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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 recognised 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.

## 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 recognised 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 Ibañez, 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

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**Fig. 1.** Sampling sites along the Italian Peninsula. The red star bullet indicates the locality (Messina) where the mussels 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.)

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 tem perature – 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 of local 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.

#### 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.

### 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 60-70 mm 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 were dissected; 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



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

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).

# 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 µg1<sup>-1</sup> kept over time.

# 2.3. 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



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

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. 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 ( ± 0.065) \* Latitude; Temperature = 29.43 $(\pm 2.78) - 0.273$ 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  $(CHL-a = 18.94 \quad (\pm 1.57) - 0.97$  $(\pm 0.09)$  \* 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 ( $\sim 3.5 \,\mu g \, l^{-1}$ ) 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.

### 4. 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 hypercaphia had a negative



Fig. 4. a. Relationship between thickness and latitude; b. Relationship thickness and temperature; c. Relationship between fragility (breaking load) and thickness.

effect on the growth of intertidal mussels, but only when there was a cooccurrence 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



Fig. 5. a. Relationship between condition index and temperature; b. Relationship between condition index and trophic condition

Fig. 6. Relationship between thickness and temperature of

the tanks.



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 mus sels. 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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### Author's contribution

MM performed the experiment and drafted the first experimental report; MCM and GS wrote the final version of the ms; GM, SM, AM and GS funded the Mesocosm experiment; GS funded the ECOTRIP latitudinal survey; all authors revised the final version of the ms. and approved it.

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