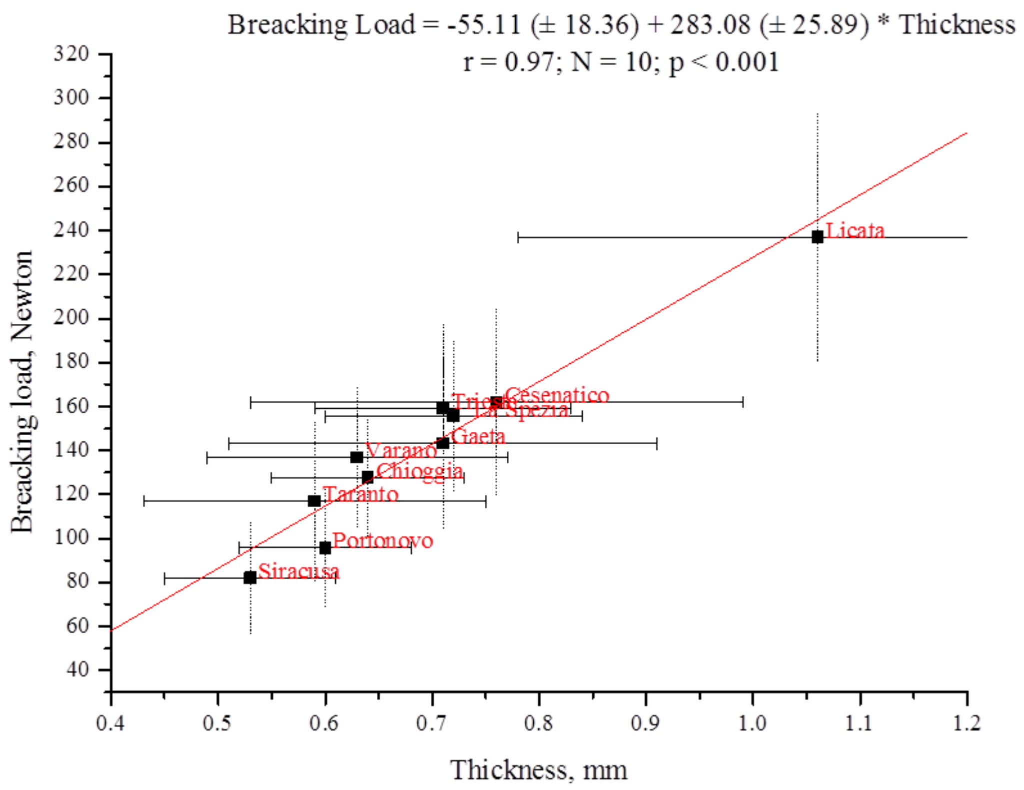

Fig. 4c. Relationship between fragility (breaking load) and thickness.

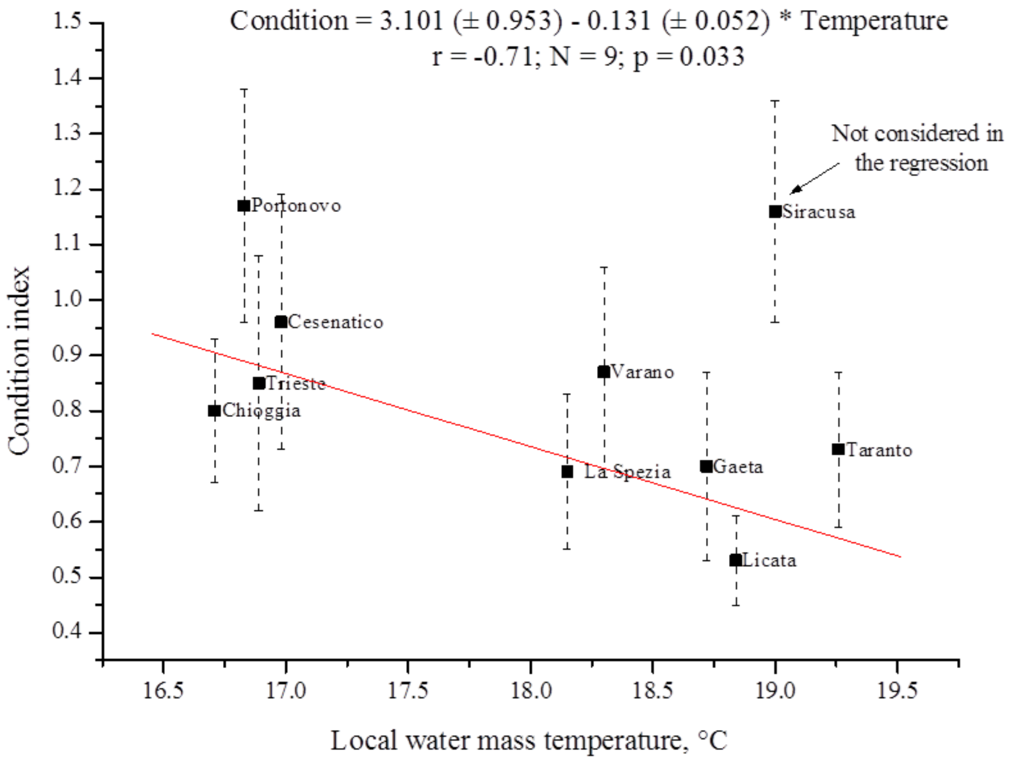

Fig. 5a. Relationship between condition index and temperature.

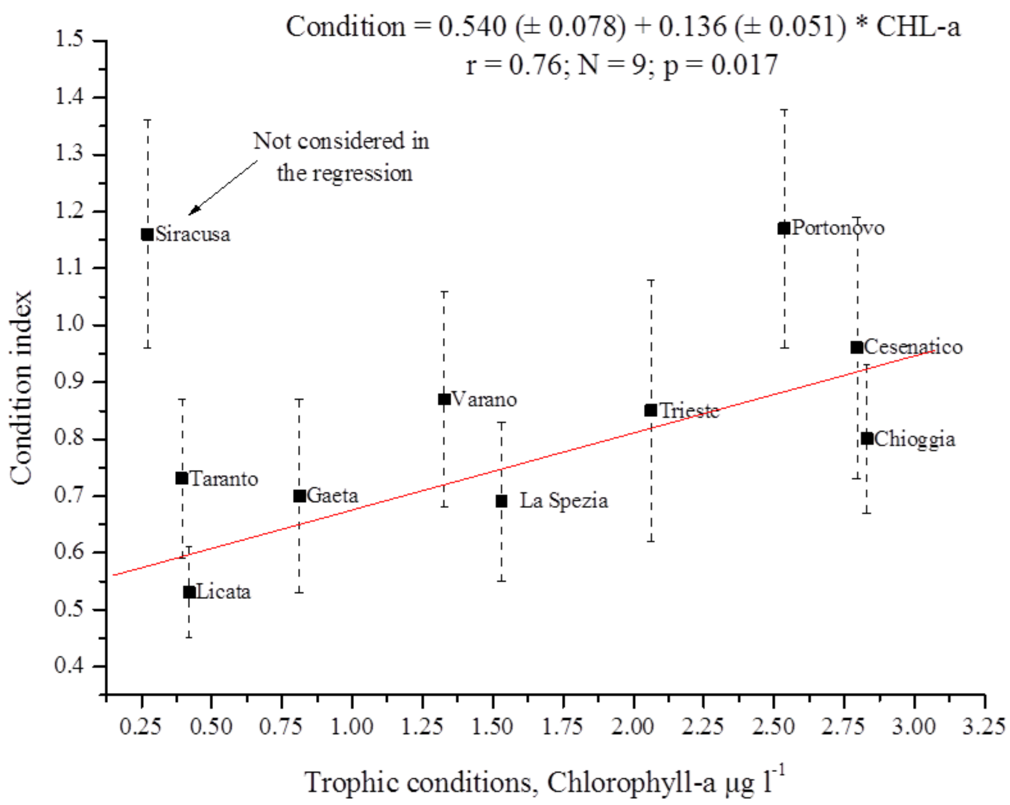

Fig. 5b. Relationship between condition index and trophic condition.

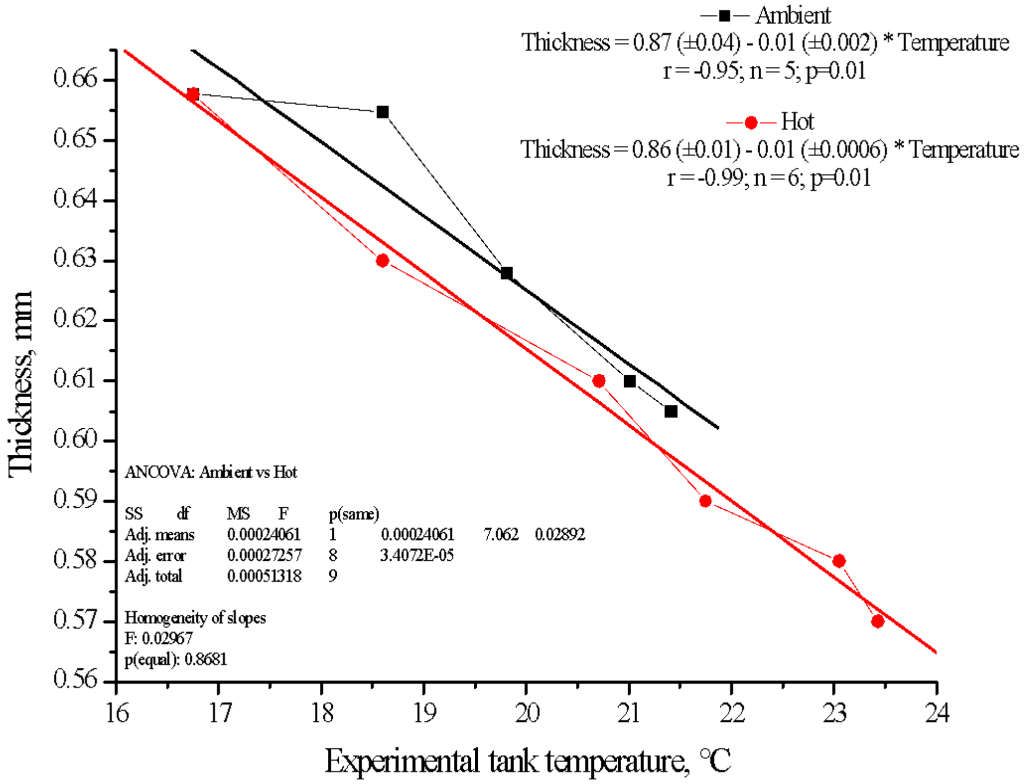

Fig. 6. Relationship between thickness and temperature of the tanks.

3.5 Discussion

The present study demonstrated a significant relationship between temperature (or latitude) and thickness in *Mytilus galloprovincialis* under temperate regimes at a large scale and between thickness and fragility (breaking load). Temperature significantly influenced the structure and composition of valves, in that, the higher the temperature, the thinner and more fragile the valves (Mackenzie et al., 2014). Such a result partially contradicts some influential past findings (e.g. Vermeij, 1993) and could be partially explained by the restricted latitudinal gradient (only 9°) of the Mediterranean Sea. More importantly, the cross-fertilisation by other factors – such as the local trophic conditions – could primarily drive those relationships. The importance of local conditions can also be corroborated by the fact that some regressions fit significantly only after having removed some sites from the analysis and this testified a mosaic of response at micro- and meso-scale level (*sensu* Helmuth et al., 2006). Trophic conditions, here expressed by satellite imagery chlorophyll-a concentrations, were negatively correlated with temperature showing that southern sites had poorer food availability than northern ones. This explains the positive correlation between conditions index and trophic conditions. Thus, the cross-correlation between temperature, nutritional state, thickness and fragility (breaking load) complicates the interpretation of the present outcome. The nutritional state seems one among the largest drivers of this complex system or relationship by exerting an effect on tolerance to environmental stress. This is, for example, in line with the findings of Kroeker et al. (2010) which showed that hypercapnia had a negative effect on the growth of intertidal mussels, but only when there was a co-occurrence between high temperature and poor food conditions. Thus, the temporal and spatial covariance of more than one factor may drive the stress response (Mackenzie et al., 2014). The key role of the nutritional state in driving vulnerability to environmental change thus provides a window of insight into how physiological impacts may affect the response of individual organisms. This is crucial in a context of aquaculture where biotic interactions are controlled by management practices and where the performance of the farm can be more easily explained through the performance of individuals with no biotic interactions involved, as happens in the wild. As a main consequence, under the present latitudinal gradient covering the whole central Mediterranean Basin (from 35° up to 44°), the simple negative significant relationship between temperature and thickness is, in the reality, masked by other factors which play interrelating roles such as the nutritional status. This could explain the possible discrepancy between results presented by other studies worldwide, which have usually been carried out assessing a simpler thickness dependency on temperature, thus failing to produce a realistic outcome. For example, this was the case of works carried out in the North Sea and in the Adriatic Sea on *Mytilus edulis* and *Chamelea gallina* (Gizzi et al., 2016; Nagarajan et al., 2006, 2008). Reporting the relationship between temperature and thickness, the study took into account factors such as salinity and solar radiation but did not report any information on the trophic condition. On the other hand, more recently, Mackenzie et al. (2014) questioned the role of trophic conditions on these types of relationships and carried out experiments under quasi-food deprivation conditions to test the combined effect of increased temperature and hypercapnia on condition index and shell morphometric in *Mytilus edulis*. They showed that food was able to compensate for the effect deriving from interacting stressful conditions, thus they were able to measure the counterweight of temperature in respect to that played by hypercapnia in determining the ultimate organismal performances. In general, our results indirectly showed that food-poorer conditions generated reduced healthy conditions of cultivated mussels (as expressed by lower condition index; Filgueira et al., 2015) and since this is recorded under warmer latitudes, the combined outcome is that the thickness of the valves was thinner. Watanabe and Katayama (2010) suggested that the effect of higher temperatures brought faster growth to Japanese clam's but which produced thinner shells. This hypothesis finds ground in the current bioenergetic theory (Kooijman, 2010) which predicts that ectotherms living under different body temperatures could have different velocities at which asymptotic size is approached (i.e. growth rate; Pecquerie et al., 2009). This should imply that higher temperature conditions produce higher growth rates (*sensu* Duarte, 2007) and then that the energetic allocation to structure may be unbalanced; this could produce thinner shells. However, as we stated previously, this picture is complicated by the different food concentrations (and mussel's nutritional status) among southern and northern sites. While in this study, we used age- and size-standardized animals, if we rely on the observation that northern mussels usually reach larger commercial size in less time but with ticker shells, we can only speculate that this depends on the fact that smaller individuals in southern sites have faster growth rates and then thinner shells (and viceversa; Kooijman, 2010). On the other hand, northern mussels – being able to rely on much more food than southern counterparts – could allocate much more energy to build structures such as shells that resulted in them being thicker and this could help to definitely explain the difference between northern and southern thickness. Thinner valves were significantly more fragile: this is crucial when assessing both the ecological and the productive implications in the shellfish aquaculture sector. While the ecological signification of thinner valves in the wild are certainly well-known (Nagarajan et al., 2006, 2008), the possible implications in productive sectors such as fishery (harvesting) and aquaculture are almost neglected. Instead, the loss of product due to breakage of shells, inducing mortality, represents an important source of economic loss, the importance of which could increase in the near future in a context of climate change. Our latitudinal gradient may be useful in studies of the effect of climate change on this aspect; in fact, there was a water temperature difference of about 3 °C between the two extreme geographic points of our study area, Trieste and Siracusa, which mirrors the IPPC predicted scenarios (2014), and is greater than the plausible COP 21 end-point (Hulme, 2016). In this context, the obtained results should be read from our Mesocosm experiment. Mesocom thermal differences produced the same outcome obtained by the field latitudinal large scale observational survey: the higher the temperature, the thinner the shells of our mussels. Thus, according to recent literature using latitudinal gradient (e.g. Watson et al., 2012), we are able to infer that in the next decades the expected "meridionalization" of the Mediterranean Sea should bring significant changes in morphometrics, structure of shells (as well as in chemical composition that unfortunately here was not investigated) and condition index which will ultimately affect bivalve production rates along the Northern Mediterranean coasts. By coupling this evidence to the expected impoverishment of trophic conditions (oligotrophication scenario IPCC, 2014) that will impact the organismal nutritional and health status (as roughly expressed by the condition index), the effects in the shellfish culture might threaten the sustainability of the shellfish culture sector in areas such as the Northern Adriatic Sea with plausible effects on the three main components: ecological, economic and social (Brander, 2007; Yohe and Strzepek, 2007; Cochrane et al., 2009; Bell et al., 2013). Further studies should be mechanistically addressed to increase our ability to predict the multi-layered effects of increasing temperature and food deprivation due to oligotrophication on the sustainability of farming activities at sea (Sarà et al., 2012, 2013a,b). The main outcome of this study may further provide the opportunity to raise awareness in public and scientific communities (*sensu* Mangano et al., 2015; Mangano and Sarà, 2017) to inform on the importance of building upon common actions and strategies to mitigate the impact of climate change on several aspects of the food chain production based on marine sectors. While some implications generated by the cross-correlation of local conditions need to be further explained and disentangled, the proposal to adopt the thickness as a proxy of healthy status of shellfish and as indicators of environmental change (including climate) could be a first feasible step (D'Alessandro et al., 2016). The thickness (and fragility) of the valves in shellfish could also be considered as a new reliable indicator when informing the Marine Strategy Framework Directive (MSFD) (2008/56/CE), which sets the overall objective of achieving or maintaining the "good ecological status" (GES) in European Marine Waters by 2020 under a context of environmental and climate change, as for example within the specific Descriptor 3 "Commercial Fish and shellfish". The need to integrate thickness and fragility as measures to consider in the shellfish aquaculture sector will represent a new challenge. A practical perceived need has been identified and needs to be addressed: we suggest future innovative best practice and solutions within a context of Blue Growth development in all sea monitoring programs and strategies (e.g. the creation of new packaging solutions; development of new cleaning and grinding equipment, as well as new modality of transportation and storage).

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Chapter 4

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The effect of the quality of diet on the functional response of *Mytilus galloprovincialis* (Lamarck, 1819): Implications for integrated multitrophic aquaculture (IMTA) and marine spatial planning

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Abstract

The integrated multi-trophic aquaculture (i.e., IMTA) is a practice combining organisms with different trophic levels with the final purpose of transforming the continuous waste of food by targeting species into nutrient input for other non-target species. This practice very often involves filter feeders, such as bivalves, by the use of which bioenergetics budgets are strongly influenced by the quality and quantity of different foods. However, to date, scant information is available, to really understand the rebounds of food availability on the growth performances of these harvested biomasses in the natural environment. By choosing the mussel *Mytilus galloprovincialis* as a model, this study aims to (1) characterize the functional response of the species to define all parameters related to food intake strategies and (2) to investigate how responses change as a function of varying food sources. Laboratory procedures have been designed to evaluate the clearance rates (CR) and assimilation efficiencies (AE) of M. *galloprovincialis* with varying food concentrations, while different diets (i.e., seagrass, phytoplankton, and pellets) have been provided to investigate how differently they reach saturation. Results show that in the presence of phytoplankton and seagrass as food sources, the feeding strategies of *M. galloprovincialis* follow a II-type Holling's curve, while it shows a I-type Holling's curve when pellet food is provided. Investigating the behavioral components of functional responses may improve our ability to predict where to place shellfish cultures, as it may be useful in the context of IMTA management and in addressing siting studies. Statement of relevance: Our paper focuses on a question central to understanding and predicting the likely impacts of one among the most important human economic activity like the aquaculture in coastal habitats. The main question deals with the possibility to combine

experimental procedures with the new mechanistic functional trait based bioenergetic models in order to effectively predict life history traits of cultivated species.

Keywords: mussels, feeding, Dynamic Energy Budget model, growth performances

4.1 Introduction

Coastal habitats are characterized by high concentrations of nutrients and organic matter and as such, they represent elective areas for the development of shellfish farms such as bivalve molluscs. In a context of marine spatial planning, integrated multi-trophic aquaculture (i.e., IMTA; Troell et al., 2003) is an increasing and largely used practice, as it combines organisms from different trophic levels with the ultimate purpose of transforming dejections and surplus food of carnivorous species into nutritive input for other non-target species (Reid et al., 2010; Troell et al., 2009, 2003). Suspension feeders such as bivalves are prominent organism species involved in IMTA (Sarà et al., 2012, 2009) and in some coastal areas, they are subjected to a continuous flux of particulate organic matter of different origin (e.g., seagrass) that dilute theenergetic value of pellets and phytoplankton, which represent the main food items for those species. The recent isotopic research supports the theory that suspension feeders such as bivalves are able to also assimilate, as secondary food energetic sources, fractions of refractory food coming from seagrass detritus (Cabanellas-Reboredo et al., 2009). Some studies have reported the possible effects of different types of food on the bivalve's growth through short-term experiments in the field and mesocosms (e.g., Sarà et al., 1998). Even as some predictive relationships between feeding rate and environmental factors such as, temperature, current speed, and food availability and composition, are available for the major aquaculture species, the information available is still complicated by low reliability in establishing the mechanistic link between the quality of diet and organismal growth processes. Nonetheless, increasing our mechanistic (*sensu* Kearney and Porter, 2009) ability to predict where and when organisms better exploit different types of food at varying densities may be particularly useful in IMTA. For example, such information could be useful to feed recent farm-scale models (Ferreira et al., 2011) or may be needed for selection of IMTA locations and optimization of layouts, to predict growth performances and site production–carrying capacity, and for assessing the potential ecological services and impacts of aquaculture operations. Here, we carried out mesocosm experiments to test the effect of three different diets (pure phytoplankton, seagrass detritus, and minced fish pellets) on the feeding behaviour of our model species, the Mediterranean blue mussel *Mytilus galloprovincialis* which represents an important economic resource for the local societies, and outside the native range area it is a highly invasive species (McQuaid and Phillips, 2000). Indeed, on a global scale, the contribution of this species to seafood production rose to about 1 million tons, with China and Spain being the most important producers (FAO, 2014). In the Mediterranean Sea, the annual production attained over 115,00 tons in 2009 mainly produced
by Italy and France (as the Spanish production is mostly Atlantic), representing 32% of the marine aquaculture production and ranking *M. galloprovincialis* in the third position after Seabass and

Seabream (Gazeau et al., 2014; Goffredo and Dubinsky, 2013). The present study was thus designed to (1) study how the feeding responses of *M. galloprovincialis* changed as a function of diet quality and quantity, and (2) use such information to fed a functional trait based model as the Dynamic Energy Budget theory (Kooijman, 2010) in order to investigate how the presence of different diets could ideally affect the growth rates of mussels cultivated under different temperature regimes (i.e., simulating six different levels, from 13 to 18 °C).

4.2 Materials and methods

4.2.1 Animal collection and maintenance

About 400 individuals of *M. galloprovincialis* (45–75 mm) were collected
in February 2014 from the Faro Lake in eastern Sicily (Lat. 38°15′39″North, Long. 15°37′02″ East), placed in containers with absorbent material, and cooled with dry ice, in order to keep them moist during the transport to the laboratory. Once in the laboratory, they were cleaned off epibionts and sediments, and then placed in special tanks (60 l) to allow them to acclimatize for approximately one week. They were fed daily with alga *Isochrysis galbana*, equal to 2–3% of the wet biomass (Sarà et al., 2013). Subsequently, the organisms were randomly divided into three equal-sized groups (n=120;mean size (±SD) 4.71± 0.45 cm), individually labeled and randomly placed into six 300-l tanks (two tanks per each treatment maintained under 12:12 h Light–Dark regime), where they were fed ad libitum three times per day. Once acclimated, the experiments were started and the specimens were constantly fed with three diets prepared to simulate three different
trophic scenarios:

1. A phytoplankton diet represented by a culture of microalgae, *I. galbana*, mimicking the natural condition of the pelagic-oceanic environment(Sarà et al., 2011) was used. To this purpose, a pure culture of *I. galbana* was prepared by inoculating a small aliquot of microalgae in beakers containing one 1 l of pre-filtered and sterilized seawater and placed in a thermal room (constant temperature of 18 °C), where the culture was maintained under a suitable period on a light table (daylight UVA heat lamps, Model Repti Zoo, 75W; Italy). Growth of the *I. galbana* strain was ensured by adding aliquots (2 ml l^{-1}) of Walne medium and vitamins (i.e., B1 and B12; 0.2 ml l^{-1}) (FAO, 1996)

2. A solution of minced pellet food and seawater was used, mimicking the incoming eutrophic downstream waters from a farm (Sarà et al., 2012, 2009). In this regard, pellet aliquots of 6.5 g (such as those used within fish farms) were ground and dried in an oven at 60 °C for

24 h. After this period, the food was placed in a beaker glass with filtered seawater and the solution was filtered through filters with a mesh size equal to 40 μm.

3. A solution of refractory organic matter with a high C/N ratio (N15–20; Vizzini et al., 2003) was obtained using fresh leaves of *Posidonia oceanica*, properly dried and minced (mimicking oligotrophic waters subsidized with highly refractory organic matter for secondary consumers). In doing so, fresh leaves of *P. oceanica* were cleaned off epiphytes and later dried in an oven at 60 °C for 24 h. Dry leaves were ground and reduced to a powder and passed through a 100 μm mesh. The solution was prepared by diluting the mince of *P. oceanica* in 1 l of seawater previously filtered (Whatman GF/F, 0.45 μm).

4.2.2 Routine laboratory measurements

According to the recent literature (e.g., Fields et al., 2012), in order to make negligible the effect of acclimation to each food treatment on *M. galloprovincialis* responses, we conditioned mussels to treatments for 4 weeks prior the physiological measurements. For each aquarium, 40 animals were weighed (0.001 g; Sartorius Inc.) every two weeks, measured for the umbonal shell length (nearest 1 mm; Vernier caliper), and the mussels growth performances were monitored for the following two months. Before measuring the wet mass (total wet weight comprising shells) they were externally dried with paper tissue and then placed on paper tissue to air dry for about 10 min in order to minimize the contribution of water content within the mantle cavity. The growth rates for each diet have been calculated taking into account the differences in both shell lengths and wet mass measured on individual organisms at the beginning and at the end of the two months period. Once the exposure period was completed, experiments to estimate the feeding behavior started and they consisted of exposing independent animals to increasing concentrations of food (from very low to very high; see below for details), to seek that value of ingestion rate corresponding to the saturation threshold for every type of diet. We carried out feeding rate experiments based on the classical estimates of clearance rate (Sarà et al., 2013) to obtain the corresponding ingestion rate (IR=CR x food) for every diet type. Solutions at different concentrations of food were prepared as follows: starting from the same initial solution (mentioned earlier in the text under the diet preparation section), seven different dilutions (from 0.1ml l^{-1} to 20 ml l^{-1})were used to estimate the feeding response of *M. galloprovincialis*. In doing so, we obtained concentrations ranging between 6.40 and 10.19 mg l^{-1} for phytoplankton, between 2.68 and 20.50 mg l^{-1} for seagrass-based diet, and between 5.76 and 12.75 mg l^{-1} when pellets were provided (see Supplementary file, Table S1). Ingestion rates (IR) and absorption efficiencies (AE) were measured in individual mussels (n=10).

The IR (mg h^{-1})was calculated through estimates of clearance rates (l h^{-1}) measured by placing single
specimens into a 1 l beaker, while a beaker was intentionally left without animals and used as the control (Sarà et al., 2013). Solutions were constantly mixed in each beaker during the experimental phases with a magnetic stirrer, by placing them onto a stirrer plates. For each experiment the organisms were left for about 5 min, to acclimatize, before adding food, and the decrease in food concentrations was monitored over 2 h by means of an electronic particle counter (Beckman Coulter Counter, Z2), fitted with a 100 μm aperture tube and set to count particles ranging between 2 and 6.5 μm. In order to estimate the hourly maximum ingestion rate, aliquots of 20 ml from every beaker were sub-sampled at 30 minute intervals. The individual maximum clearance rate was then calculated following the equation:

$$CR(l\, h^{-1}) =(Vol) *(\ln C1 - \ln C2)/\text{time interval}$$

where Vol is the volume of the beaker used in the experiments and C1 and C2 are the initial and subsequent concentrations minus the decline in the cell concentrations in the control tank in each time interval (Ezgeta-Balic et al., 2011; Sarà et al., 2013; Widdows and Staff, 2006). Accordingly, the decreasing cell concentrations were converted in terms of mass concentration and the resulting maximum ingestion rate was then calculated as the maximum density of food (particulate organic matter, mg l^{-1} POM) ingested per hour.

Following the measurement of CR, the mussels were placed into new 1 l beakers containing filtered seawater and left undisturbed overnight (12 h), after which the faeces produced by each individual as separately collected and filtered onto pre-weighed glass-fiber filters (Whatman GF/F). After filtration, filter papers were accurately washed by alternating solutions of 0.5 Mm ammonium formate and distilled water for three times. The absorption efficiency (AE) for each treatment was then calculated using the Conover ratio (Conover, 1966), which took into account the ratio of the amount of organic matter in the faeces and that in the food. Accordingly the AE was estimated using the following formula:

$$AE=(F-E/[(1-E)F]$$

where F is the ratio between the dry weight (DW) and ash-free dry weight of food (AFDW), and E is the ratio between the dry weight (DW) and ash-free dry weight of the faeces (AFDW). When estimating, the filters containing the food and faeces were dried in an oven at 90 °C, re-weighed, and then placed into a furnace at 450 °C, for 4 h, after which the filters were weighed again (Ezgeta-Balic et al., 2011, Sarà et al., 2013).

4.2.3 Estimating the functional response and modeling of the effects on an individual's ultimate fitness

Ecological theory offers strong principles which, if used in a reliable modeling framework, are able to increase our understanding of how to predict growth performances of bivalves under varying conditions of food and temperature. Particularly the Scaled Functional Response (SFR; Holling, 1959), has been recently called as a prominent mechanism to describe how the consumption rate of a predator changes with prey density. SFR involves information about the trade-off between two important behavioral components, i.e. searching and handling, together being used as an estimate of the ability to get food from the environment (Lang et al., 2012). In some species, handling time may prevent or limit subsequent food acquisition; however in bivalves, both processes occur simultaneously (Saraiva et al., 2011) and in the particular case of filter feeder modeling, several studies have highlighted the need to investigate the magnitude of the half saturation coefficient - which correspond to the searching: handling ratio- to improve our ability to predict where to place shellfish cultures (e.g., Ren et al., 2012). Here, we estimated the differences in the functional responses Here, we estimated the differences in the functional responses (f) of *M. galloprovincialis* as a proxy of different intake strategies, under natural environmental conditions. Specifically, the f values for each diet were estimated according to Sarà et al. (2014), according to which f = x / (Xk+x) where x was the food density (mg POM l^{-1}),while the parameter Xk corresponded to the concentration where the value of the ingestion rate was equal to half of the maximum. Such information to gether with the manner in which *M. galloprovincialis* managed the energy from different types of food (i.e., IR and AE), have been used in combination with the bioenergetic parameters of the species (http:// www.bio.vu.nl/thb/deb/deblab/add_my_pet), based on the recent Dynamic Energy Budgetmodeling (DEB, Kooijman, 2010), to perform simulations aimed to investigate the potential variations in growth performance and the individual ultimate fitness. Certainly, being based on individual bioenergetics, the DEB theory provided a general framework that could be utilized to describe physiological mechanisms by which temperature and food availability combine to drive growth and reproductive performances in organisms (Monaco et al., 2014). The present DEB model allowed us to quantify some of the most important response variables, such as, Maximal Habitat Individual Size (MHIS; cm), Maturation Time (MT; day), which refers to the time required to reach the minimal size threshold for sexual maturity and first spawning, the Number of Reproductive Events per life span (RE; #), and the Total Reproductive Output (TRO, #),which is the total number of eggs per life span (Montalto et al., 2015). To incorporate the effects of different food sources, we performed a sensitivity analysis, to predict the dynamics of the mussel's energetic fluxes by combining the experimentally-derived feeding functional responses with six different levels of body temperatures (from 13 °C to 18 °C, chosen as representatives of the average annual BT experienced by this species throughout its entire biogeographic distributional range; Marshall and Gofas, 2015) constantly maintained throughout the life span. For the sake of simplicity, models were run by putting in a fixed food density (i.e., 10mg l−1 h−1) such that the f value was always above a threshold of 0.5 for each diet, and the entire simulated period was cut off when the individual growth reached commercial size (here fixed to 5 cm according to the Food and Agriculture Organization (FAO) statistics).

4.3 Statistical analysis

A two-way analysis of variance (ANOVA; Underwood, 1997) was carried out to test the differences in the feeding responses and in the assimilation efficiencies of *M. galloprovincialis* fed with diets varying both qualitatively and quantitatively. Both factors (DIET, three levels and CONCENTRATION, seven levels) were chosen as fixed factors and ten replicates were used for each experimental session. The assumption of the homogeneity of variance was tested a priori by mean of the Cochran's test. When significant differences were observed, the appropriate means were compared using the Student-Newman-Keuls (SNK) tests (Underwood, 1997). ANOVA was carried out by using the GMAV software (version 5.0). Also, to establish whether the relationship between prey density and the number of prey eaten is best described by type I, a type II or a type III response, a phenomenological approach focusing on the overall shape of the response curve was used. Specifically, we compared the relationship between food density and the number of cells eaten for each diet by means of an integrated package (frair) for functional response analysis in R (v. 3.0.3), as recently done in Paterson et al. (2015). Lastly, the differences in the shell and mass growth of *M. galloprovincialis* exposed to different food sources at the end of the two-month period, were instead tested by mean of a PERMANOVA due to the ease of use, with unbalanced design and to avoid the usual normality assumptions (Perkol-Finkel et al., 2012). PERMANOVA was carried out by using the PRIMER software (version 6.0).

4.4 Results

The IR values ranged between 3.94 ± 0.30 mg l^{-1} h^{-1} with pellet diet and 4.85 ± 0.34 mg l^{-1} h^{-1} when the mussels were fed with *P. oceanica* detritus, and the ANOVA showed significant differences between the IR when response with the pellet was compared with the IR estimated in the presence of seagrass and algae diets ($p < 0.05$ and $p < 0.01$ for *P. oceanica* and *I. galbana*, respectively; Fig. 1 and Table 1).

AE differed significantly ($p < 0.01$) and contrary to IR, the lower value was estimated for the *P. oceanica* diet (AE = 0.31 ± 0.02) compared to the other two diets (mean AE = 0.51 ± 0.01) (Fig. 2 and Table 1). Overall the IR not changed significantly when different phytoplankton concentrations were provided, while significant differences resulted among IR of organisms fed with seagrass and pellet; indeed as showed by the pairwise comparison, *Mytilus'* responses differed respectively at lowest and intermediate levels of the seagrass diet and from the intermediate to the highest concentrations of pelleted food provided in this study (Table S2). No evident patterns resulted by comparing AE at different concentrations of pellet and phytoplankton while they differed significantly when elevated concentrations of seagrass-base diets were available (Table S2). The phenomenological analysis revealed significant evidences of a Holling type II curve in the presence of algae ($p < 0.05$) and seagrass ($p < 0.001$), while mussel's SFR met the Holling type I curve with the pellet (p b 0.001). The saturation coefficient was about 6 mg l^{-1} with algae and seagrass and 8.04 mg l^{-1} with pellet (Table 2). Our analysis did not show significant differences (PERMANOVA, p N 0.05; see Supplementary file Table S3) in the growth rate of individuals fed with the three different diets. Indeed, within the experimental period Mytilus increased on average (±S. E.) it’s size of 1.02 ± 0.11 mm, 1.06 ± 0.09 mm, and 1.41 ± 0.28 mm when fed with *I. galbana*, *P. oceanica*, and pellet, respectively. On the other hand, the estimated increase in weights was similar for organisms fed with *I. galbana* and pellet, being equal to 1.21 ± 0.17 g, and 0.91 ± 0.17 g while it resulted significantly lower for organisms fed with seagrass (i.e., 0.47 ± 0.07 g). Finally, DEB allowed us to get information of rebounds of the three different diets and the SFR values. Indeed, as shown in Figs. 3–5, for the entire range of simulated body temperatures, the amount of time needed to reach the commercial sizes varied between about four years in the presence of both phytoplankton and pellet and up to eight years in case of seagrass. Also, changes in the reproductive features were detected within the simulated period; specifically under all the trophic conditions (food quality) tested, the results showed that when the BT increased, *M. galloprovincialis* was able to invest more energy (about 25%) to reach its maturation size and to reproduce (Table 2). Such a pattern was observed when the temperature range fell between 13 °C and 17 °C, while a failure was evident under the maximum simulated body temperature.

Table 1. ANOVA carried out on IR; and AE under different diet regimes and at varying food concentrations; Sqrt(X + 1)= data transformed; ns= not significant.

Source	CR			AE	
	DF	MS	P	MS	P
DIET	2	0.766	**	0.9914	***
CONC	6	2.6371	***	0.212	***
DIET × CONC	12	0.7327	***	0.0483	***
RES	189	0.1535		0.0152	ns
TOT	209				
Cochran's test			Sqrt(X + 1)		ns

*P < 0.05.
**P < 0.01.
*** P < 0.001.

Table 2. Table reporting the experimental results for each diet and used as input of modelling exercise and list of the outputs obtained through the DEB simulations carried out at varying organismal body temperatures. IR, ingestion rate; AE, assimilation efficiency; a, saturation constant; f, simulated functional responses at constant food availability (density). For each simulated body temperature (BT) we modelled i) the days to reach commercial size (Comm. Time), ii) size of first reproduction (MT) and iii) the reproductive ability in terms of numbers of eggs (TRO) produced and of number of spawning events (RE)

Diet	**IR**	**AE**	**f**	**density**	***Xk***
I. galbana	8.07	0.53	0.63	10	5.75
P. oceanica	7.88	0.31	0.64	10	5.57
Pellet	8.48	0.49	0.55	10	8.04
BT	**Diet**	**Comm. Time**	**MT**	**TRO**	**RE**
13	***I. galbana***	1496	947	311,623	4
	P. oceanica	3382	1,892	723,691	11
	Pellet	1811	1,121	358,101	5
14	***I. galbana***	1427	903	319,423	5
	P. oceanica	3225	1,804	728,320	14
	Pellet	1727	1,069	444,886	7
15	***I. galbana***	1362	862	331,624	6
	P. oceanica	3079	1,722	748,040	17
	Pellet	1648	1,020	416,389	8
16	***I. galbana***	1301	824	346,037	7
	P. oceanica	2942	1,646	709,547	19
	Pellet	1575	975	403,435	9
17	***I. galbana***	1245	788	361,832	8
	P. oceanica	2814	1,574	689,576	21
	Pellet	1507	933	398,806	10
18	***I. galbana***	1195	755	0	0
	P. oceanica	2695	1,508	0	0
	Pellet	1443	893	0	0

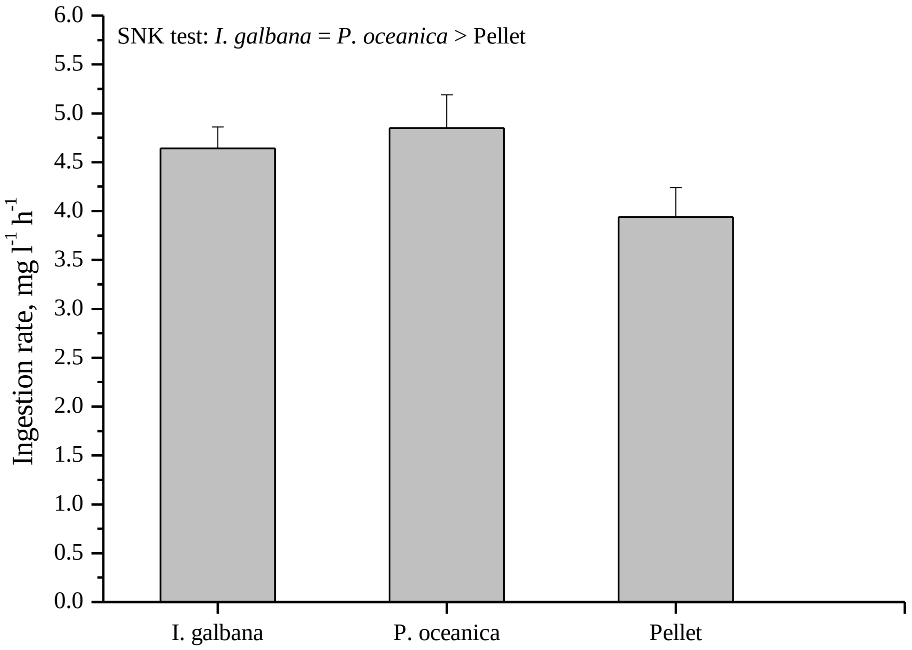

Fig. 1. Ingestion rates (IR, mg l^{-1} h^{-1}) of *M. galloprovincialis* experimentally derived under three different trophic conditions.

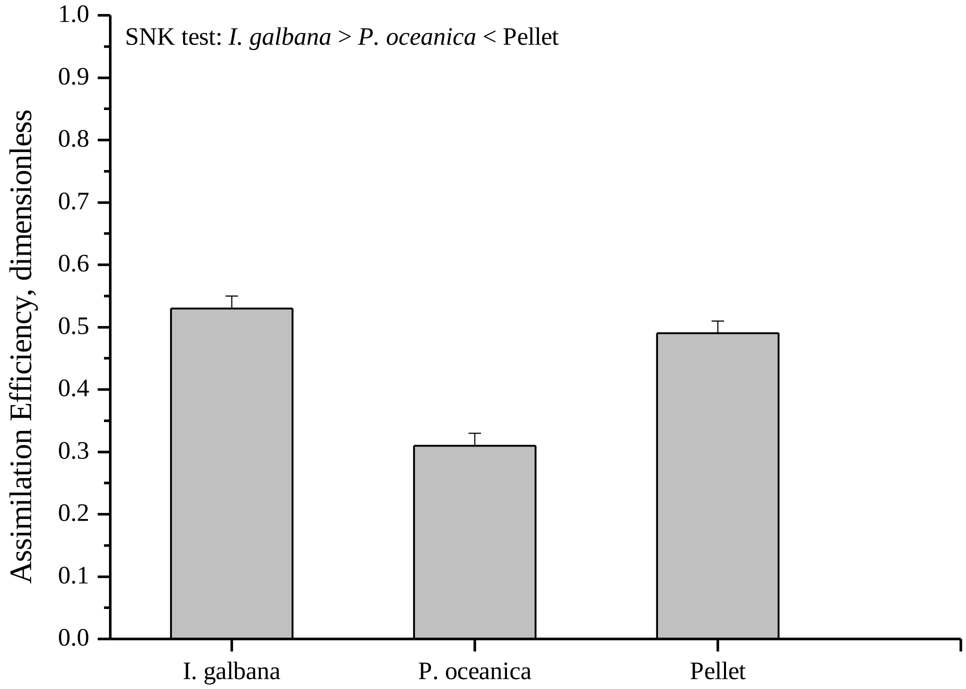

Fig. 2. Assimilation efficiencies (AE) of *M. galloprovincialis* experimentally derived under three different trophic conditions.

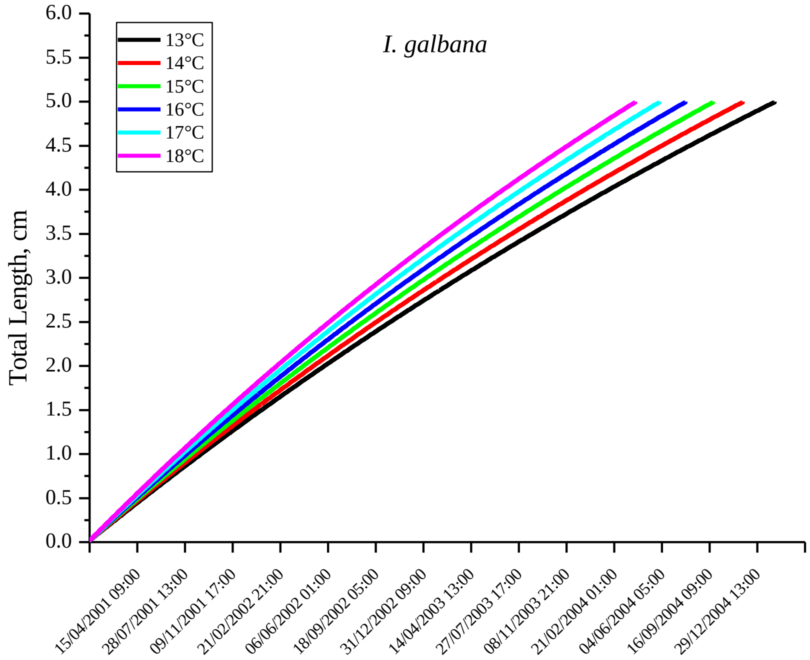

Fig. 3. Simulated growth performances resulting from intake energetic strategies adopted by individuals fed exclusively with *I. galbana.*

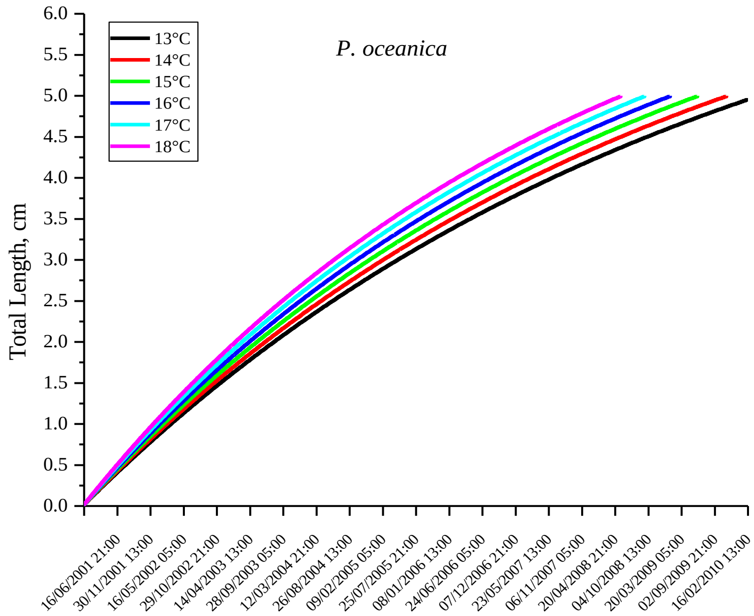

Fig. 4. Simulated growth performances resulting from intake energetic strategies adopted by individuals fed exclusively with *P. oceanica.*

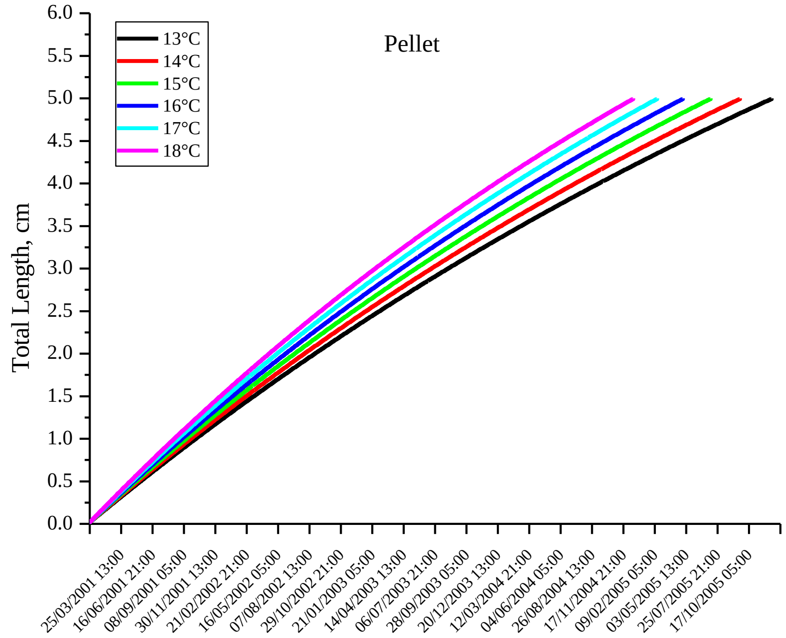

Fig. 5. Simulated growth performances resulting from intake energetic strategies adopted by individuals fed exclusively with pellets.

4.5 Discussion and conclusions

According to previous studies, our results showed that *M. galloprovincialis* might be able to ingest food—in the form of particulate matter—of different origins (Manganaro et al., 2009; Sarà, 2006), although, as shown here, the response could be different depending on the food source provided. Indeed, based on the significantly higher ingestion rates, our results suggested that the mono-algal and the seagrass diets were the best energy providers for the mussel's intake compared to pelleted food. The increase in ingestion rates on increasing food densities could be partly explained by the differences in dimension of the microalgae species (4–7 μm), which perfectly match the size range at which they realise maximum filtration efficiency of microalgal component (Dame, 1993) and confirmed a non-selective filter-feeding of this species when fed with algae (e.g., Vahl, 1972). Also, when analysed in terms of optimal foraging (*sensu* Lehman, 1976) mussels would benefit from the constancy of higher IR to cope with the low quality diet. After all, the existence of an upper limit to ingestion in bivalve molluscs has been generally acknowledged (Bayne and Newell, 1983; Navarro et al., 1992) and demonstrated, either in bivalves fed with algae (Sarà et al., 2013; Saraiva et al., 2011) or in those fed with vascular plants (Arambalza et al., 2010; Charles, 1993).

However, similar to what was observed by several authors (Martìnez-Fernàndez et al., 2004; Saraiva et al., 2012), such as having higher ingestion rates, it does not imply that all energy ingested would be also digested. Indeed as showed in Fig. 2, organisms fed with P. oceanica increased their ingestion rates and this resulted in significant lower absorption efficiency when compared with the phytoplankton diet. Such a fact could be a probable consequence of constraining the capacity of the digestive system and the residence time of food (Navarro et al., 1994). As showed for other bivalves, by assuming the same probability to process different types of food, differences in the overall retention relate not to gut passage times but to the processing and release strategies of the food material (Saraiva, 2014). Indeed, it is likely that the low digestibility (low nitrogen content) of the phanerogamic organic matter and the high content of phenolic compounds could be the two primary factors that affected the organismal functioning and reduced the efficiency of the digestive enzymes (Charles et al., 1996). Also, our results showed that when supplied with pelleted food, *M. galloprovincialis* was able to modify feeding strategies by meeting the type I functional response, which is conventionally thought to be the more frequent model adopted among filter feeders (Bontes et al., 2007). According to the categorization provided by Holling (1966) such response is described by a region of linear increase up to a certain threshold of food abundance which is determined by the incipient limiting level; therefore compared to the type II response, the expression of the type I response gave the advantage of increasing the consumption rates at intermediate food availability and not showing a saturation plateau of digestive processes.

However, a recent review revealed that the majority of filter feeders did not show the type I functional response and the reasons were likely to be found in the conditions that mussels must fulfill in order to show this type of response (Jeschke et al., 2004). Anyway, our results suggested that all the conditions have been satisfied in organisms fed with fish-farmfood pellets. Certainly, the digestibility of dietary components being improved (Khater et al., 2014), the presence of pellets would guarantee both the handling and satiation conditions (*sensu* Jeschke et al., 2004), while the dimension of the detrital particles was homogenized during their preparation, so that the sizes were such that they allowed organisms to meet the digestion condition. Results of the feeding strategies also seemed to be mirrored by the biometric data collected during the growth experiments and by the modeling exercise carried out within this study. Our feeding trials showed significant increases in biomass of organisms fed with pellets and monoalgal diet compared to those fed seagrass diets and although not statistically different better growth performances in presence of pellets, confirming the nature of bivalves to be generalists (e.g., Dame, 1996; Lehane and Davenport, 2006). The DEB models allowed to demonstrate that growth trajectories could vary among diets and under our simulated body temperature (BT) conditions. Comparisons based on different food sources revealed that regardless of the simulated BT, the microalgae and pellet diets generated the best growth performances, compared to those that resulted from models simulating *P. oceanica* as a food proxy. Indeed, as shown in Table 2, in an hypothetical culture system placed close to seagrass meadows, the amount of time estimated for an individual to reach the commercial size was more than twice than that spent in areas where the main food proxy was represented by both phytoplankton and pellets (as in IMTA). Also, our results were consistent with other studies where mussel growth was related to the organic content of particulate matter rather than the phytoplankton abundance or chlorophyll-a. Results from those studies indicated that chlorophyll-a was part of bivalves food in most coastal are as worldwide, although they were usually able to rely on detritus and particulate matter, such as, organic waste from uneaten pelleted feed, fish, and bivalve faeces in fish-farming impacted areas (Mazzola and Sarà, 2001; Saraiva et al., 2011). Apart from the sensitivity of the individual's growth and environment interactions to global changes, a vein of sustainability of IMTA is crucial to understand how environmental conditions combine to drive the ultimate fitness of the cultivated species. In this context, the DEB model helps in estimating the fecundity potential of cultivated organisms. With few exceptions (i.e., simulated BT of 18 °C), the results showed that *M. galloprovincialis* was able to allocate energy in a reproductive buffer, which was converted to eggs at the time of reproduction, under every simulated body temperature and every food source. The presence of gametes, as part of the somatic weight, and the magnitude of each spawning event, could have a significant impact on the success of aquaculture practices being responsible for both the price of the harvested shellfish as well as the negative impacts associated with gamete release (*sensu* Sarà et al., 2007). Our results suggested that the energetic balance of organisms maintained under feeding regimes based on microalgae and pellets showed lower reproductive outputs (MT, TRO, and RE) than those obtained with simulations carried out with feeding performances of mussels relying on a diet exclusively of seagrass detritus, probably as a result of a longer simulated time period to reach the size of 5 cm, which was two-fold in case of growth trajectories performed in the presence of one among the two other diets. Indeed, with the exception of feeding performances, the same model and parameter set were used to predict mussel growth under different food and temperature scenarios, underlying the idea that a generic mussel model should represent an average (growth) performance of mussels under given environmental conditions. However, further research on the optimal feeding and digestive behaviour obtained for mussels fed with mixed diets as proxies of natural environments, might provide a better coverage for the changes in an individual's metabolic requirement during growth, which depending on the feeding capacity of mussels might vary between species and life stages, larvae to adults (Fernandez-Reiriz et al., 2011). The need for adopting an ecosystem approach to site selection and framing the allocation of areas dedicated to aquaculture activities within the broader context of the Marine Spatial Planning requires the use of modeling tools as a support for decision-making in aquaculture. The integrated model described in this study can provide a useful means to design responsible aquaculture production systems for tomorrow. The mechanistic nature of such models combined with broad applications to other species, allows the consideration of the effects of different environmental drivers such as water temperature and food availability inexplicitly calculating the metabolism of the cultivated species, increasing our ability to prevent impacts and to assist with site selection, moving toward the sustainability of integrated multitrophic aquaculture (Diana et al., 2013; Klinger and Naylor, 2012).

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4.7 Tables supplementary material

Table S1. List of food concentrations (CONC, mg l^{-1}) used to perform feeding experiments under the three different treatments (DIET).

#	Diet	Conc	Diet	Conc	Diet	Conc
1	*I. galbana*	6.40	Pellet	5.76	*P. oceanica*	2.68
2	*I. galbana*	6.83	Pellet	6.06	*P. oceanica*	7.40
3	*I. galbana*	6.85	Pellet	6.76	*P. oceanica*	9.20
4	*I. galbana*	7.73	Pellet	7.92	*P. oceanica*	10.50
5	*I. galbana*	8.25	Pellet	8.26	*P. oceanica*	10.90
6	*I. galbana*	8.87	Pellet	8.75	*P. oceanica*	14.50
7	*I. galbana*	10.19	Pellet	12.75	*P. oceanica*	20.5

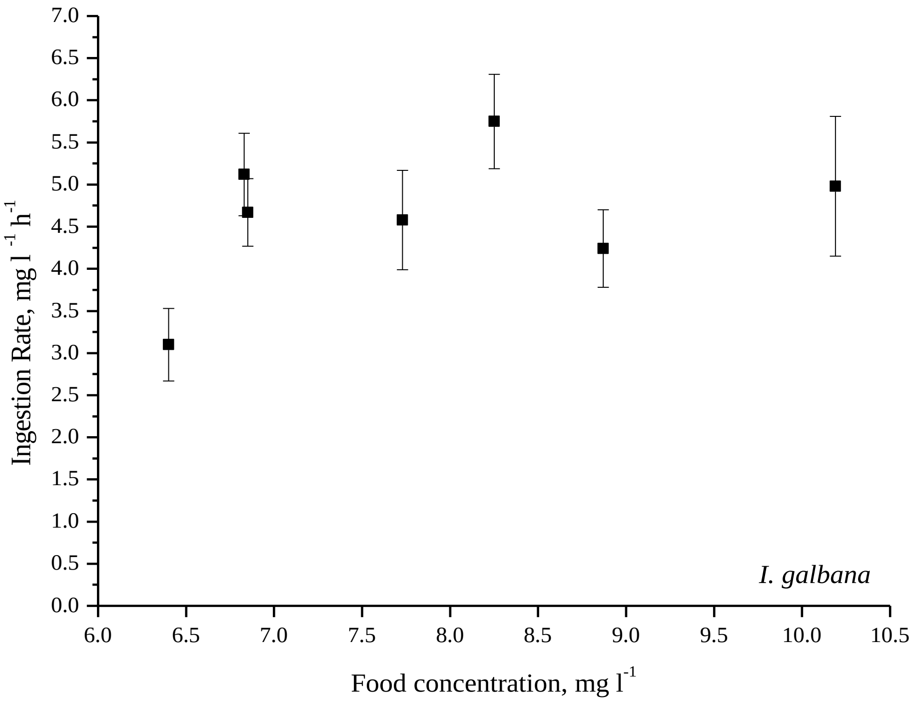
Table S2. Post-hoc comparison SNK outcome to verify differences in Ingestion rates (IR) and Assimilation Efficiencies (AE) as a function of varying food densities; numbers refer to concentrations for each diet listed in Table S1; *= P ≤ 0.05; **= P ≤ 0.01; ***= P ≤ 0.001; ns = no significant difference (P > 0.05).

I. galbana															
IR	1	2	3	4	5	6	7	AE	1	2	3	4	5	6	7
1	-							1	-						
2	ns	-						2	ns	-					
3	ns	ns	-					3	ns	ns	-				
4	ns	ns	ns	-				4	**	**	**	-			
5	*	ns	ns	ns	-			5	ns	ns	ns	**	-		
6	ns	ns	ns	ns	ns	-		6	ns	ns	ns	**	ns	-	
7	ns	ns	ns	ns	ns	ns	-	7	**	*	ns	ns	ns	*	-
P. oceanica															
IR	1	2	3	4	5	6	7	AE	1	2	3	4	5	6	7
1	-							1	-						
2	**	-						2	ns	-					
3	**	ns	-					3	ns	ns	-				
4	**	ns	ns	-				4	ns	ns	ns	-			
5	**	**	**	**	-			5	ns	ns	ns	ns	-		
6	**	ns	ns	ns	**	-		6	ns	ns	ns	ns	ns	-	
7	**	ns	ns	ns	**	ns	-	7	**	**	**	**	**	**	-
Pellet															
IR	1	2	3	4	5	6	7	AE	1	2	3	4	5	6	7
1	-							1	-						
2	ns	-						2	*	-					
3	ns	ns	-					3	**	ns	-				
4	ns	ns	ns	-				4	**	ns	ns	-			
5	**	*	**	ns	-			5	ns	ns	ns	**	-		
6	**	*	**	ns	ns	-		6	ns	ns	ns	*	ns	-	
7	**	**	**	**	**	**	-	7	*	ns	ns	ns	ns	ns	-

Table S3. PERMANOVA table of results and group analysis for shells (a) and mass (b) growth rates of *M. galloprovincialis* fed with different diets (* $p<0.05$; ** $p<0.01$; *** $p<0.001$; ns not significant); Phyto = *I. galbana*, Pel = pellet, Pos = *P. oceanica*

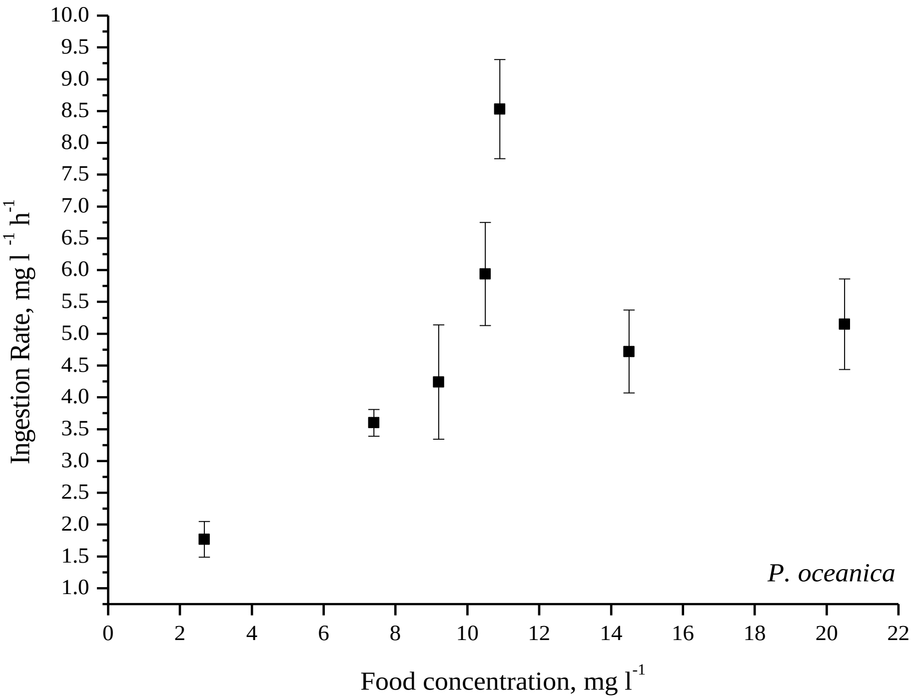
a								
Source	df	MS	Pseudo-F	P(perm)	Groups	t	P(perm)	Unique perms
Di	2	0.39331	1.816	ns	Phyto, Pel	1.580	ns	426
Res	33	0.21662			Phyto, Pos	0.289	ns	367
Total	35				Pel, Pos	1.540	ns	401
b								
Source	df	MS	Pseudo-F	P(perm)	Groups	t	P(perm)	Unique perms
Di	2	2.2314	10.620	**	Phyto, Pel	1.138	ns	982
Res	33	0.21012			Phyto, Pos	4.563	***	984
Total	35				Pel, Pos	3.106	**	978

Figures



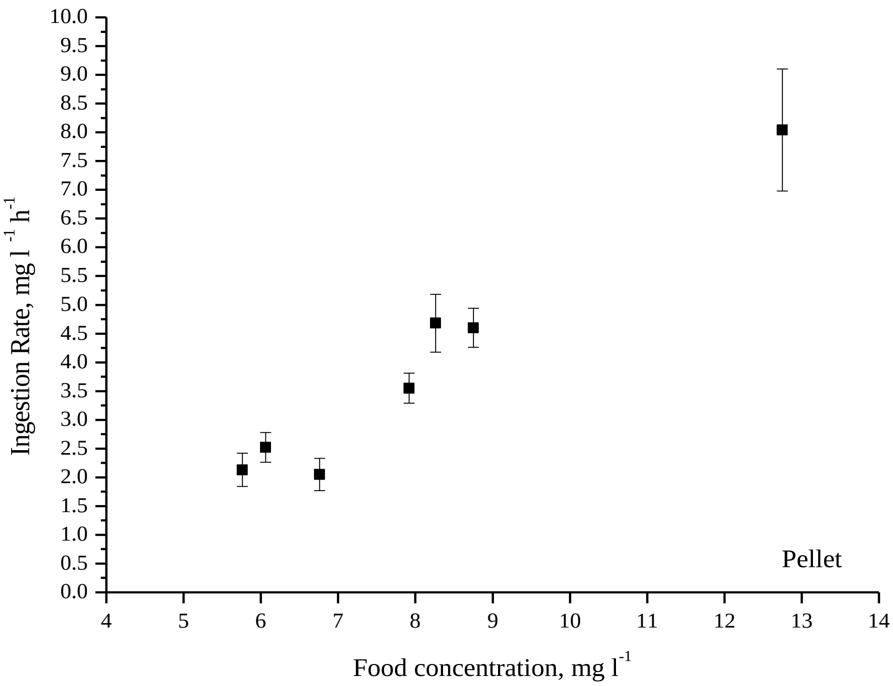
Ingestion Rate, mg l -1 h-1
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
10.5
Food concentration, mg l-1
I. galbana

a)



Ingestion Rate, mg l -1 h-1
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
2
4
6
8
10
12
14
16
18
20
22
Food concentration, mg l-1
P. oceanica

b)



c)

Figure S1. Ingestion rates of *M. galloprovincialis* estimated at varying food concentrations; a) phytoplankton, *I. galbana*; b) seagrass, *P. oceanica*; c) pellet

Chapter 5

5.1 General discussion and conclusions

The general objective of this research was to determine the cumulative pressures caused both by the increase in temperature and by human activities, so as to provide useful information for the destination of the GES and for a sustainable planning of the maritime space, using an ecosystem-like approach.

The results of this study have been useful to understand and broaden the current basic knowledge as well as to clarify the different approaches in relation to the comprehensive and complex marine strategy framework directive (MSFD), and specifically the study of the D2 (non-indigenous species) and D3. (Commercial species) descriptors.

The results of **chapter 1** have defined a complete picture of the marine strategy, but above all have revealed important gaps on the determination of a correct analysis of the state of the environment through the use of the ecosystem approach. Specifically, as claimed by De Jonge et al. (2012) it is necessary to consider the evaluation of ecological, economic and social aspects of fundamental importance. This is to allow the sustainable use of goods and services, while maintaining a good ecological status and preventing marine deterioration (Borja and Elliott 2013).

Specifically, the main results that are highlighted in this study are:

1) Involve interested parties.
2) Aggregation of multiple indicators, descriptors and spatial scales effectively.

Therefore, I recommend consolidating scientific knowledge, in particular of ecological terminologies and concepts, in order to reach and maintain the “good environment status”.

Chapter 2 underlined the importance of understanding the complex interactions between the increase in temperatures and the distribution of non-indigenous species (NIS) in the entire Mediterranean basin, where complex and fundamental alterations that are still on going, have already affected the structure and functioning of the sea, and the consequent supply of goods and services (Galil et al., 2016).

The continuous arrival of NIS and their increasing presence and stabilization in the Mediterranean basin reveal a profound and continuous alteration in the distribution models of the species, which seem to have increased significantly in recent years (Galil et al., 2014).

Therefore, two general conclusions arise from the results of this study:

1) a strong relationship between the number of occurrence and the temperature difference.

2) a shift of alien species in the Mediterranean.

In fact, there is a growing need to define preventive measures to control biological invasions and thus mitigate the related impacts on biodiversity, ecosystem services and human activities. (Katsanevakis et al., 2013).

The flow regulation of exotic species is particularly relevant for future actions concerning the main maritime strategic objectives, such as the Marine Strategy Framework Directive (MSFD) (2008/56 / EC). This directive specifically considers the introduction of alien marine species as a serious threat to the biodiversity and the health of European ecosystems, and calls for Member States to include NIS in the definition of "Good Environmental Status" (GES), setting environmental objectives to reach it. (Katsanevakis et al., 2013).

The results described in **chapter 3** have shown the effects that climate change can inflict on shellfish aquaculture, which extends along the Mediterranean coast. Adopting a study along the Italian peninsula, we studied the effects of the temperature increase on the model species, *Mytilus galloprovincialis*, measuring the characteristics of the fragility of the valve (thickness) and the condition index. Furthermore, our experiment in mesocosmo corroborated the relationship between temperature and thickness, and showed that temperature could be the main factor behind the relationship, as demonstrated by the significant difference in the intercepts between thickness and load.

Specifically, the main results of this study may have the following implications:

1) Considering the proposal to take the thickness (and fragility) of shells in molluscs as a new reliable indicator for the Marine Strategy Framework Directive (MSFD, 2008/56 / EC, European Commission, 2008).
2) Offering an important basis to help the next generation of managers and stakeholders in assessing the reliability and feasibility of shellfish aquaculture economic activities.

Furthermore, this study could provide an opportunity to raise awareness among public and scientific communities (*sensu* Mangano et al., 2015) to inform about the importance of building common actions and strategies to mitigate the impact of climate change on different aspects of production.

In the last chapter of the thesis (**chapter 4**) it was shown the possibility of combining experimental procedures with new bioenergetics models based on mechanistic functional traits in order to effectively predict the life history traits of the species cultivated in a context of integrated multi-trophic aquaculture (IMTA).

By choosing the *Mytilus galloprovincialis* mussel as a model, the results of this study have defined:

1) The functional response of *Mytilus galloprovincialis.*

2) Parameters related to food intake strategies.

The integrated model described in this study provides useful means to design and pre-assess production systems for aquaculture. Hence, the mechanistic nature of these models combined with extensive applications to other species, allows to consider the effects of various environmental factors such as water temperature and food availability by not explicitly calculating the metabolism of the cultivated species, increasing our ability to prevent site impacts and selection, towards the sustainability of integrated multi-trophic aquaculture.

The series of studies presented here underlines the importance of identifying and selecting ecosystem-based techniques and approaches based on a macro-ecological vision to reduce the effect of global warming, the impact of human activities and their consequences on the marine environment. In fact, over the last few decades we have witnessed rapid ecological changes that have occurred in the world's oceans, affecting above all the capacity for resilience and resistance of ecosystems and the vulnerability of the communities living there. These interactions can generate effects that impact on the functioning of the oceanic world and consequently on goods and services, such as fishing and aquaculture production. In fact, these activities need to be managed and administered responsibly to avoid competition for space, which causes numerous conflicts and increases the human impact on ecosystems.

Part of the present findings wanted to respond to a call from the European community, in being able to build a common road to all European countries, on the determination of the GES. Proposing the use of a macro-ecological approach based on large and small scale studies, to mitigate the impact of anthropogenic climate change.

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