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MESTRADO EM CIÊNCIAS DO MAR - RECURSOS MARINHOS
ESPECIALIZAÇÃO EM BIOLOGIA E ECOLOGIA MARINHA

Too warm for the sea urchin? The effect of temperature on the metabolism and fitness of the European purple sea urchin, *Paracentrotus lividus*

Ana Filipa Moreno Contente Costa

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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Too warm for the sea urchin? The effect of temperature on the metabolism and fitness of the European purple sea urchin, *Paracentrotus lividus*

Dissertação de Candidatura ao grau de Mestre em Ciências do Mar – Recursos Marinhos, Especialidade Biologia e Ecologia Marinha submetida ao Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto

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I would like to dedicate this work,

to my family and friends who were deprived of me for a time.

Thank you for your understanding and for helping me to achieve such special goal.

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Abstract

The European purple sea urchin *Paracentrotus lividus* (Lamarck, 1816) is a very abundant species in the tidal rocky shores of Portugal. Due to the high economic value of its roe, regular harvests of sea urchins for food industry have abruptly increased in the North of Portugal in the recent years, but with an unappropriated regulation it may lead to a depletion of the natural populations. To define a sustainable management plan requires prior basic knowledge on the population response to environmental conditions. This is particularly important in the context of climate change, since rising temperatures may affect the metabolism and fitness in ectothermic organisms.

In this study, we investigated the physiological performance of the European sea urchin in terms of mortality, growth, feed intake and respiration rates in relation to nine constant temperatures from 8 to 30ºC. The general geographic distribution of the species was also modeled in accordance with the worst predicted climate change scenario for the Mediterranean-Atlantic zone.

The results show a) a LT_{50} of 29.9˚C in three months; b) a similar thermal optimum for growth and feed intake rates, of about 17˚C; c) the respiration rate exponential increase with the temperature up to 30˚C; d) a lower activation energy of well fed organisms and e) a decrease in habitat suitability for *P. lividus* from 0.5 to 0.2 in Portugal in 2100, with consequences not only at the population level but also for the commercial harvesting. We then anticipate that only a very cautious stock management plan based on scientific monitoring can assure a sustainable harvesting of the Portuguese sea urchin population and avoid its collapse under the most dramatic climate change scenario previewed by the IPCC.

Key words: sea urchin, *Paracentrotus lividus*, growth, feed intake, respiration, thermal performance, activation energy.

Resumo

O ouriço-do-mar Europeu *Paracentrotus lividus* (Lamarck, 1816) é uma espécie muito abundante no intertidal rochoso de Portugal. As suas gónadas têm um elevado valor comercial para a indústria alimentar, o que tem levado nos últimos anos a uma procura/captura intensiva dos stocks naturais na região norte de Portugal. Para evitar o declínio irreversível das populações são necessários planos de gestão dos recursos e estes devem ser apoiados em estudos científicos sobre a resposta da espécie aos diferentes stressores ambientais. Isto é particularmente importante no contexto das alterações climáticas onde o aumento da temperatura da água do mar pode provocar desequilíbrios no metabolismo e fitness destes organismos assim como alterar a sua distribuição geográfica.

Neste estudo vamos avaliar a performance do ouriço-do-mar Europeu, tendo em conta a sua taxa de mortalidade, crescimento, ingestão e respiração para nove níveis de temperatura, entre 8 e 30ºC. A previsão da distribuição geográfica da espécie num futuro cenário de alterações climáticas também foi modelada.

Os resultados indicam: a) uma TL_{50} de 29.9˚C ao longo de três meses; b) uma temperatura ótima, tanto para a taxa de crescimento, como para a de ingestão de aproximadamente 17˚C; c) um aumento exponencial da taxa de respiração com o aumento da temperatura até 30˚C; d) energias de ativação mais baixas para os ouriços-do-mar saciados, quando comparados com ouriços em jejum; e) a redução em Portugal do habitat adequado ao ouriço-do-mar de 0.5 para 0.2 em 2100, com consequências não só ao nível das populações mas também para a exploração comercial. Por esta razão, prevê-se que só um plano de gestão rigoroso e apoiado na monitorização científica contínua das populações de ouriço-do-mar possa assegurar a sua exploração de forma sustentável e prevenir o declínio da espécie na nossa costa, em particular no cenário mais dramático de alterações climáticas previsto pelo IPCC.

Palavras-chave: ouriço-do-mar, *Paracentrotus lividus*, crescimento, ingestão, respiração, performance termal, energia de ativação.

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1. Introduction

1.1. Background

The European purple sea urchin *Paracentrotus lividus* is an important species both from an ecological and an economic point of view. However, little is known about the population structure, density and fluctuation over time on the North coast of Portugal (Bertocci et al. 2018). The species has an increasing commercial interest (Andrew et al. 2003), leading to the overexploitation and sometimes the collapse of populations (Ouréns et al. 2015). Harvesting of sea urchins in Portugal has also increased in last years: from no register at all in 2000 (INE 2001), the register of sea urchin captures raised to about 3ton in 2010 (INE 2011), and to 67ton in 2016, (INE 2017), which represents an increase of near 2200%.

Local overfishing is probably not the only stressor of sea urchin populations in Portugal. The sea surface temperature has been increasing in the North Atlantic since decades, and climate change scenarios point to higher temperatures in the late of the 21st century (IPCC, 2014). In the Portuguese coast, increasing seawater temperature trends have been clearly identified (Lima et al. 2006). On the other hand, it is well known that temperature influence physiological processes of living organisms (Angilletta 2009). In echinoderms the temperature influences physiological processes such as respiration, feeding, fertilization and larvae development (Shpigel et al. 2004; Byrne et al. 2009), and may pose risk to its biogeographic distribution (Schulte 2015).

Thus, the evaluation of the temperature effect in a key species with commercial interest is the starting point for a study that aims in the future to have a solid background about the local sea urchin population dynamics to mitigate the impacts of anthropogenic pressures. This achievement will give a solid contribution to support the goal number 14 “Life Below Water” of the Sustainable Development Agenda of the United Nations, that aims to implement effective harvesting regulation plans, based on scientific data, by 2020, and to enhance the conservation and sustainable use of oceans and their resources.

1.2. *Paracentrotus lividus*: ecology and threats

The European sea urchin *Paracentrotus lividus* (Family: Perechinidae) is a marine invertebrate that belongs to the phylum Echinodermata like sea lilies, starfish, brittle stars and sea cucumbers. *Paracentrotus lividus* (Figure 1) is one of many species of sea urchins within the class Echinoidea. It has a very large geographic distribution range in the north-eastern Atlantic from Morocco to Scotland, including the Macaronesian archipelagos, and in the Mediterranean Sea, in isotherms that range from 8°C of water temperature in the winter, to 28°C in the summer (Boudouresque and Verlaque 2001). They can be found in a wide variety of benthic habitats such as seagrass meadows, erect macroalgal assemblages, shallow subtidal reefs, littoral lagoons and intertidal rock pools, where they have an important ecological role (Benedetti-Cecchi and Cinelli 1995; Eklöf et al. 2008; Hernández et al. 2008) due to its grazing activity.

Figure 1 - European purple sea urchin, Paracentrotus lividus*. Author's photo.*

Paracentrotus lividus is the most common echinoid in Portugal (Gago et al. 2001), being present in the rocky intertidal or shallow subtidal habitats commonly up to 10-20 meters depth (Boudouresque and Verlaque 2001).

In the rocky pools, as in other type of habitat, *P. lividus* plays a key role in the ecosystem since they have the potential to modify the structure of the benthic community through its grazing activity (Girard et al. 2008; Bertocci et al. 2018). Sea urchins are among the most efficient grazers in marine environments (Jacinto et al. 2013), able to change erect macroalgal communities into encrusting coralline barrens (Bertocci et al. 2018). Its ability to create burrows (Figure 2) in the substratum increases its complexity and associated biodiversity (Otter 1932; Trudgill 1987; Boudouresque and Verlaque 2007). Hence, small variations in the relative abundance of sea urchins may lead to drastic composition changes in its ecosystems (Himmelman et al. 1983). Moreover, these burrows created by the mechanical action of the spines and mouthparts of the sea urchin provide shelter and avoid the dislodgment due to hydrodynamic forces (Otter 1932; Boudouresque and Verlaque 2007).

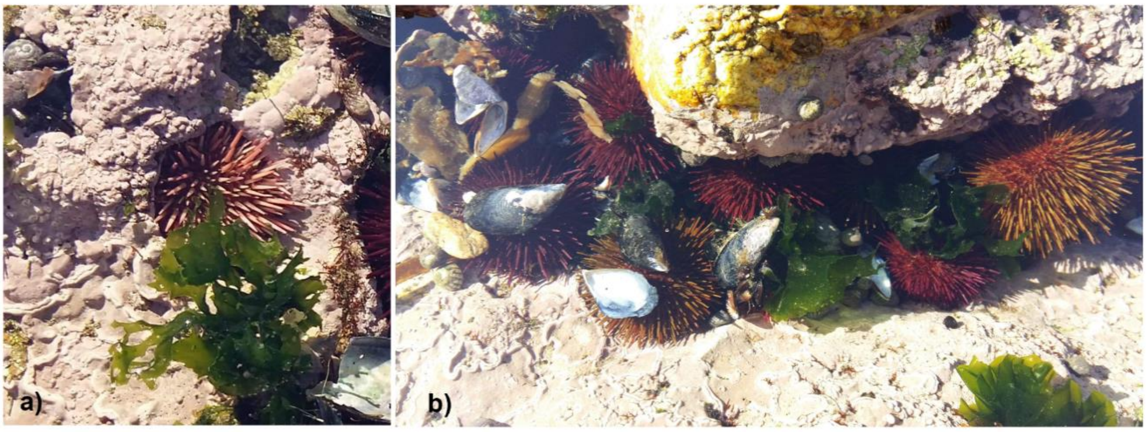

Figure 2 - a) Paracentrotus lividus *in a burrow; b)* P. lividus *barren in a rocky pool. Author's photo.*

Changes in the communities of sea urchins are thriven by both biotic and abiotic factors along with human pressures. The natural fluctuation of the populations of sea urchins is related to factors like predation, larval development, settlement and recruitment, food availability and disease outbreaks, which are processes environmental-dependent (Sala et al. 1998; Shears and Babcock 2002; Boudouresque and Verlaque 2007; Clemente et al. 2007). Therefore, the abiotic unbalance caused by rising water temperature and decreasing pH fostered by climate change will have an impact on the species behavior (Miller et al. 2018).

Moreover, the sea urchins' overfishing has been rising concern in the last years and can severely affect the natural stocks. In fact, recently Bertocci et al. (2018) found a drop of about 90% in the commercial-size of sea urchins in the post-harvesting period in the North of Portugal.

Hence, climate change and commercial harvesting are the main threats faced by sea urchins.

1.3. Climate change: the effect of temperature rising in ectotherm organisms

Greenhouse gases emissions have risen since the beginning of the industrial revolution with larger absolute increases between 2000 and 2010, despite a growing number of climate change mitigation polices (IPCC, 2014). Thus, greenhouse effect has been warming both the earth surface and the oceans due to its direct absorption of heat (Harley et al. 2006), which is traduced in a decrease of cold temperature, an increase of warm temperatures and an increase of heat waves frequency in large parts of Europe (IPCC, 2014). In the north Atlantic, sea surface temperatures have been increasing 0,21°C per decade since 1978 and the future predictions for the global average sea surface temperature points to further increases (EEA, 2016). The last Intergovernmental Panel for Climate Change's (IPCC) report (IPCC 2014), shows that future projections for the worst scenario, the Representative Concentration Pathway (RCP) 8.5, present a global temperature increases of 3.7°C in average by 2100.

Through the thermodynamic law, is the temperature that determines the rate of biochemical and physiological reactions. Hence, the temperature is one of the most pervasive state variables affecting the metabolism and the biological processes, being able to modulate the organism performance (Angilletta et al. 2006; Angilletta 2009; Atkinson et al. 2011). Moreover, for aquatic ectotherms, changes in the water temperature mean changes in their body temperature too.

The species response to warming temperatures has increasing concerned marine ecologist. Indeed, every single species live within a limited range of body temperatures and there are already some known effects of temperature-rise in sea urchins. Larvae development is usually favoured by warmer temperatures (Byrne et al. 2009; Brennand et al. 2010) but gonad somatic index drop when the temperature rises (Shpigel et al. 2004).

Thermal performance curves are then an useful tool to describe the response of traits across a range of temperatures (Dell et al. 2011). Performance curves are assumed to be a proxy for fitness, where the physiological rate rises slowly with the temperature up to a maximum level, considered as the optimal temperature (Sinclair et al. 2016). A further temperature increase leads to the critical thermal maximum (Somero 2010) and the Arrhenius breakpoint temperature (Marshall et al. 2010), followed by a fast drop . The Figure 3 depicts an example of a thermal performance curve and its relationship with the activation energy in the Arrhenius plot.

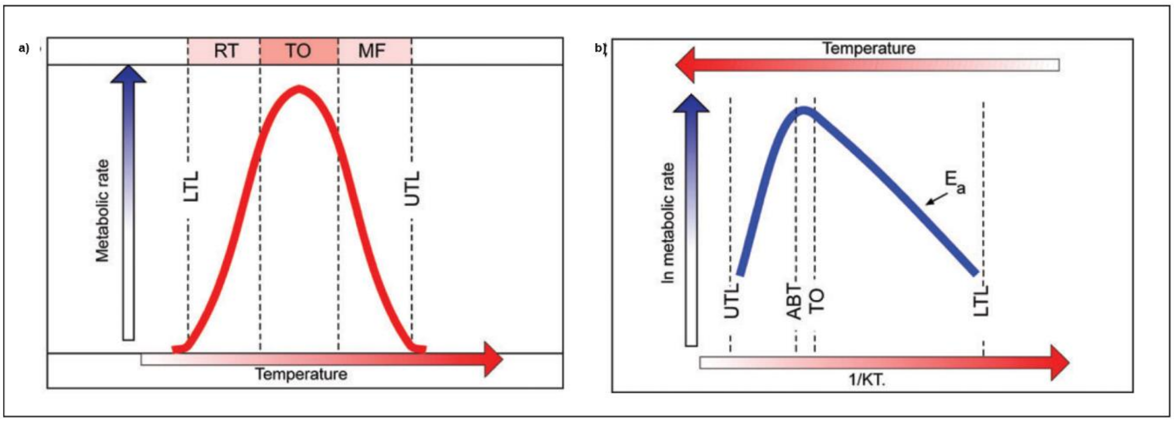

Figure 3 - a) Thermal performance curve relating temperature and metabolic rate from the lower thermal limit (LTL) compatible with functional integrity, the rate increases with temperature (RT), until reaching the optimal temperature (TO). Further warming leads to a monotonic fall (MF) and the upper thermal limit (UTL) compatible with functional integrity. b) Arrhenius plot showing the relationship between the natural log of the metabolic rate and the reciprocal of the absolute temperature (kT); ABT is the Arrhenius breakpoint temperature; E_a *is the mean activation energy. Source: Alcaraz et al. 2013 (adapted).*

The overlap between rising temperature and the biological processes increase follow the Botlzman-Arrenhius relation, where the Boltzman constant specifies how temperature affect the rate of the reaction by changing the proportion of molecules with sufficient kinetic energy (Brown et al. 2004).

The Metabolic Theory of Ecology is a mathematic model to translate the physical, chemical and thermodynamic principles that govern the metabolism and other physiological rates in general, giving the activation energy (Brown et al. 2004). Although Gillooly et al. (2001) argued that the majority of the biological rates, show a similar temperature dependence and have a similar activation energy among all the processes, with a mean of 0.60-0.70 eV, several studies (Pörtner 2002; Sokolova and Pörtner 2003; Seibel and Drazen 2007; Lemoine and Burkepile 2012) pointed to another results of activation energy and also for mismatches in the optimal temperature.

1.4. Objectives

The present dissertation aims to respond to the following questions:

1) What is the water temperature impact on the growth, ingestion and respiration rates of our model species *P. lividus*?;
2) Do the thermal optimum vary depending on the measured rates (growth, feed intake and respiration)?;
3) What are the (predicted) fitness and ecosystem consequences of temperature rising for these species in a context of climate change?

4) Will the distribution be affected in the extreme conditions previewed by climate change scenarios? Will it compromise the commercial harvesting in the North of Portugal?

2. Materials and methods

2.1. Collection and acclimation of living organisms

Purple sea urchins with about 1.50 – 6.00 cm test diameter (TD) were collected from the intertidal zone during low tide, in a rocky beach in Porto (41.164238N -8.688091W), Portugal (Figure 4) in early Spring (March) 2018. The sea urchins were immediately transported up to CIIMAR bioterium in Matosinhos, in plastic cool boxes filled with seawater.

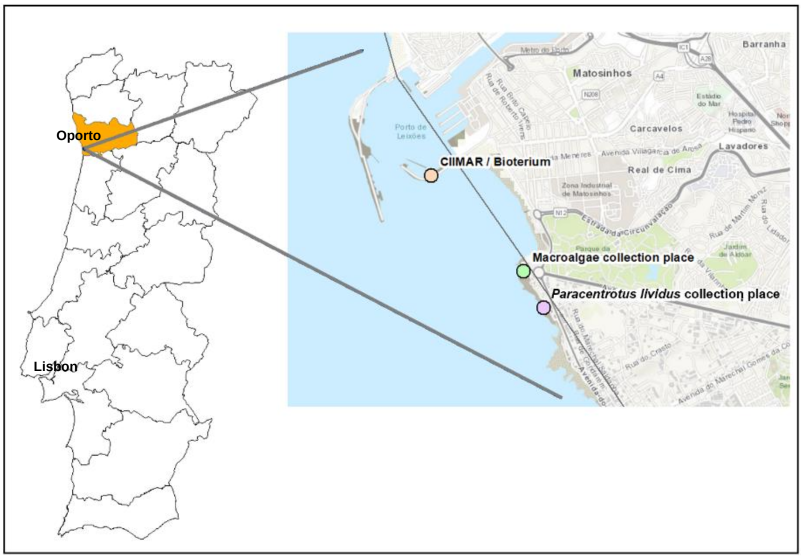

Figure 4 – Location of the sampling places and the research centre, CIIMAR.

The Table 1 shows the characterization of the samples according to the size of the animals.

Table 1 – Characterization of the samples according to the size (TD, test diameter) of the sea urchins Paracentrotus lividus.

Size class	TD	Number of sea urchins
Small	≤2.99cm	35
Medium-sized	3.00 to 4.99cm	259
Large	≥5.00cm	13
Total		306

Once at the laboratory, the sea urchins were assigned to quarantine for 3 days at 16ºC. After the quarantine, sea urchins were moved to the experimental room where they were placed in different tanks at the same temperature for two weeks to allow for acclimation to the new conditions (first acclimation period) such as a reduction of available area and water volume. Prior to the start of the experiment, the temperature of each tank was gradually changed from ambient by 1ºC every day until all the specimens reached the final target temperature (second acclimation period) (following Yeruham et al. 2015).

As food supply, the green macroalgae *Ulva spp.* was offered. The algae were collected every 2 weeks in a rocky pool at Castelo do Queijo beach (Porto, Figure 4) and stored in an aerated tank inside the experimental room, at ambient temperature (~16 ˚C).

2.2. Experimental setup

A system containing 18 tanks at nine different temperature levels - 8˚C, 12˚C, 16˚C, 18˚C, 22˚C, 24˚C, 26˚C, 28˚C and 30˚C - was set up as shown in the Figure 5. There were two tanks per temperature level, connected between them, used as water bath. Five cylinders of 2 litters each were placed inside each tank. All the cylinders contained a plastic mesh to better manage the sea urchins, an air bubble stone and a regulated flow of water controlled automatically. The cylinders were identified with the letters "G", "I", "R1", "R2" and "R3", corresponding to the different physiological rates to be tested (G – Growth; I – Ingestion; R – Respiration), and a total of 306 sea urchins were distributed among them as follows: 4 sea urchins in each G, and I, and 3 sea urchins in each R1, R2, R3, and the same in the respective replicas, which represents a total of seventeen sea urchins per tank (plus a total of seventeen in the replicates). The sea urchins were assigned to each cylinder according to their test diameter, with the small ones in the G, and medium-sized and large sea urchins in the Rs and I (for size class dimensions see Table 1). An attempt was made to individually mark the sea urchins from the G tanks in order to follow individual growth, using plastic beads. However, this procedure was unsuccessful as the individuals released the beads and showed clear signs of stress.

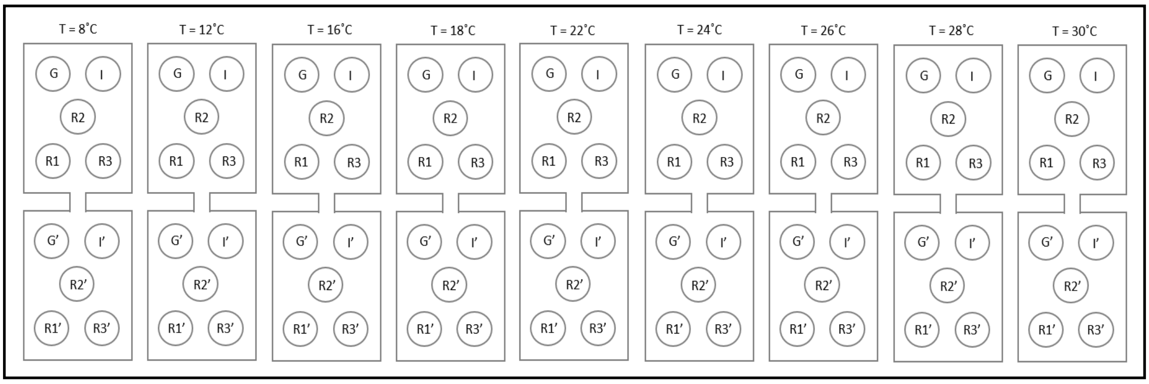

Figure 5 - Explanatory scheme representing the five cylinders in the various water baths at each experimental temperature level. G: n=4 sea urchins; G': n=4 sea urchins; I: n=4 sea urchins; I': n=4 sea urchins; R1: n=3 sea urchins; R1': n=3 sea urchins; R2: n=3 sea urchins; R2': n=3 sea urchins; R3: n=3 sea urchins; R3': n=3 sea urchins. The letters "G", "I", "R" correspond to the different physiological rates being tested, i.e. G – Growth; I – Ingestion; R – Respiration. The " ´ " represents the replicate.

2.3. Routine procedures

The salinity and the water temperature were recorded daily, from the acclimation period until the end of the experiment. The water and air flow were checked twice a day.

The sea urchins were fed with fresh *Ulva spp. ad libitum* unless the experiment required a fasting period (see details for each biological rate in sections 2.4.2, 2.4.3 and 2.4.4).

The cleaning routines were implemented three times a week. On Monday, we changed the total amount of water and washed every cylinder and mesh. On Wednesday and Friday, we changed about 90% of the water and removed as many faeces as possible. The sea urchins that died were removed from the system.

At the end of the experimental assays, all living sea urchins were released back to the same place of collection.

2.4. Biological rates

2.4.1. Mortality

The mortality along the experiment was recorded and survival was estimated as the proportion of live/dead sea urchins over the total number of individuals at each temperature level. The lethal temperature for 50 % of population (the temperature at which 50% mortality occurs, LT_{50}) was estimated using survival data from the sixty-and ninety-days experiments in relation to temperature.

2.4.2. Growth

The test diameter (TD, cm) and the live weight (LW, 0.001g) of all the experimental sea urchins were measured at the beginning of the experiment (during first acclimation period) and after two months (sixty days experiment), with a calliper (±0.02mm) and a RADWAG PS 1000/C/2 scale, respectively. The sea urchins were wrapped in paper towel before weighing to reduce the water content in their tests.

The growth rate (g $LW.day^{-1}$) of the sea urchins placed in G and G' cylinders was calculated as:

$$Growth = \frac{Wt - Wi}{t} \qquad (equation\ 1)$$

where Wi was the sea urchin live weight at the beginning of the experiment, W_t was the living weight after two months, and t was the number of days. Since individual identification of each organism was not possible, it was used the mean live weight per cylinder at the beginning and at the end of the trial, respectively as *Wi* and as W_t.

The same formula was used to assess the growth rate in centimetres (cm.day^{-1}), replacing the values of the weight by the measurements of TD.

The organisms assigned to this experiment were fed *ad libitum* since the acclimation period until its release back to the sea.

2.4.3. Feed intake

Five assays were conducted over one month with organisms from I and I' cylinders of each temperature level. The I cylinders had two sea urchins with TD > 4.00cm and the I' cylinders had six sea urchins with TD between 2.90 and 3.99cm. This size distribution resulted in similar conditions in both tanks as the total biomass was equivalent between temperature levels (Table 2).

Table 2 – Total biomass (g) of the sea urchins Paracentrotus lividus *in each cylinder I (each cylinder with 2 large sea urchins) and I' (each cylinder with 6 medium-sized sea urchins) during feed intake experiments.*

Treatment	Sea urchins live weight (g)	
	I	I'
Temp. 8˚C	99.733	99.128
Temp. 12˚C	99.581	109.544
Temp. 16˚C	120.221	116.93
Temp. 18˚C	115.405	109.234
Temp. 22˚C	103.966	109.105
Temp. 24˚C	121.075	133.014
Temp. 26˚C	109.18	129.504
Temp. 28˚C	101.495	142.307
Temp. 30˚C	137.433	159.847

One extra cylinder was added to every tank (water bath) to have an autogenic control with algae solely.

Prior to the experiments, the animals were deprived of food for two days, and afterwards, fresh *Ulva* sp. was provided at a ration of 4% of the sea urchins' live weight. The leftovers were removed after 2 days and feed intake per gram of sea urchin was calculated as:

$$Feed\ intake = \frac{\frac{(Wi - Wl)}{gU}}{t} \qquad (equation\ 2)$$

where *Wi* was the wet weight of the offered feed, *Wl* was the wet weight of the leftovers, *gU* was the live weight of the sea urchins and *t* was the number of days. The average of the controls was calculated for each temperature level and subtracted to the respective feed intake.

The controlled feeding regime with periods of starvation was limited to one month, while the ingestion assay was conducted. Prior and after that, all the animals that were assigned to this experiment were fed with *Ulva spp. ad libitum*.

2.4.4. Respiration rates

The respiration rates were estimated from oxygen consumption. The oxygen consumption rates were measured at three stages along the experiment, varying the food condition:

1) after the 3 days of quarantine, at ambient temperature (16ºC), where twelve sea urchins were randomly chosen to assess its initial condition;

2) after five days at the target treatment for every temperature level (16˚C excluded) with organisms fasted for two days; for the specific temperature level of 16˚C, the sea urchins in those tanks spent the whole time at the same temperature, and the incubation started in the ninth day since the beginning of second acclimation period;

3) after two weeks at the target temperature level with animals fed *ad libitum.*

So, over the whole time that we kept the organisms (three months), the sea urchins assigned to respiration traits had a unique fasting period of two days, prior to the trials in starvation condition.

Three blank controls for each temperature level were done to determine the microbial oxygen background consumption, and the average was used to calibrate the data. The controls that showed up some degree of abnormality (e.g. the slope of the control was three or more times larger than the others), were treated as outliers. This was the case of one replicate of the controls at 12, 24, 28ºC (fasted period), 12 and 26ºC (fed period). For these cases, the average of the control was calculated with the measurements given by the other two regular values. Moreover, the output file of the three controls at 16ºC (fasted conditions) was corrupted and, for the same temperature during the fed period the control was abnormal (mean = -19µmol O_2 l^{-1}), both being replaced with the control made during the initial condition respirometry with a mean consumption of -5µmol O_2 l^{-1}.

For each temperature level, three incubations were run with sea urchins plus one control. Each incubation consisted in three independent respirometry chambers with a known volume, filled with filtered sea water and sealed (Figure 6a). The water temperature inside the chamber was controlled through a water bath and peristaltic pumps that were used to create a current in each chamber and to homogenize the water. Each incubation lasted forty minutes to avoid oxygen depletion and the initial ten minutes of measurements were discarded to reduce the effect of stress due to the manipulation of the organisms.

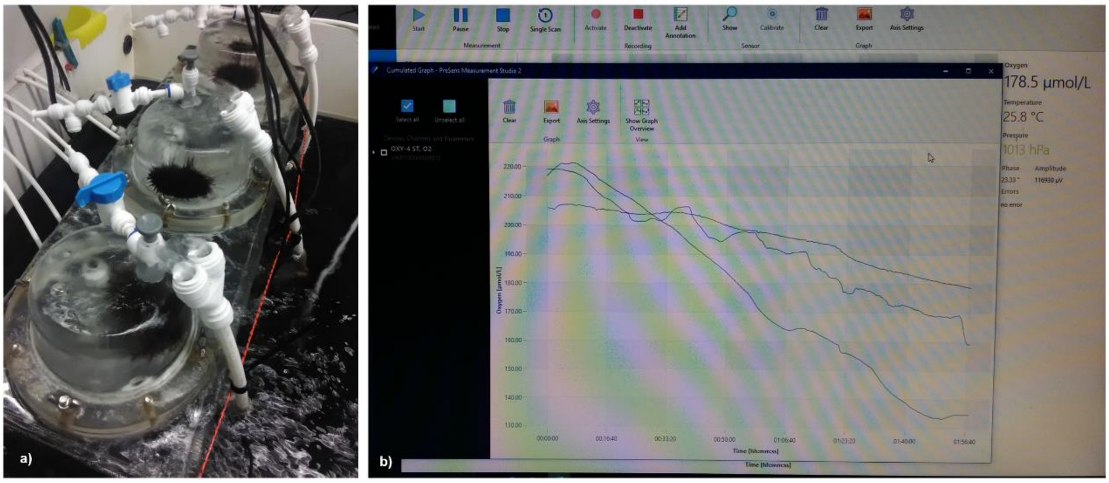

Figure 6 - Respirometry assay with Paracentrotus lividus. a) incubation of three sea urchins at 26ºC; b) real-time dynamic graph with oxygen decreasing inside each chamber measured with PreSense software. Author's photo.

For each incubation, three sea urchins were picked randomly from R1, R2, R3, R1', R2' or R3' cylinders resulting in nine respiration rates for each temperature level for trials number 2) and 3). The oxygen concentrations (µmol.l^{-1}) were measured every twenty seconds with an OXY-4 ST PreSense Measurement device, connected to optical oxygen sensors into the respirometer chambers and recorded with the software PreSense Measurement Studio 2 version 1.1.2.0 (Figure 6b). At the end of the incubation, the chambers were flushed and cleaned with filtered sea water. The weight and volume of each sea urchin were also determined. The volume (ml) of all sea urchins was calculated by measuring the weight of the fluid displaced and by taking in account that 1ml of sea water was equivalent to 1gram. The weight of the fluid was determined with the same scale as for the sea urchins and converted into liters.

The oxygen consumption per sea urchin was then determined using the volume of water (L) and the oxygen concentration measured in each chamber during the incubation. The slope of oxygen concentration as function of time gave us the individual respiration rate per hour (µmol O_2.h^{-1}) after subtracting the average of the blank controls (µmol O_2.h^{-1}). Since the metabolic rate is dependent on body-mass, each individual respiration rate was divided by the live weight (g) of the organism to obtain the respiration rate per gram of sea urchin (µmol O_2.h^{-1}.g^{-1}).

2.5. Effect of temperature on metabolism

2.5.1. Activation Energy

The calculation of the activation energy (E_a) was based on the equation described by Brown et al. (2004) under the Metabolic Theory of Ecology (MTE), which corresponds to a simple linear regression, with data represented through Arrhenius plots with ln($IM^{-3/4}$) against 1/kT:

$$\ln(IM^{-\frac{3}{4}}) = -Ea\left(\frac{1}{kT}\right) + \ln(i0) \qquad (equation\ 3)$$

Where *I* is the individual metabolic rate, *M* is the sea urchin body weight (g), T is the temperature (in Kelvin) and k is the Boltzman constant. The E_a is the activation energy (eV) obtained as the (minus signed) slope coefficient of the linear regression, and i_0 is a normalization constant independent of body size and temperature, which is given by the intercept coefficient.

The activation energy E_a was determined for respiration (µmol $O_2.h^{-1}$) in fasted and fed sea urchins, and for growth (g.d^{-1}) and feed intake (g *Ulva*.d^{-1}) rates also in well fed sea urchins, with *I* being replaced for the respective rate. The range of temperatures chosen to draw the plot was selected according to each rate since the Arrhenius plot only applies to the near-exponential increasing phase (Sibly et al. 2012).

Through the same equation, the body-mass dependence can be described as well. The respiration rates as a function of body mass for initial conditions (after 3 days of quarantine, at 16˚C) and for fasted and fed sea urchins (at each temperature level) were also investigated by plotting ln(IM^{-1}) against ln(M). Then, a new attempt to calculate the activation energy of fed sea urchins were done in accordance with the new exponent.

2.5.2. Thermal Performance Curves

Thermal performance curves (TPC) for a range of temperatures between 8 and 30˚C of biological rates such as growth (in grams) and feed intake were fitted to a quadratic and to a Gaussian function, both plausible models (Angilletta Jr 2006).
Taking into account the general equation of the quadratic model:

$$I(T) = aT^2 + bT + c \qquad (\ equation\ 4)$$

The optimal temperature (˚C), T_{peak} is given from equation 5, corresponds to the vertex of the fitted parabolic curve given by:

$$Tpeak = \frac{-b}{2a} \qquad (equation\ 5)$$

The peak performance, with the units in accordance with the taxa, is given by:

$$I_{peak} = I(T_{opt}) = a\left(\frac{-b}{2a}\right)^2 + b\left(\frac{-b}{2a}\right) + c = c - \frac{b^2}{4a} \qquad (equation\ 6)$$

Fitting the same data to a Gaussian curve (see equation 7), it is also possible to find the performance breadth, which consists in the optimal range of temperatures over which physiological performance is at least 69% of the peak (van der Veer et al. 2006). In other words, the thermal breadth corresponds to the temperature interval, centred around the optimal temperature ± one standard deviation.

$$I(T) = \frac{A}{\sqrt{2\pi\cdot\sigma^2}} \cdot \exp\left(-\frac{1}{2}\left(\frac{T-T_{peak}}{\sigma}\right)^2\right) \qquad (equation\ 7)$$

Where we have:

$$I_{peak} = I(T_{peak}) = \frac{A}{\sqrt{2\pi\cdot\sigma^2}} \qquad (equation\ 8)$$

2.6. Species distribution model

Paracentrotus lividus data occurrence was collected from existing online databases such as Ocean Biogeographic Information System (OBIS - http://iobis.org/) and Global Biodiversity Information Facility (GBIF - https://www.gbif.org/). Additional records were added from a literature review using the Web of Science. Duplicate records were eliminated using R code. A total of 266 georeferenced occurrence records were used in the Species

Distribution Models (SDM), for a given distribution area that considered European and North Africa shores. These coastal areas were masked using a bathymetric raster to include only a depth range from 0 to 200 meters (Bertocci et al. 2010).

The data about environmental predictors were downloaded as raster layers from the repository BIO-Oracle (http://www.bio-oracle.org/) and then environmental predictors were selected in accordance with the potential influence on the distribution of *P. lividus*. Since correlation among environmental drivers is a potential problem in species distribution modelling (Elith et al. 2010), thus we only used predictors for which pairwise Pearson correlations between variables were less than 0.85: salinity, pH, maximum annual seawater temperature, minimum annual seawater temperature and seawater temperature range.

The species distribution models were constructed using two different algorithms. First, we used Maximum Entropy Modelling (MaxEnt software) (Phillips 2005) where the MaxEnt algorithm aims to maximize the entropy of the species probability distribution (Merow et al. 2013). This algorithm fits complex models as linear combinations of basic functions and we ran the models using the linear and quadratic features (Elith et al. 2011). Additionally, we built a generalized linear model (GLM) using the R package Biomod2, a regression-like method that relates presence records and pseudo absences with environmental layers (Guisan et al. 2017).

The contribution of each predictor was examined using the permutation importance and percent contribution coefficients from MaxEnt software, as well as with the variable importance function of Biomod2. In the first case, the performance of the model was evaluated in accordance with the predicted area under the curve (AUC), where values higher than 0.85 indicated a good discrimination power (Phillips et al. 2006). Internal data-splitting validation was applied to confirm the variable importance of the final predictors in the training data (70% of presence = points) and the consistency of the above evaluation metric (AUC).

MaxEnt was used to determine the habitat suitability index for all the study areas with the environmental conditions registered from 2002 to 2009, as well as to obtain future distribution projections by using rasters of forecasted physical conditions under the Representative Concentration Pathways (RCP) 8.5, the pathway with the highest greenhouse gas emissions (IPCC 2014). The layers extracted from Bio-Oracle contained the information from the UKMO-HadCM3 model, which represented the most severe among those provided by Bio-Oracle (Meehl et al. 2007).

2.7. Statistical analyses

Statistical analyses were performed with RStudio version 1.1.383 (http://www.rstudio.com/).

The relationship between survival and temperature was evaluated through a logistic regression model, using the glm (General Linear Model) function and LT_{50} was calculated with dose.p function (package *mass*).

A simple linear regression model (function lm) was used to assess the relationship between $\ln(IM^{-3/4})$ and 1/kT for respiration (fed and fasted sea urchins), ingestion and growth rates. A one-way ANOVA test (package *car*) was used to determine the significance of the biological rates depending on the temperature, followed by a pairwise post-hoc Tukey test (package *multicomp*) in order to show the significant differences between each temperature level.

Thermal performance curves were drawn through poly function for the quadratic model and nls (Nonlinear Least Squares) function for the Gaussian model.

Visual inspection of residual plots did not reveal any obvious deviation from homoscedascity or normality except for growth data in the linear regression. The p-values were obtained by likelihood ratio tests of the full model, for a significance level $\alpha=0.05$.

3. Results

3.1. Sample characterization

The distribution of the sea urchins in each cylinder was in accordance with the goals. Hence, G cylinders (Figure 7), specific for growth measurements, had the smallest organisms (TD = 3.06cm ± 0.30/ LW = 12.350g ± 3.108).

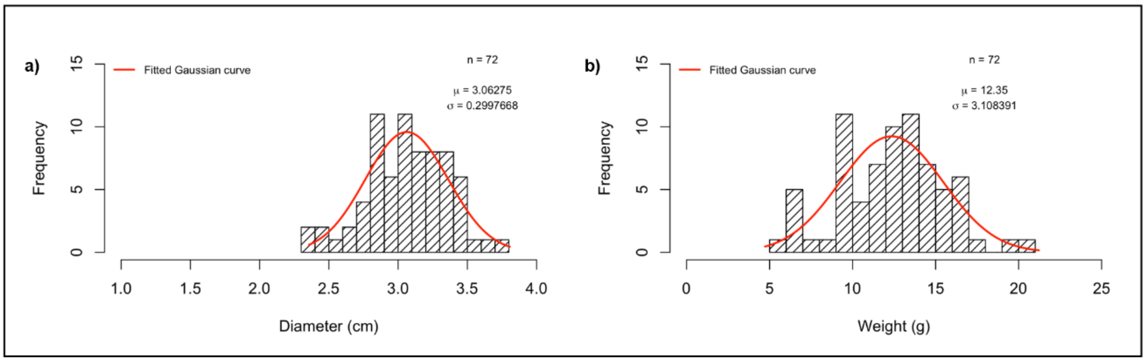

Figure 7 - Histogram of a) test diameter (cm) and b) live weight (g) of the sea urchins Paracentrotus lividus *in the G/G' cylinders.*

The size and weight composition of the sea urchins used in the ingestion experiments was slightly different. We had medium-sized sea urchins with an average TD = 3.65cm ± 0.41 and LW = 20.530g ± 5.128, and large sea urchins with TD = 5.19cm ± 0.38 and LW = 56.005g ± 8.473 in average (Figure 8), distributed in different cylinders.

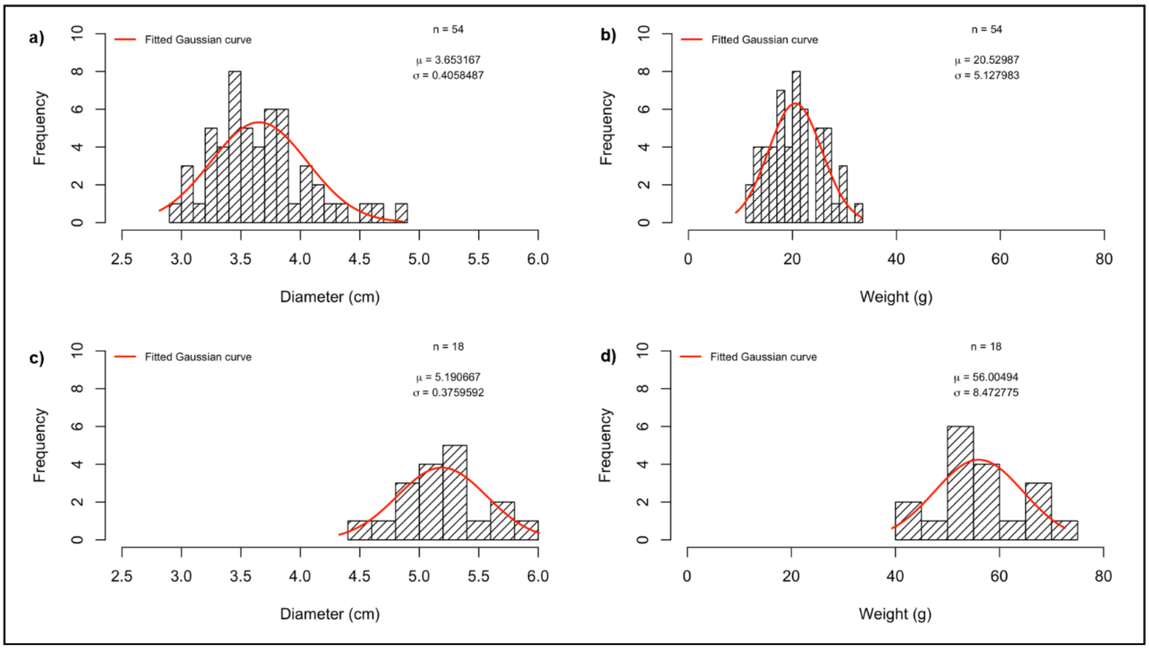

Figure 8 - Histogram of test diameter (cm) and live weight (g) of the sea urchins Paracentrotus lividus *in the I/I' cylinders. a) and b) correspond to the medium-sized sea urchins placed in the I' cylinders; c) and d) correspond to the large sea urchins placed in the I cylinders.*

In the R cylinders (Figure 9), the ones used for respirometry assays, there were mainly medium-sized sea urchins (TD = 3.94cm ± 0.47/ LW = 25.414g ± 7.910).

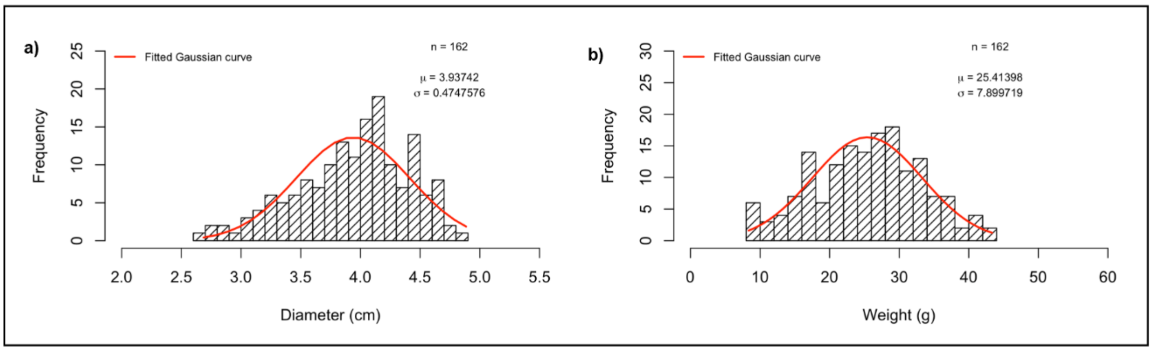

Figure 9 - Histogram of a) test diameter (cm) and b) live weight (g) of the sea urchins Paracentrotus lividus *in R/R' cylinders.*

3.2. Seawater physical parameters

The water temperature was maintained at the target level over the whole experiment (Table 3). Lower and upper temperatures were the most difficult to maintain due to the heat transfer between tanks and the surroundings. The tanks at 28 and 30˚C were the most affected, with the mean water temperature below the expected value by approx. 1˚C. On the other hand, the tanks at 8˚C were almost 0.5˚C higher than expected. Despite using water directly pumped from the sea and not modified, the salinity was a bit lower than reference values (approx. 35 ppt). The highest salinity values were recorded at temperatures above 24˚C which can be associated to higher evaporation rates.

Table 3 – Physical parameters of seawater measured during the experiment (Mean ± Standard deviation).

Treatment	Measured Temp. (˚C)		Salinity (ppt)	
	Mean	± SD	Mean	± SD
Temp. 8˚C	8.451	0.537	32.031	1.193
Temp. 12˚C	12.080	0.366	32.249	1.143
Temp. 16˚C	15.724	0.345	32.665	1.050
Temp. 18˚C	17.772	0.401	32.742	1.069
Temp. 22˚C	21.815	0.565	32.902	1.276
Temp. 24˚C	23.465	0.525	33.139	1.159
Temp. 26˚C	25.518	0.376	33.412	0.992
Temp. 28˚C	26.868	1.797	32.505	1.219
Temp. 30˚C	28.656	1.101	32.810	1.204

3.3. Effect of temperature on mortality

The Figure 10 shows the evolution of the mortality at every thirty days, in a total of three months.

The sea urchins showed 100% survival along the first month, which corresponded to the whole acclimation period. In the second month, it was recorded twenty six deaths and more twenty four during the last month, corresponding to the ninety-days experiment.

This pattern lead us to a LT_{50} curve of 29.9˚C at the end of the ninety-days experiment (Figure 11).

The logistic regression suggested a strong association between mortality and temperature, with p-values <0.001 for sixty- and ninety-days experiments.

Figure 10 - Number of dead (orange)/alive (blue) sea urchins Paracentrotus lividus *for each temperature level. a) sea urchins at the end of thirty days; b) sea urchins at the end of sixty days; c) sea urchins at the end of ninety days.*

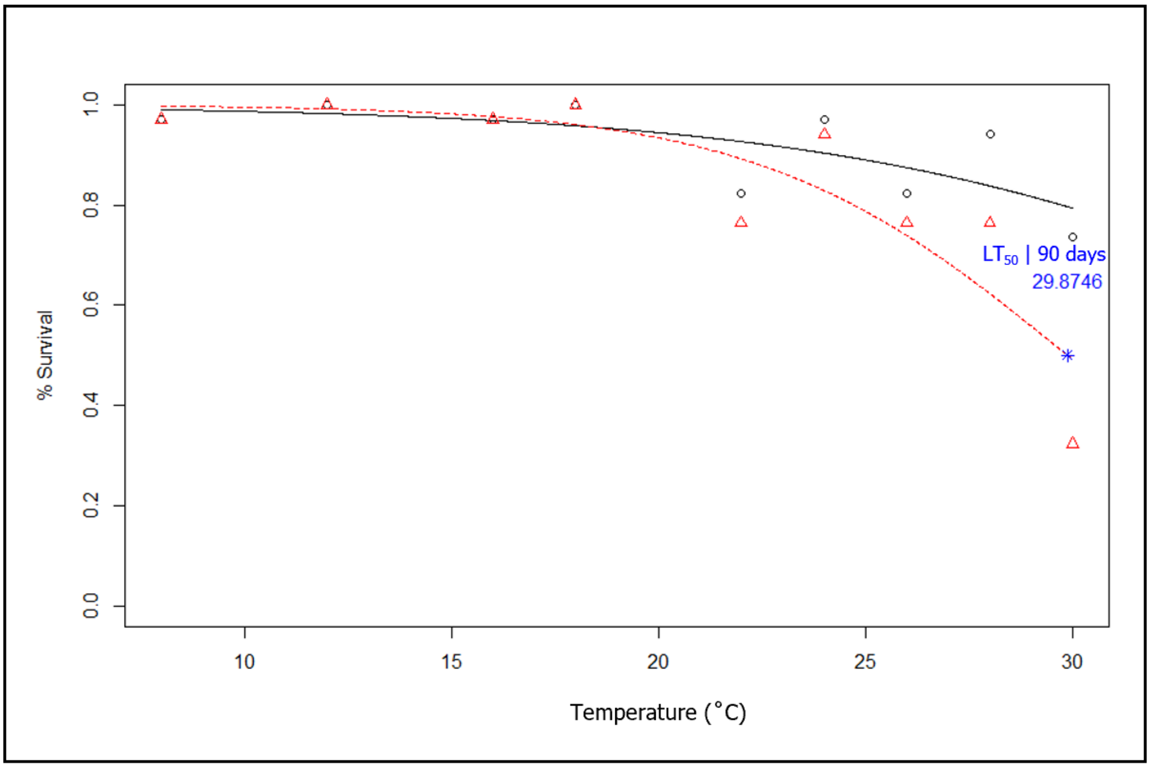

Figure 11 - Differences in LT_{50} curves for the sea urchins Paracentrotus lividus *experiments under laboratory conditions along different times. Red line represents the LT_{50} for the ninety-days experiment. Black line represents the curve trajectory at the end of sixty days where LT_{50} were estimated to be beyond the test gradient used in our experiment.*

3.4. Effect of temperature on biological activities

3.4.1. Growth and feed intake rates

The Table 4 highlights the results of the growth, both in diameter and in weight, and feed intake experiments.

The growth rate ranged from 0.0017 to 0.0298 g d^{-1} and from 0.0001 to 0.0040 cm d^{-1}. When we analyze the results for growth rate in weight, the only sea urchins that did not increase in weight were the ones at the two higher temperatures, 28 and 30ºC, and the sea urchins in G cylinder at 22ºC. Since the mean TD of the same sea urchins also decreased, both biological variables tracking the size of the sea urchins (weight and diameter) show coherent results. The highest growth rates were recorded 18 and 26ºC. Growth measured in centimeters is harder to obtain, prone to human error and was almost imperceptible, sometimes negative.

Table 4 - Effect of temperature on the growth (in centimetres and grams per day) and feed intake (per gram of sea urchin per day) of the sea urchins Paracentrotus lividus *maintained in laboratory conditions. For growth rates, each G/G' cylinders represent the mean growth of 4 sea urchins along two months. Feed intake, evaluated in cylinders I/I' counted with 5 trials (one month) and the results are expressed by mean ± standard deviation, per temperature level.*

Treatment	Cylinder	Growth (cm d^{-1})	Growth (g d^{-1})	Cylinder	Feed intake (gSU d^{-1}) Mean	Feed intake (gSU d^{-1}) ± SD
Temp. 8˚C	G	0.0018	0.0026	I	0.8065	0.1724
Temp. 8˚C	G'	0.0026	0.0050	I'	0.4826	0.4260
Temp. 12˚C	G	0.0040	0.0113	I	1.1840	0.1481
Temp. 12˚C	G'	-0.0003	0.0075	I'	1.5341	0.2012
Temp. 16˚C	G	0.0001	0.0105	I	1.3102	0.2171
Temp. 16˚C	G'	0.0003	0.0146	I'	1.9857	0.4402
Temp. 18˚C	G	0.0015	0.0229	I	0.7699	0.4270
Temp. 18˚C	G'	0.0008	0.0298	I'	1.5776	0.6650
Temp. 22˚C	G	-0.0008	-0.0006	I	1.2623	0.2407
Temp. 22˚C	G'	0.0003	0.0017	I'	2.2513	0.6990
Temp. 24˚C	G	-0.0022	0.0155	I	0.4277	0.0725
Temp. 24˚C	G'	0.0002	0.0124	I'	1.6811	0.1324
Temp. 26˚C	G	-0.0007	0.0078	I	0.2303	0.2167
Temp. 26˚C	G'	-0.0005	0.0230	I'	1.0365	0.3983
Temp. 28˚C	G	0.0003	-0.0090	I	0.7785	0.2732
Temp. 28˚C	G'	-0.0024	-0.0068	I'	1.4331	0.3882
Temp. 30˚C	G	-0.0105	-0.0294	I	0.0920	0.1678
Temp. 30˚C	G'	-0.0025	-0.0187	I'	0.0596	0.1548

In terms of temperature influence, the Table 5 describes the significance among treatments for growth rates (in grams), through a Tukey test.

The results for growth rate show that:

- Significative differences were found for growth rates in the range of temperatures 8-26ºC compared with the growth rate at 30ºC, where the sea urchins did not increase their body mass;
- The highest difference in growth was at 16, 18, 24 and 26ºC, when compared with 30ºC (p-value <0.001);
- There were also differences between the growth rates at 8 and 18ºC (p-value <0.05). On the other extreme, differences in the growth rates at 24 and 26 compared with 28ºC (p-value <0.05) were also significative.

Table 5 - Post-hoc Tukey's p-values between the growth and feed intake rates of the sea urchins Paracentrotus lividus *at each temperature level. NS: not significant. Blue colour represents data from growth and Grey corresponds to feed intake.*

Temperature (˚C)	8	12	16	18	22	24	26	28
12	NS NS							
16	NS <0.05	NS NS						
18	<0.05 NS	NS NS	NS NS					
22	NS <0.01	NS NS	NS NS	<0.05 NS				
24	NS NS	NS NS	NS NS	NS NS	NS NS			
26	NS NS	NS NS	NS <0.01	NS NS	NS <0.01	NS NS		
28	NS NS	NS NS	<0.05 NS	<0.01 NS	NS NS	<0.05 NS	<0.05 NS	
30	<0.01 NS	<0.01 <0.01	<0.001 <0.001	<0.001 <0.05	<0.05 <0.01	<0.001 NS	<0.001 NS	NS <0.05

The feed intake ranged from 0.0596 ± 0.1548 to 2.2513 ± 0.6990 gSU d^{-1}. The feed intake results suggest a difference between cylinders (Table 4). Except for the thermal extremes of 8 and 30ºC, the sea urchins on I' cylinder had higher intake rates with two maximum peaks at 22 and 16ºC. Following the same pattern, even with lower ingestion rates, in I cylinders there were also two peaks at the same temperature levels.

In general, we observed higher rates of *Ulva* sp. ingestion in the beginning of the experiment, followed by an ingestion decrease in the next trials. The sea urchins at 12 and 16ºC were the only ones showing a homogenic feeding behavior for the whole length of the experiment. The graphs presented in the Annex 1 give the detailed information.

The significance among treatments for feed intake rates, are described through a Tukey test (Table 5).

For the feeding intake rates:

- The temperature levels 22 and 16ºC that corresponded to ingestion peaks have significative differences when compared against temperature levels 8, 26 and 30ºC;
- The feed intake rates among extreme temperatures did not show significant differences (p-value NS).

Fitting a quadratic and a Gaussian curve to growth and feed intake data (Figure 12), allowed us to estimate the optimum temperature (*Tpeak*) which was approx. 17ºC in both rates. The performance breadth for growth rate was inside the interval 13.07 to 21.92ºC, and for feed intake it moved a bit forward, in the range of temperatures 9.61 to 25.38ºC. All the parameters are described in the Table 6.

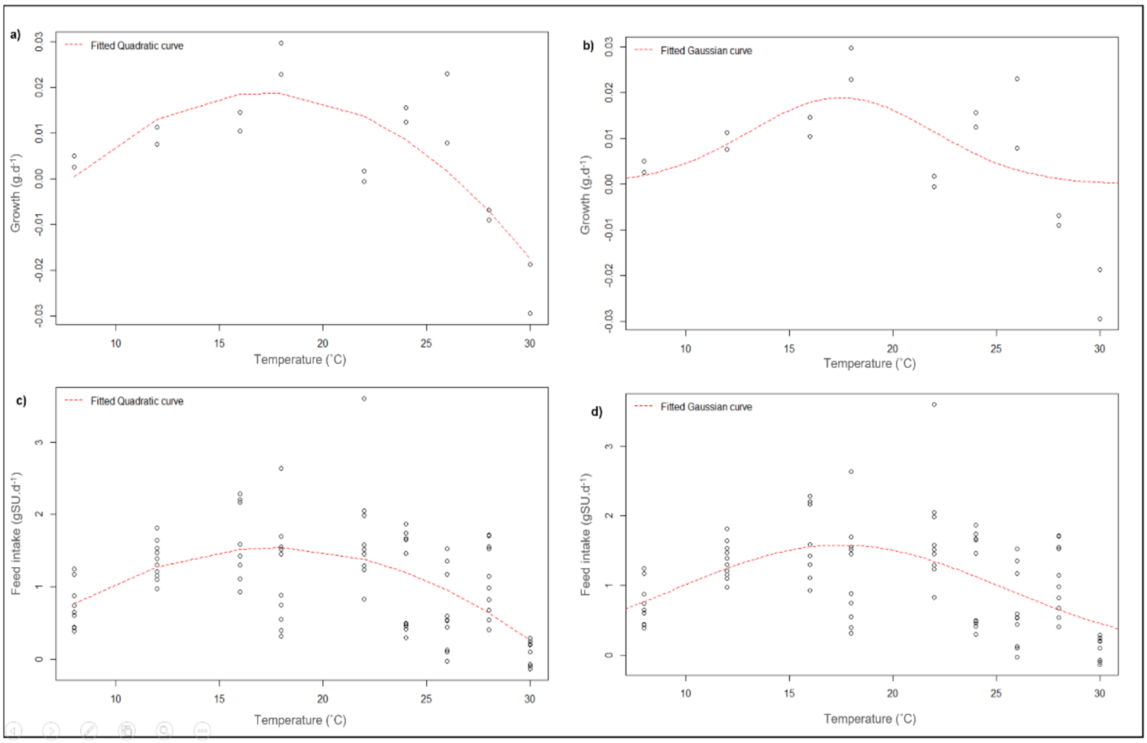

Figure 12 - Thermal performance curves for growth and feed intake rates of the sea urchins Paracentrotus lividus*. a) thermal performance curve for growth rate fitted to a quadratic function; b) thermal performance curve for growth rate fitted to a Gaussian function; c) thermal performance curve for feed intake rate fitted to a quadratic function; d) thermal performance curve for feed intake rate fitted to a Gaussian function.*

Table 6 - Temperature of peak performance (Tpeak), biological activity at the Tpeak (Ipeak, in the same units as the rate) and performance breadth (PB) for growth and feed intake of the sea urchins Paracentrotus lividus*, obtained through quadratic and Gaussian functions.*

Parameters	**Growth**	p-value	**Feed intake**	p-value
Tpeak (˚C) (Quadratic)	17.16	<0.001	17.64	<0.001
Tpeak (˚C) (Gauss)	17.53 (±1.98)	<0.001	17.49 (±0.71)	<0.001
Ipeak (Quadratic)	0.02 g d^{-1}	<0.05	1.54 gSU d^{-1}	<0.05
Ipeak (Gauss)	0.02 g d^{-1}	<0.05	1.58 gSU d^{-1}	<0.001
PB (˚C) (Gauss)	13.07 - 21.92 (±1.76)	<0.05	9.61 - 25.38 (±0.85)	<0.001

3.4.2. Respiration rate

After the sensor corrections, the temperature 22ºC still presented two points that indicated abnormal data showing as outliers which were removed.

We assisted to a quite consistent increase in the respiration rates of the sea urchins as the temperature rise, until the maximum test temperature of 30ºC. The Figure 13 depicts the variation of the mean oxygen consumption rates for each temperature level, in fasted and fed conditions, showing that the fed sea urchins exhibit higher respiration rates in almost every temperature level, with a peak at 28ºC. The mean respiration rate of the sea urchins analyzed after quarantine (initial condition) at 16ºC was between both conditions. The comparison between respiration rates in fasted and fed sea urchins appear to be significantly different (one-way ANOVA p-value <0.05). The major difference seen between conditions occurred at the lower temperatures, mainly 8 to 12ºC. At 22 and 30ºC, the respiration rate of fasted sea urchins is superior than that of fed animals but with a slight difference.

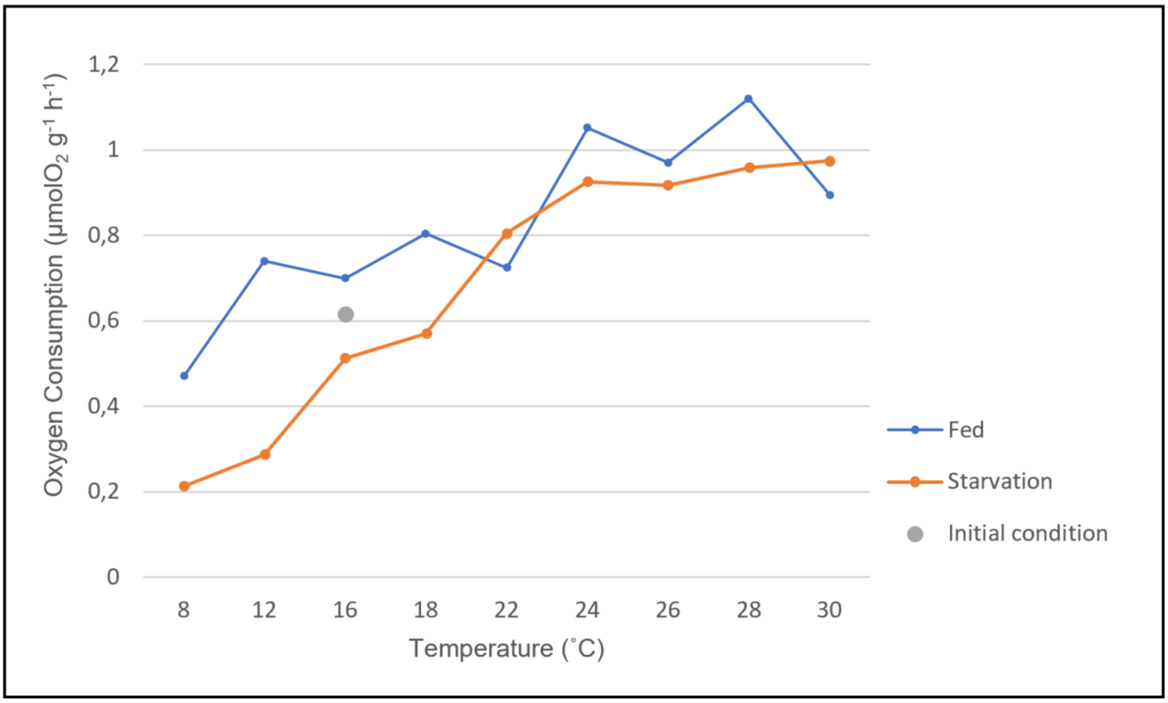

Figure 13 - Mean oxygen consumption rate (μmol O_2 $g^{-1}h^{-1}$) depending on temperature (ºC) for the sea urchins Paracentrotus lividus *at the initial condition at ambient temperature and for acclimated sea urchins in fasted and fed conditions.*

The Figure 14 compares the oxygen consumption rates, through an exponential trend line across the mean respiration rate per gram of fasted and fed sea urchin as function of temperature.

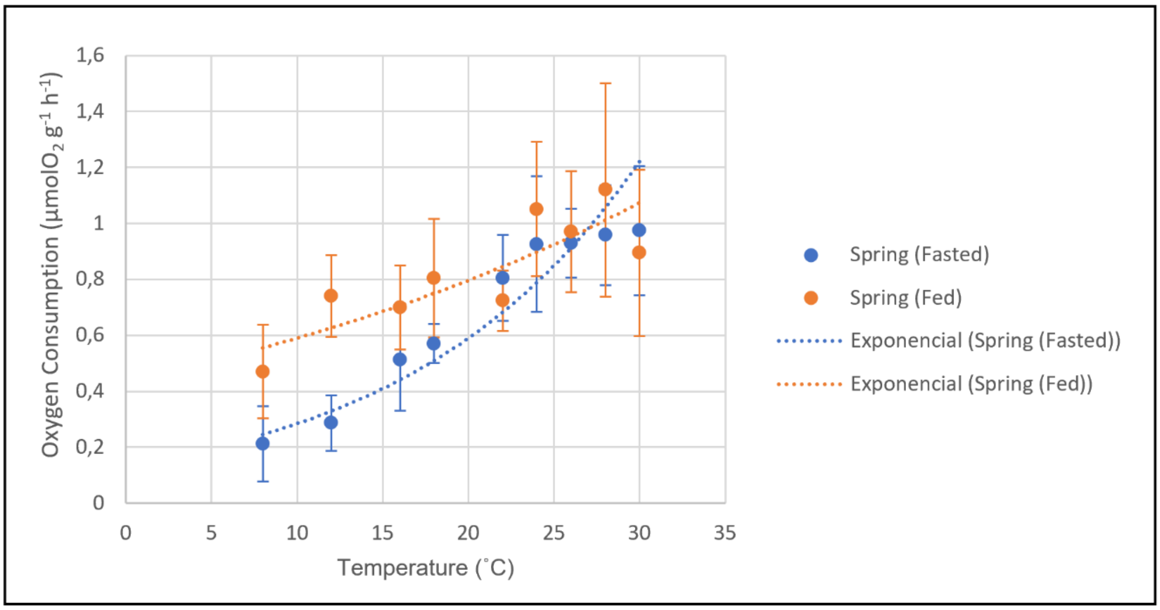

Figure 14 - Mean oxygen consumption rate (µmol O_2 $g^{-1}h^{-1}$) depending on temperature (ºC) in fasted (blue) and fed (orange) sea urchins Paracentrotus lividus. *Error bars representing the standard deviation (SD). Exponential trend curves with R^2 = 0.92 (fasted) and 0.72 (fed).*

Despite the respiration rate in fed sea urchins has a good value of R^2, we can see that the exponential phase stops at the temperature 28ºC, when the respiration has its peak.

Considering the patterns of fasted and fed sea urchins, the next table (Table 7) describes the significant differences in the respiration rates among treatments. The results show that:

- The respiration rate in the fasted sea urchins increases significantly between 8 and 16ºC (p-value <0.01) being significantly different afterwards between every treatment. The respiration rates at 12, 16 and 18ºC levels were also significantly lower than measured rates above 24ºC;
- The sea urchins in fed conditions exhibit major differences in respiration rates mainly between extreme temperatures like 8 and 24 up to 30ºC, as well as between 28 and 12-16ºC (p-value <0.05).

Table 7 - Post-hoc Tukey's p-values between the respiration rates of sea urchins Paracentrotus lividus *at each temperature level. NS: not significant. Blue represents the fasted sea urchins and light grey corresponds to the sea urchins in fed condition.*

Temperature (˚C)	8	12	16	18	22	24	26	28
12	NS NS							
16	<0,01 NS	NS NS						
18	<0,001 NS	<0,01 NS	NS NS					
22	<0,001 NS	<0,001 NS	<0,01 NS	NS NS				
24	<0,001 <0,001	<0,001 NS	<0,001 NS	<0,001 NS	NS NS			
26	<0,001 <0,01	<0,001 NS	<0,001 NS	<0,001 NS	NS NS	NS NS		
28	<0,001 <0,001	<0,001 <0,05	<0,001 <0,05	<0,001 NS	NS NS	NS NS	NS NS	
30	<0,001 <0,05	<0,001 NS	<0,001 NS	<0,001 NS	NS NS	NS NS	NS NS	NS NS

3.4.3. Activation energy

In the Figure 15, the inverse of the slope in the Arrhenius plot represents the activation energy. We found that the slopes are quite similar in both the growth and the feed intake rates, with an activation energy of 0.29 for growth and of 0.24 eV for ingestion as depicted in the Table 8. However statistical references indicated a weak linear relationship in both biological rates, with p-value >0.05, which will be discussed in detail in the next section.

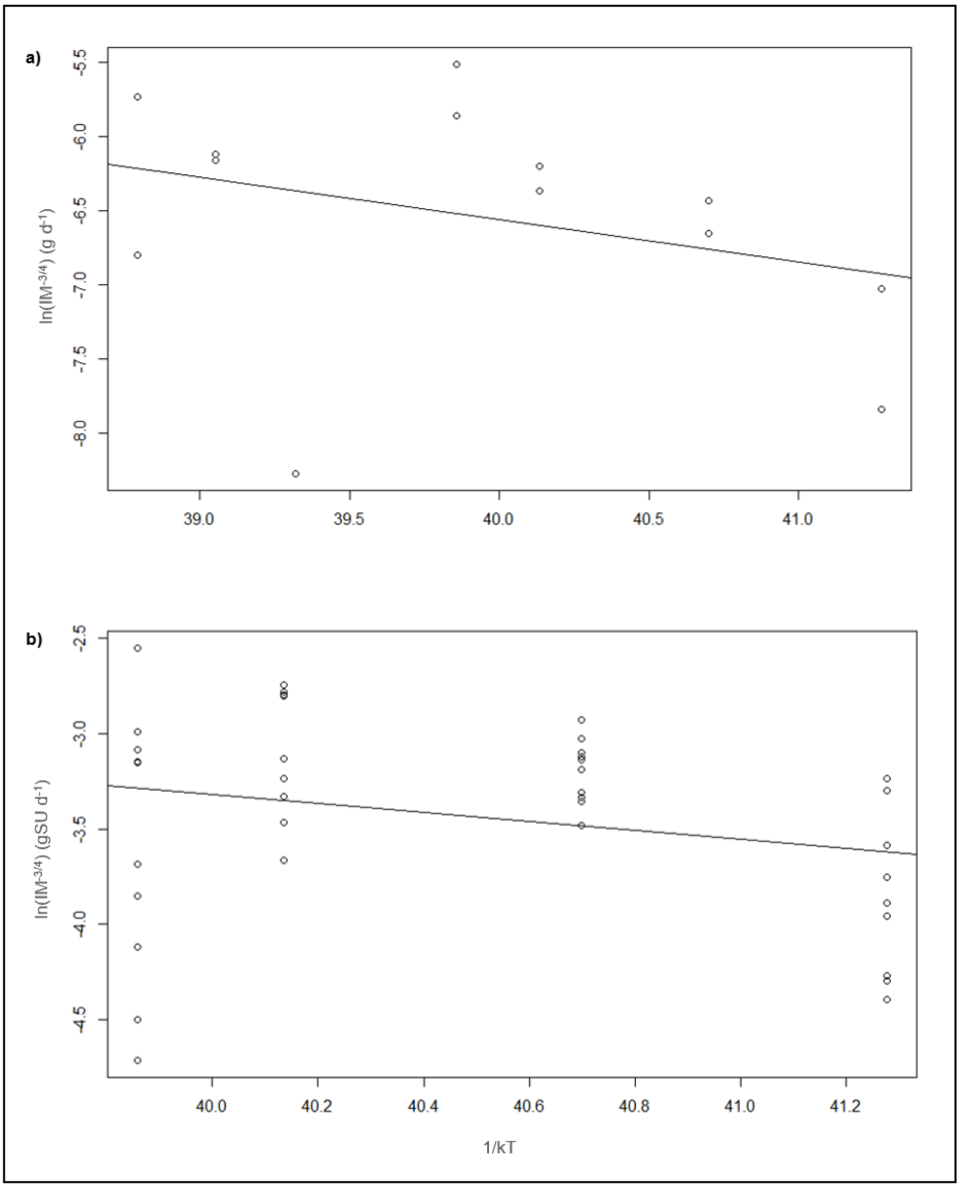

Figure 15 - Plots of the biological rates as a function of temperature and mass for the sea urchins Paracentrotus lividus. *a) Arrhenius plot of the growth rate (ln growth rate vs reciprocal of absolute temperature) with Ea = 0.29; mass correction coefficient =0.75 and linear relationship p-value >0.05. The near-exponential increasing phase for the plot was considered until the second peak of growth; b) Arrhenius plot of the feed intake rate (ln feed intake rate vs reciprocal of absolute temperature) with Ea = 0.24; mass correction coefficient =0.75 and linear relationship p-value >0.05. One negative value at 22ºC (growth rate) and another negative value at 8ºC (ingestion rate) were removed.*

Table 8 - Activation energy (eV) ± standard deviation and p-values for all the tested biological rates of the sea urchins Paracentrotus lividus. *The selected range of temperatures corresponds to the near-exponential increasing phase of each biological rate.*

Biological rate	**Temp. range (°C)**	**E_a (eV)**	**±SD**	**P-Value**
Growth	8 - 24	0.29	0.23	0.30
Ingestion	8 - 18	0.24	0.15	0.14
Respiration (fasted)	8 - 30	0.52	0.04	<0,001
Respiration (fed)	8 - 28	0.28	0.05	<0,005

The next Arrhenius plots (Figure 16) were drawn for the respiration rate of the sea urchins in fasted and fed conditions. The obtained activation energy was 0.52 ± 0.04 eV and 0.28 ± 0.05 eV respectively.

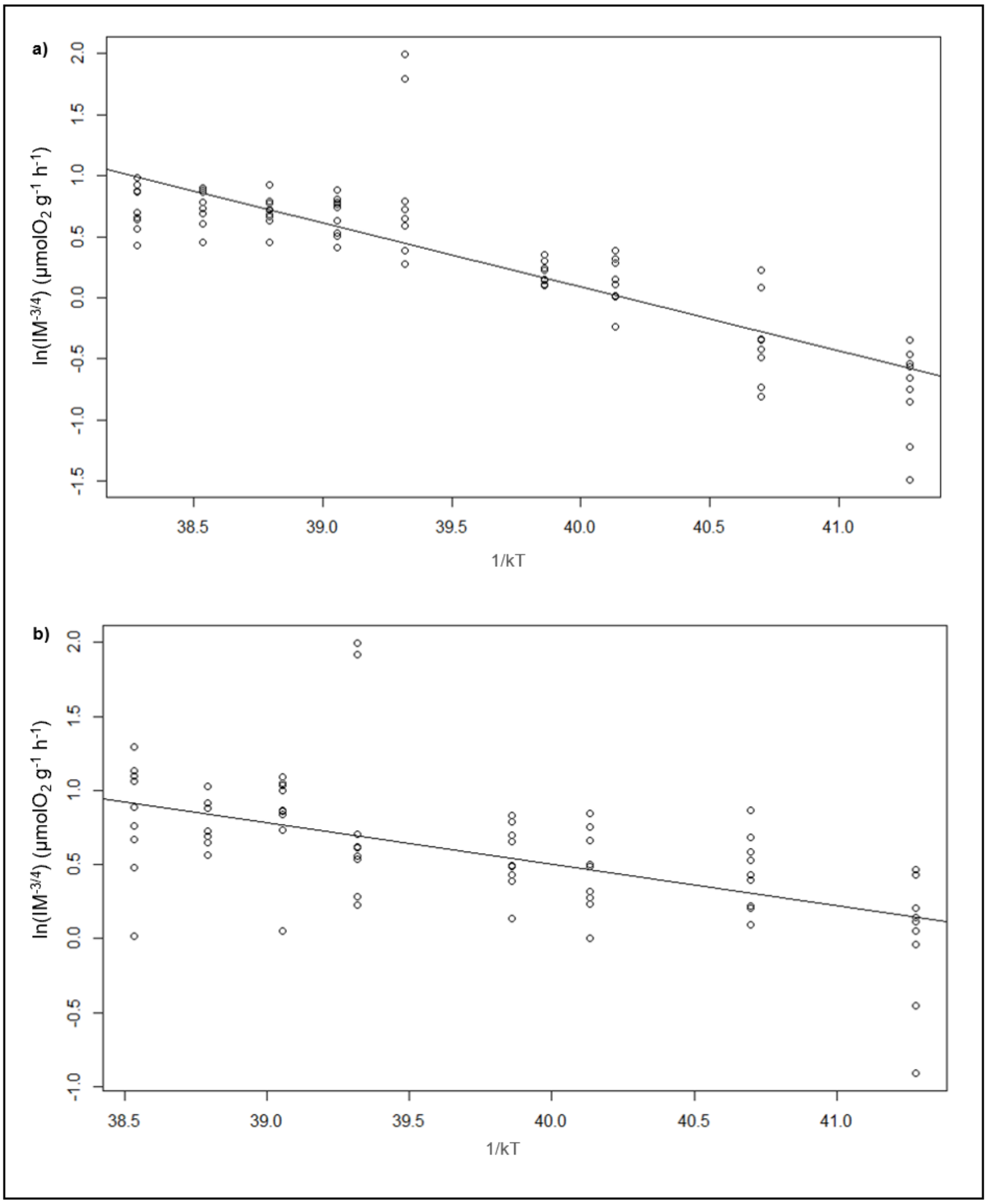

Figure 16 - Plots of metabolic rate as a function of temperature and mass for the sea urchins Paracentrotus lividus *in fasted and fed conditions. a) Arrhenius plot of respirometry activity of fasted sea urchins (ln respiratory oxygen consumption rates vs reciprocal of absolute temperature) with Ea = 0.52; mass correction coefficient = 0.75 and linear relationship p-value <0.001.One point at 12°C was removed because the rate was negative and there is no logarithmic correspondence; b) Arrhenius plot of respirometry activity of fed sea urchins with Ea = 0.28; mass correction coefficient = 0.75 and linear relationship p-value <0.001.*

A summary of all the values of activation energy obtained for the tested sea urchins is represented in the Figure 17.

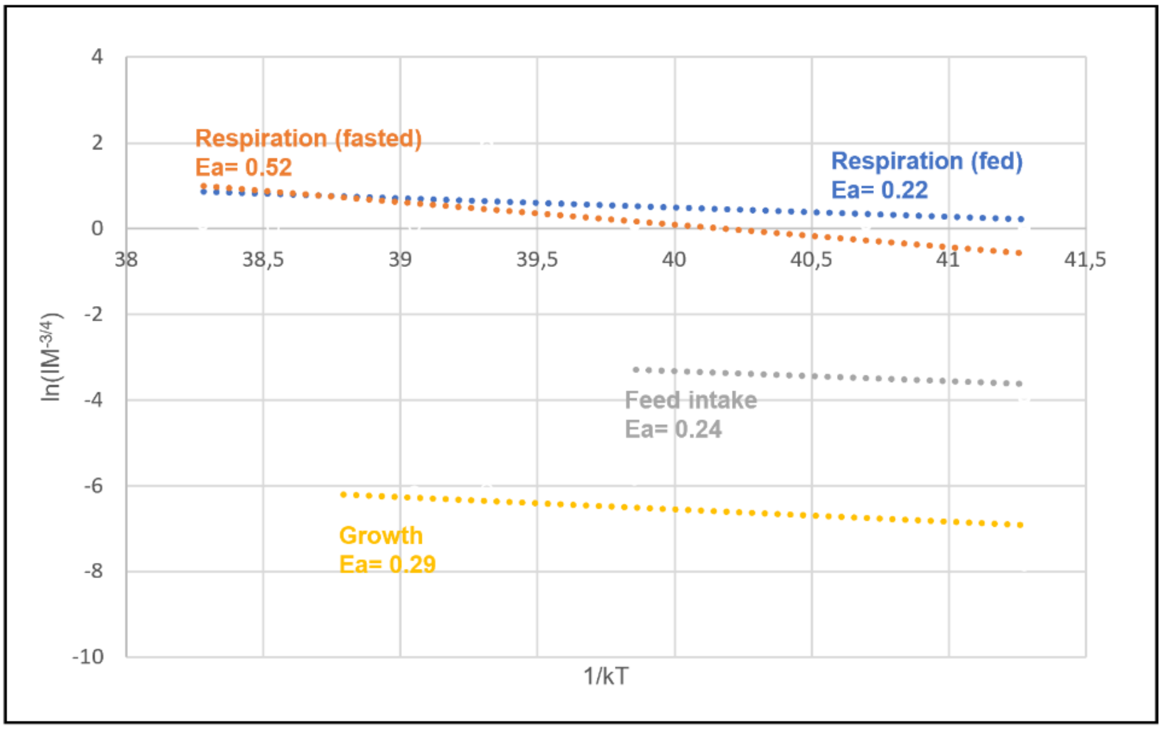

Figure 17 - Arrhenius plot of the different biological rates obtained with the sea urchins Paracentrotus lividus *in fasted and fed conditions, along with the activation energy results (Ea, eV) for growth (g d^{-1}), feed intake (gSU d^{-1}) and respiration (µmol O_2 $g^{-1}h^{-1}$).*

Based in the MTE model, we also looked for the body-mass dependence which is the other pillar of the theory. The respiration rates as a function of body mass at the initial condition (after collection at 16ºC) and for fasted and fed sea urchins (at each temperature level) are depicted in the plots below (Figure 18 and 19). The mean of fasted sea urchins slopes for mass correction exponent (-0.31 ± -0.12) are in agreement with MTE predicted value of (-1/4) for mass specific rates (IM^{-1}). The mass correction presumed for the sea urchins under the initial conditions is similar, showing a value of (-0.16).

However, for the fed sea urchins (Figure 19), the mass correction exponent was not as predicted by theory and the mean value for mass specific rates after removing positive slopes is approx. (-0.63 ± 0.23), indicating a probable fraction of (-2/3). The positive slopes at 16 and 24ºC (in fed condition) were associated to sensor error since the blank controls also gave abnormal measurements.

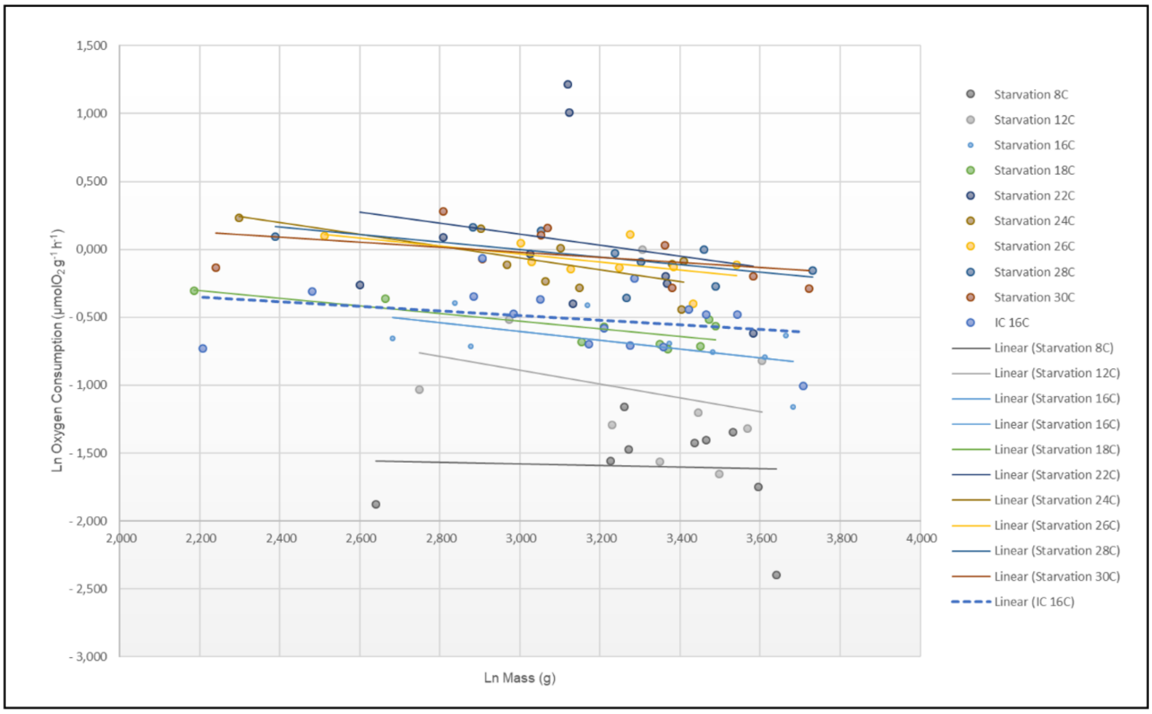

Figure 18 - Log-log plot of the respirometry activity versus mass at each temperature level for the sea urchins Paracentrotus lividus *in fasted conditions. Dashed line represents the sea urchins at the initial conditions at ambient temperature (16ºC).*

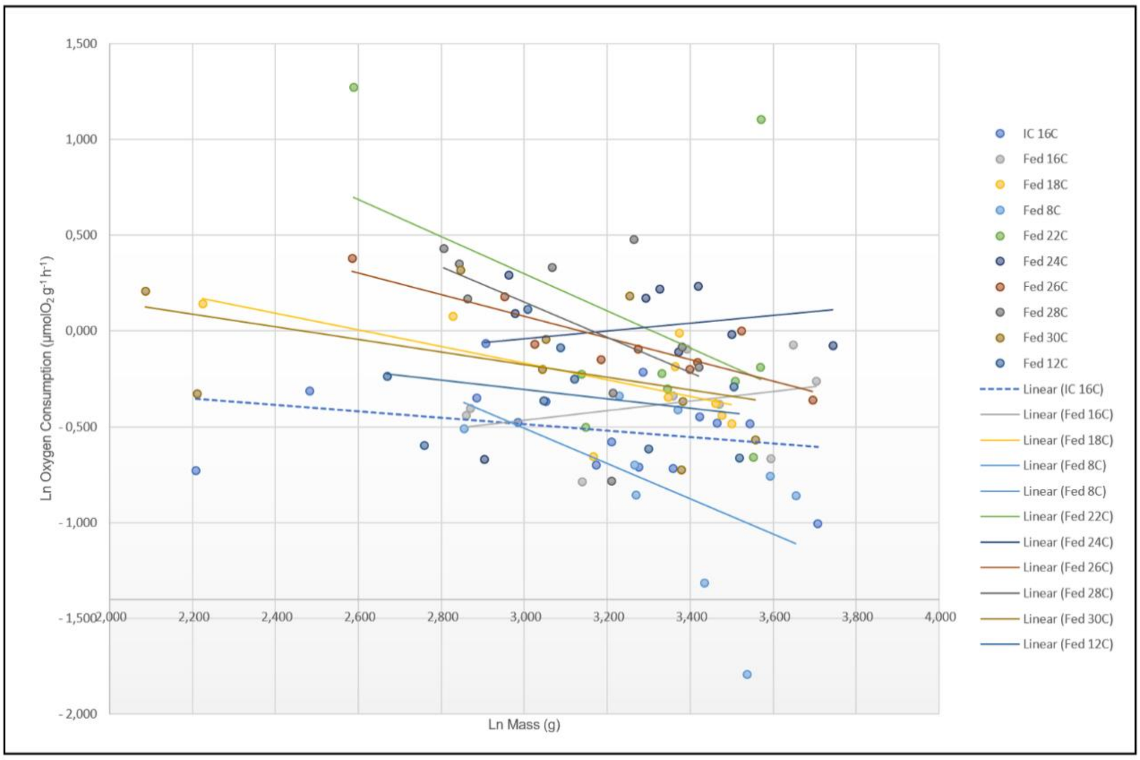

Figure 19 - Log-log plot of the respirometry activity versus mass at each temperature level for the sea urchins Paracentrotus lividus *in fed conditions. Dashed line represents the sea urchins at the initial conditions at ambient temperature (16ºC).*

Taking this in account, replacing the exponent (-3/4) by (-1/3) for the whole organism rate, a new activation energy was found (Ea = 0.19 eV) for the respirometry rate of fed sea urchins, although, the results were similar, and the original exponent was kept for the whole data treatment.

3.5. Species distribution models

The Figure 20 shows the present georeferenced species distribution, plotted on the bathymetry raster (0-200 m).

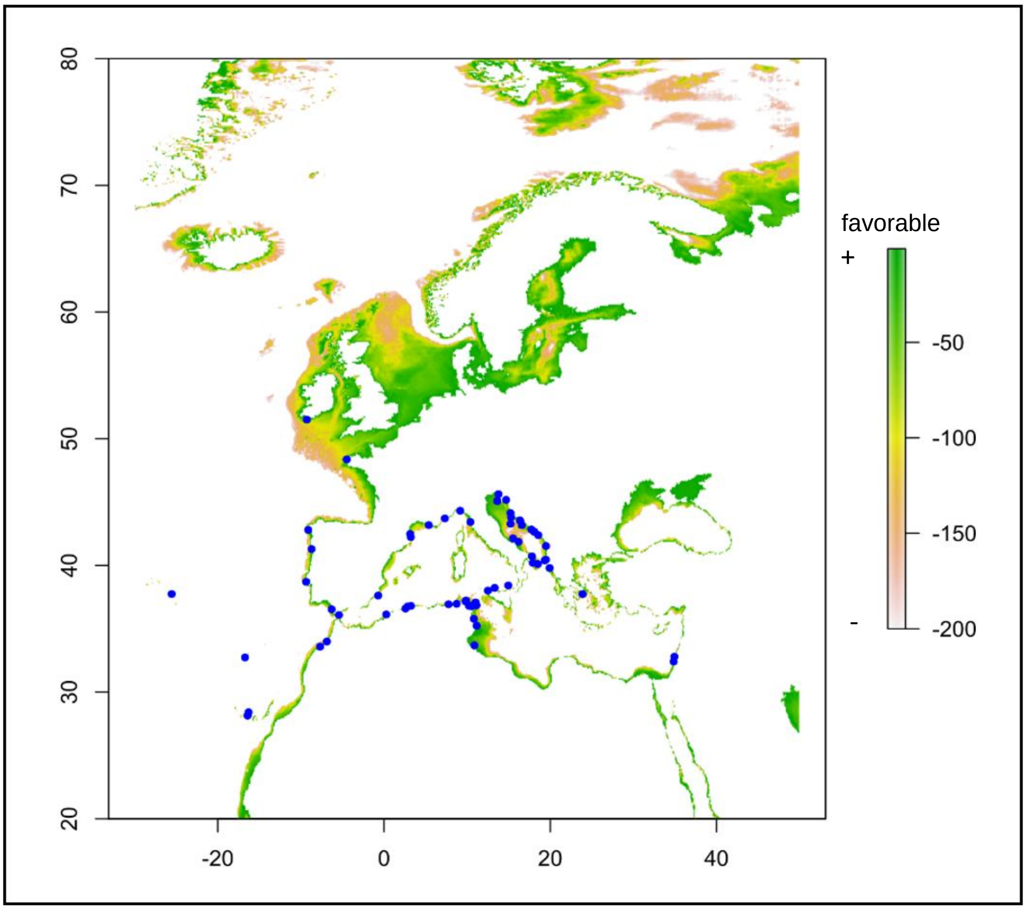

Figure 20 - Records of the geographic distribution of the sea urchin Paracentrotus lividus*, used in this model.*

The species distribution models included five initial predictors (i.e. salinity, pH, maximum annual seawater temperature, minimum annual seawater temperature and seawater temperature range). However after the selection of the most relevant predictors (using permutation procedures from MaxEnt and the variable importance algorithm in Biomod2), we selected three predictors as the more relevant in the modelling distribution of this species: salinity, seawater minimum temperature and seawater maximum temperature (Table 9).

Table 9 - Percent contribution, permutation importance (MaxEnt) and variable importance (GLM Biomod2) of the predictors used in the models. SSTmin, max and range correspond to sea surface temperatures' minimum, maximum and the range between them respectively. Variables in bold were selected for the final model.

	MaxEnt		Biomod2
	Percent contribution	Permutation importance	Variable importance
SST(min)	**8.2**	**44.7**	**0.2316**
SST(max)	**55.5**	**20.2**	**0.2805**
Salinity	**31.4**	**30.7**	**0.2837**
SST(range)	1.1	0	0.1333
pH	3.8	4.5	0.0596

Functional responses for the several runs done to the three selected predictors help to identify the thresholds of the species for each variable (Figure 21). The functional responses to minimum and maximum temperatures were not able to identify clear thresholds. In the Figure 21a, below 5˚C the presence of the species clearly reduce but no upper threshold was identified for maximum temperatures (Figure 21b). In the case of salinity, the functional response shows that the species is marine with a low probability of occurrence in salinities below 30 ppm.

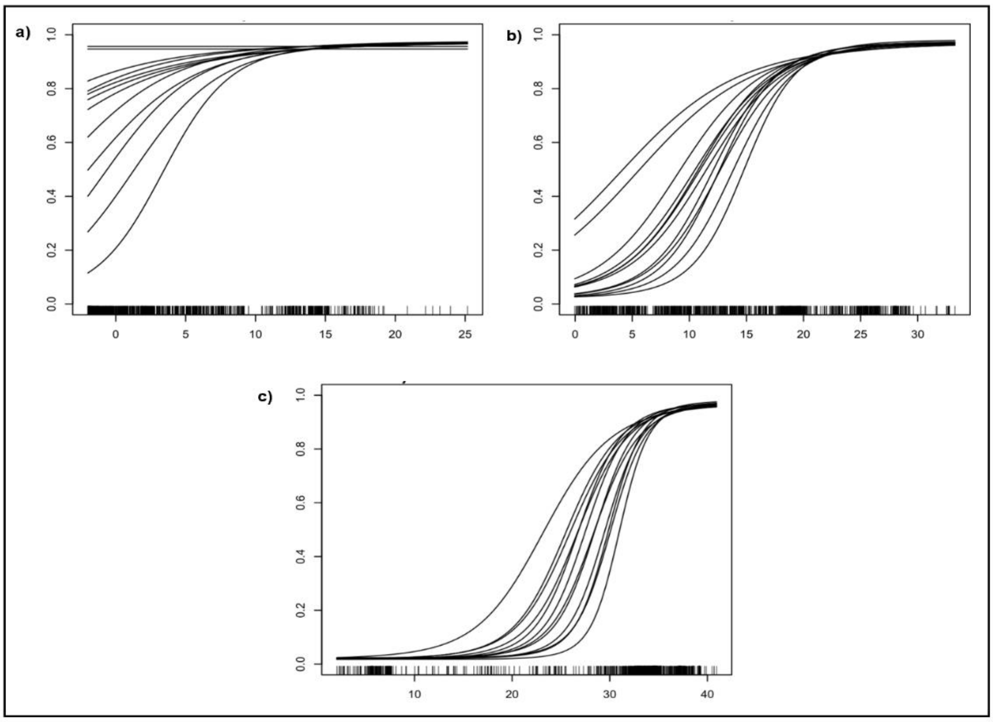

Figure 21 - Functional responses estimated from the different runs of the three predictors used in the SDM models. a) seawater minimum temperature; b) seawater maximum temperature and c) salinity.

The predictive accuracy of the modes was excellent, with a high evaluation score in both models (MaxEnt AUC = 0.897, Biomod2 AUC > 0.95).

The model prediction for the current distribution shows a species with affinities for fully marine warm waters with higher habitat suitability in the Mediterranean and south shores of the Atlantic regions (Figure 22a). Future projections using the worst Representative Concentration Pathways (RCP) scenario (>900 CO_2 ppm) suggest a reduction of habitat suitability for the species in Portugal from a favourable index of 0.5 to 0.2 by 2100 - Figure 22b - where the overall European population also found a decrease in habitat suitability.

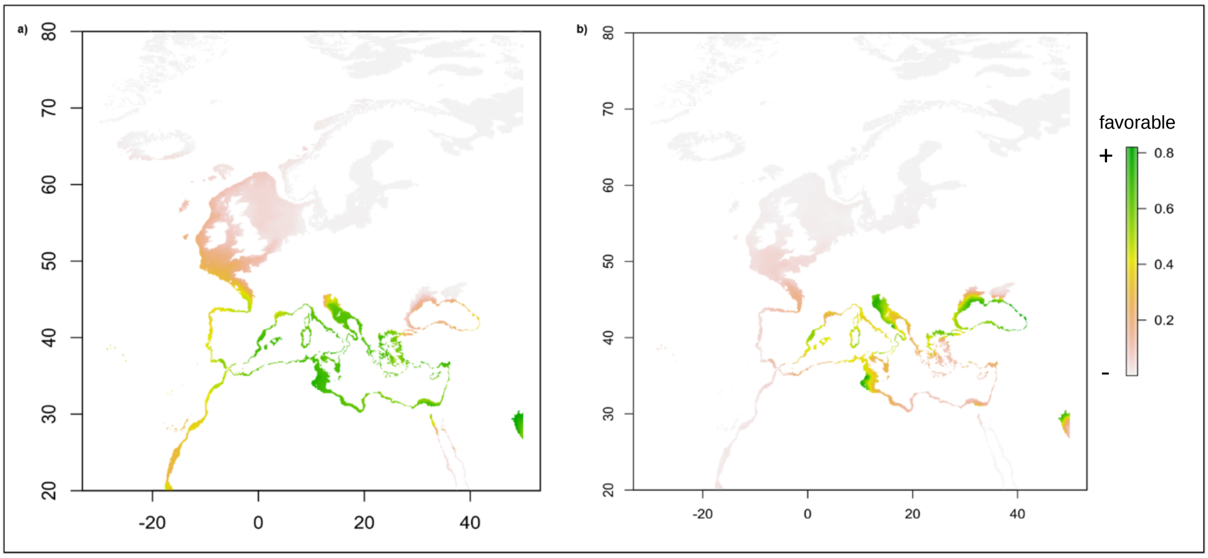

Figure 22 - MaxEnt projections of habitat suitability for Paracentrotus lividus*. a) habitat suitability for the current conditions (year 2002-2009); b) predicted habitat suitability in 2100 with the worst RCP scenario (8.5).*

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4. Discussion

In the present work the growth, the feed intake and the respiration of *Paracentrotus lividus* were investigated for the first time in a population from the north of Portugal where commercial harvesting is intensively increasing. The influence of temperature was depicted allowing to successfully determine the activation energy also for the first time for this population. The activation energy was found to be within the lower range of observations on other marine species and biological processes. In addition, the optimum temperature for growth and ingestion was found to be similar and close to 17ºC, also close to the thermal optimum of Mediterranean populations. The results enabled to apply the species distribution model to the species and to map its future geographic distribution in a global warming scenario, which allowed to draw conclusions on climate change impacts on the Portuguese population and to point advertences on the commercial harvesting.

4.1. Effect of temperature on mortality

A strong relationship between mortality and temperature was found in our investigations. The lethal temperature (obtained from the ninety-days experiment) was relatively high (29.9˚C), considering the only available findings for another Atlantic population: in the English Channel, the upper lethal temperature is 29.0ºC (Mortensen 1943; Le Gall et al. 1990). In contrast, the lethal temperature found for the Portuguese population of sea urchins is similar to the findings carried out by Yeruham et al. (2015). On that study, Mediterranean *P. lividus* maintained in lab conditions at 28.4 – 31.5˚C, also experimented a high mortality when the temperature was above 30.5˚C. The same way, a tremendous decline of the wild stocks of *P. lividus* in Israel was also associated to an increase of water temperature that rose from 29.0˚C (in the ninety's) up to 30.5/31.5˚C in recent years. This suggests that the Mediterranean population lives close to its upper thermal limit. Girard et al. (2012) reported a mass mortality in the population of *P. lividus* in Teneriffe by the year of 2003. As the causes for that mortality, the authors referred to an outbreak of bald disease which occurred during the highest SST (sea surface temperature) and the lowest wave heights of the year, and that both abiotic stressors led to an increase of the sea urchins' vulnerability.

The purple sea urchin *P. lividus* presents two distinct populations, one from the Mediterranean basin and another one from the Atlantic basin, each of them showing panmixia within these two areas (Duran et al. 2004). Since the Mediterranean Sea is warmer, the local populations might present local adaptations and tolerate higher temperature than northern Atlantic populations.

Our results on the lethal temperature, then, suggest that Portuguese *P. lividus* populations are closer to the Mediterranean population than to the Atlantic one. However, a long-term study is required in order to be sure of that since in our ninety-days experiment, the sea urchins had only two months at 30˚C and the mortalities started to pop up at the end of the assay. It would also be interesting to compare the thermal response of other physiological parameters along with the survival between these two populations in the same experimental conditions.

A comparatively minor issue that arise from these results is about the duration of the period of acclimation to temperature, whether it could influence or not. In other parts of the world, subtidal sea urchins from the species *Strongylocentrotus droebachiensis* (Norway) and *Heliocidaris erythrogramma* (Australia) were allowed to adapt at different temperatures for a longer period of time than ours (1ºC changed per day) (Siikavuopio et al. 2008; Carey et al. 2016). Even Yeruham et al. (2015) changed the water temperature by 1˚C per day but in a narrower range of temperature levels that varied between 28.4 to 33.5˚C.
The sea urchins used in our experiment were caught in the intertidal, being naturally adapted to water temperature variation. Due to the proper schedule of the experiment, it was not possible to increase acclimation time but we consider that it should be assessed in future experiments.

4.2. Effect of temperature on biological rates

4.2.1. Growth

Growth in echinoderms means change in mass, diameter, and shape of the test, which requires expansion, calcification, and production of soft tissues (Ebert 2007). Indeed, the body size is one of the most fundamental properties of the organism, affecting several aspects of its biology and ecology (Brown et al. 2004).

Our results suggest the existence of significant differences among growth rates (in weight) recorded for *P. lividus* at different temperature levels. The main factors related with sea urchins growth in the wild are water temperature, food quality and gonadal development

(Fernandez and Pergent 1998), whereas for Spirlet et al. (2000) the temperature is the most relevant factor that governs the growth rates in sea urchins when food supply is unlimited.

The observed growth rates, though apparently small, were consistent with the duration of our experiments which were short (only two months) considering the longevity of sea urchins of about 13 years. Cirino et al. (2017) reported no growth occurrence in a four-month experiment with *P. lividus* and Siikavuopio et al. (2008) considered that somatic growth of *S. droebachiensis* was negligible along a two-month experiment. Two aspects might have interfere with the growth study contributing to the low growth rates: 1) though the animals were fed *ad libitum*, the diet consisted in a single green algae (*Ulva spp.)* species, instead of a nutritionally richer diet as available in the natural habitat. 2) The size of the animals and the season of collection do not guarantee that they were not in sexual maturation and hence allocating less energy to growth. We even observed negative growth in part of the trials, as in a growth study of another sea urchin species *S. purpuratus* (Ebert 2007), suggesting the reabsorption of calcite. Yet, in this previous study a different measurement methodology was used which can be considered more precise because it included the five possible diameters of each sea urchin. We took only one test measurement due to the small test diameter of the organisms to avoid stress and injuries such as breaking spines, but for sure it will increase the measurement error since the test diameter is not a perfect circle.

Even with a slow growth rate, the results clear show a growth peak at 18˚C and a second peak at 26˚C according to weight gain. We are not able to explain what happened at 22˚C – despite the results were not associated with measurement errors, the observed loss of weight at that temperature was not expected and seemed unrealistic because a) the given algae was consumed and b) sea urchins at 24 and 26˚C continued to gain weight as such in the study of Spirlet et al. (2000), where *P. lividus* showed a growth peak at 24˚C. A decline of herbivore biomass is also common when the temperatures increase beyond the optimal range (Lemoine and Burkepile 2012).

Once again, our population of *P. lividus* seemed to be closer to Mediterranean populations than to the Atlantic ones, with its growth peak occurring at 17.53˚C (Gaussian curve). The maximum growth rate registered in populations of the English Channel occurred between 18 and 22˚C (Le Gall et al. 1990) while in the Mediterranean it was between 12 and 18˚C (Azzolina 1988; Fernandez and Caltagirone 1994; Turon et al. 1996).

4.2.2. Feed intake

The amount and frequency of ingestion of food by sea urchins is affected by physical and chemical characteristics of the food, physiological state of the individual, and abiotic environmental conditions (Lawrence et al. 2013).

Several studies have been done to investigate the feed intake in sea urchin species (Lawrence 1987; McBride et al. 1997; Klinger et al. 1998; Siikavuopio et al. 2007; Siikavuopio et al. 2008), but the majority of them focused on commercial food since its scope was aquaculture optimization. Hence, it was hard to find literature on the effects of temperature in feed intake rates when having a natural diet.

As the first assessment of the effects of temperature in an interval between 8 and 30˚C, corresponding to the thermal distribution of *P. lividus* of both Atlantic and Mediterranean populations, our results provide new insights on feed intake across two different body size classes.

Here, as in the study carried out by Spirlet et al. (2000), the water temperature has a relevant factor that influences feed intake rates. Similarly, their study pointed out for high rates of feed intake in a range of 16 to 24˚C, and in our case the feed intake peaks occurred at 16 and 22 ˚C; yet, they were only significant different from ingestion rates obtained at 8, 26 and 30˚C.

The optimal temperature for ingestion in *P. lividus* was in accordance with the one for growth rate, which was around 17˚C as determined through both quadratic and Gaussian thermal performance curves. The decreased feed intake at higher temperatures such as 28 and 30˚C can be a response to thermal stress, as it was reported by Watts et al. (2011).

Surprisingly, the results obtained between the different body-size classes are different than the ones obtained in the past, highlighting that feed intake increase with test diameter (Nedelec et al. 1983; Siikavuopio et al. 2008). Indeed, our results showed a contrary pattern that was coherent along the experiment, with medium-sized sea urchins eating more than the large ones. We cannot assure however if the animals were under sexual maturation, which could interfere in food requirements and hence with feed intake.

Since we have a different pattern of feed intake for each temperature level along the trials (see Annex I), generally with higher levels of food consumption in the beginning, and a stable decrease in the following trials, it seems that the effect of temperature change is dependent on the thermal history of the sea urchin, also described by Watts et al. (2011). In fact, several studies have pointed out for the effect of short-term changes in temperature on the rate of ingestion in several sea urchins' species (Moore and McPherson 1965; Klinger et al. 1986; Hofer 2002), suggesting a feed acclimation.

4.2.3. Respiration rates

The metabolic rates of ectotherms usually increase with temperature, within their natural thermal maximum (Cossins and Bowler 1987). The size of the animals (Brown et al. 2004) and the feeding condition (Brody 1945) are fundamental matrices in its performance.

Brockington and Clarke (2001) concluded that a few percentage of the summer rise in metabolism in the wild sea urchin *Sterechinus neumayeri* was caused by the temperature but mostly, due to the increase of the physiological activity associated with feeding, growth and spawning. Actually, our study confirms these relationships. Both water temperature and nutritional condition of the sea urchins were evaluated and were considered statistically significant on influencing the oxygen consumption.

The oxygen consumption rate of *P. lividus* increased exponentially in the whole range of test-temperatures for fasted sea urchins. Similar conclusions have been found in other species of sea urchins such as *Eucidaris tribuloides*, *S. droebachiensis*, *S. purpuratus* and *Arbacia stellate* (McPherson 1968; Percy 1973; Siikavuopio et al. 2008; Díaz et al. 2017). This pattern suggests that high thermal tolerance may come with a significant fitness and energetic costs (Díaz et al. 2017). For the fed set of sea urchins, the respiration rate was even higher, which is due to a phenomenon called Specific Dynamic Action (SDA). The SDA is characterized by a rapid increase in the metabolic rate after feeding because of the energy expended on all the activities of the body regarding ingestion, digestion, absorption and assimilation of a meal (Secor 2009). This feeding status is known to lead to an increase in the respiration rate of many organisms, where aquatic crustaceans (Robertson et al. 2001) and bivalves (Sarà et al. 2013) are also included.

4.3. Activation energy and the consequences of different thermal optimums among the studied rates

The activation energy determines the strength of the temperature dependence (Cornish-Bowden 2004) or, in other words, it represents the sensitivity of a given process to changes in temperature. In this study the activation energy was determined using three biological rates: growth, feed intake and oxygen consumption rates. Values of the activation energy were all within the expected range of 0.2 to 1.2 eV suggested by Gillooly et al. (2001), but lower than the average of about 0.60-0.70 eV referred by Brown et al. (2004) as the typical activation energy observed for most biochemical reactions of metabolism. More recently, the range was enlarged to 0.08 to 3.52 eV through a meta-analysis on consumption rates as function of temperature (Norman 2012). The average proposed by Brown et al. (2004) was also criticized because most results (about 87%) were out of the predicted range and the value of 0.77 eV was obtained as the average (Norman 2012), which is still higher than all the values obtained for *P. lividus*.

In the present work, the activation energy for the respiration rates of the sea urchins ranged from 0.28 to a maximum of 0.52 eV, respectively in fed and fasted animals. This suggests an influence of feeding activity on the activation energy, with temperature dependence being weak when the organisms were in good feeding conditions. Childress and Letcher (2017) even considered that organismal performance under lab conditions with unlimited food can underestimate the impacts of high temperatures. Rocky shore snails *Echinolittorina malaccana* have also an activation energy for the oxygen consumption rate in the lower range (0.05 to 0.43 eV) (Marshall et al. 2010).

The activation energy obtained from feed intake and growth rates were very similar between each other: 0.24 and 0.29 eV respectively. But, the linearity was not significant maybe due to: a) utilization of an equation specifically for respiration rates; b) few observations and c) short term experiments.

Although the metabolic theory of ecology, MTE (Gillooly et al. 2001; Brown et al. 2004) predicts equivalent thermal responses (i.e. similar Ea) for all biological functions, the experimental evidence not always support this hypothesis. Many examples exist of differences in the activation energy, or of mismatch in optimal temperature for closely related metabolic processes. This is the case of the activation energy and optimum temperature of haemolymph oxygen tension, ventilation and heart rates of the decapod crustacean *Maja squinado* (Frederich and Pörtner 2000). Indeed, if we considered the entire range of test-temperatures for every studied rate, we will also find a mismatch (see Annex 2 and 3). These deviances are particularly important in the context of climate change since higher metabolic rates relative to other biological rates can result in an overall reduction in fitness (Lemoine and Burkepile 2012).

Though the activation energy for the respiration rate of fed sea urchins is almost half of that of fasted sea urchins, in the present study, this parameter was very close between the three evaluated biological rates. Even more consistent was the optimum temperature determined for growth and feed intake rates which was 17.53 and 17.74ºC, respectively.

Unfortunately, it was not possible to determine the critic temperatures which define the upper and the lower tolerance limits for the sea urchins. The thermal response of various biological processes is often unimodal within the limits of functional integrity and displays an increasing pattern when temperature increases, until reaching a temperature optimum at which the velocity of the rate of the biological process is maximal. The present results on growth and feed intake rates allowed to fit to a quadratic function and to Gaussian (normal) curve and both enable the determination of a maximum value for the rates (the optimum temperature). Yet, data was insufficient to allow an accurate determination of the critical temperatures.

4.4. Future of sea urchin populations

The response of species to global warming depends on how different populations are affected by increasing temperature throughout the species' geographic range (Gardiner et al 2010).

Physiological thresholds and correlative functional responses from species distribution models performed quite accordingly in shaping the response of *Paracentrotus lividus* to temperature. Thus, our confidence in the performance of our modelling exercise is high. However, the Species Distribution Model (SDM) used did not take in account biological predictors such as food availability, which can also compromise the existence/ absence of species. Unfortunately, there were no available projections for algae distribution as well as other biological stressors.

The projections from our model showed some worrying evidences of a decrease of the suitability of Portuguese coastal habitats for the sea urchin populations. Contrary to what was expected, apparently the species will not prefer higher latitudes, where the increase in temperature was expected to be favourable, moving to East instead, probably as a result of a combination of the other environmental factors considered in the model. Obviously, uncertainty of these models is high but the results are consistent with similar predictions for other coastal species (Martinez et al. 2015; Assis et al. 2016).

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5. Conclusions

The response of a poikilothermic organism to an increase of the temperature has negative effects at the molecular level, with obviously consequences in their fitness. Hence, the temperature is an important environmental factor affecting the physiology, behaviour and distribution of aquatic ectotherms.

The present study provided new insights on the effect of the temperature in a common ectotherm species in the rocky shore of Portugal, the European Purple Sea Urchin *Paracentrotus lividus*, which is presently under an increasing pressure of commercial harvesting. Additionally, we also found a relationship with feeding conditions, seeming that the thermal plasticity of the sea urchins may be dependent on its nutritional condition, since the activation energy in fed sea urchins was lower than that of sea urchins deprived of food for two days.

In summary, our findings suggest that an increase in the temperature promote growth and increase feed intake and oxygen consumption until an optimum level is reached. There was an overlap of the thermal optimum for growth and ingestion rates (~17˚C) whereas respiration rate reached its peak at higher temperatures. The higher metabolic rates relative to other biological rates can result in an overall reduction in fitness for the organism but mainly, higher metabolic rates and warmer water favour the reduction of oxygen availability which may pose a risk for the entire marine ecosystem, as it happened in the past, 252 million years ago.

The physiological activity of the sea urchin here presented is the base for the study of the predicted impacts of climate change in the fitness of a marine key species. We anticipate through modelling a reduction on the area of habitat suitability for the species due to climate change. In particular for the Portuguese population, the reduced suitability might highly compromise the commercial exploitation. This emphasises the need for a proper stock management, based on scientific monitoring, to assure a future sustainable harvesting of sea urchins.

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Annex

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Annex 1 - Feed intake rates per trial for each temperature level

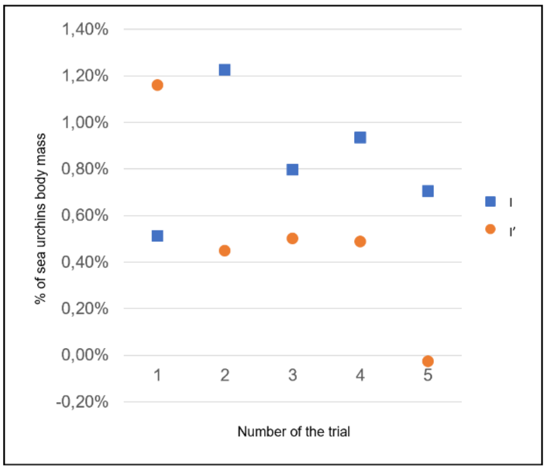

Figure 1 - Feed intake rate as a percentage of body mass of Paracentrotus lividus *at 8˚C, for trials 1, 2, 3, 4 and 5. Blue for cylinder I and orange for cylinder I'.*

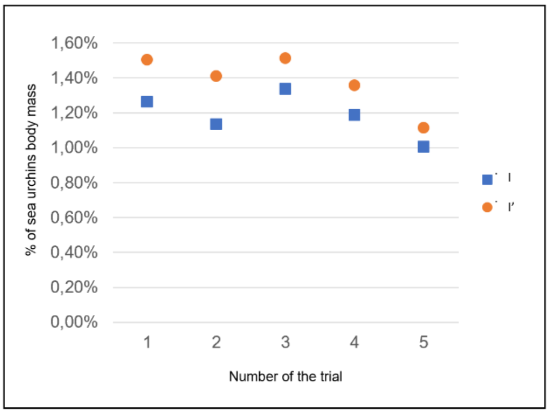

Figure 2 - Feed intake rate as a percentage of body mass of Paracentrotus lividus *at 12˚C, for trials 1, 2, 3, 4 and 5. Blue for cylinder I and orange for cylinder I'.*

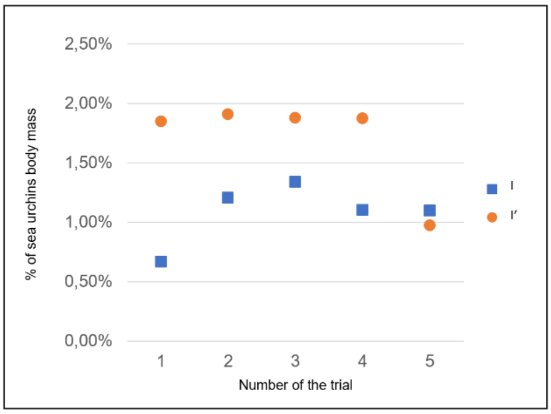

Figure 3 - Feed intake rate as a percentage of body mass of Paracentrotus lividus *at 16˚C, for trials 1, 2, 3, 4 and 5. Blue for cylinder I and orange for cylinder I'.*

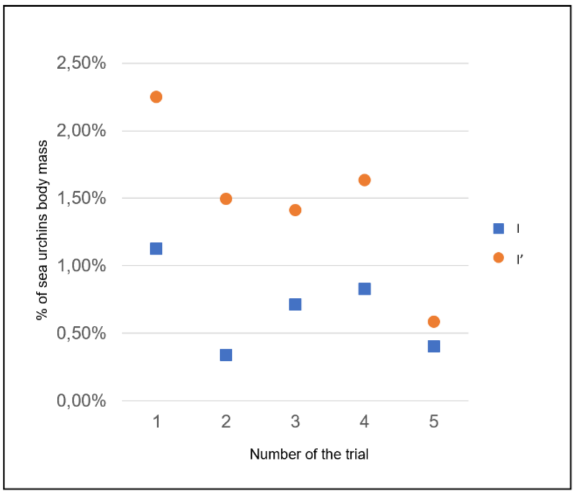

Figure 4 - Feed intake rate as a percentage of body mass of Paracentrotus lividus *at 18˚C, for trials 1, 2, 3, 4 and 5. Blue for cylinder I and orange for cylinder I'.*

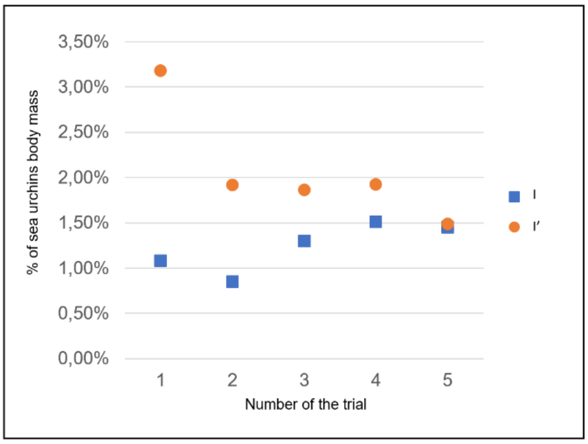

Figure 5 - Feed intake rate as a percentage of body mass of Paracentrotus lividus *at 22˚C, for trials 1, 2, 3, 4 and 5. Blue for cylinder I and orange for cylinder I'.*

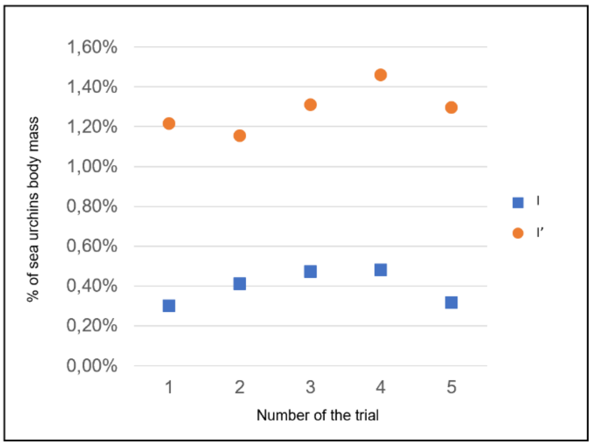

Figure 6 - Feed intake rate as a percentage of body mass of Paracentrotus lividus *at 24˚C, for trials 1, 2, 3, 4 and 5. Blue for cylinder I and orange for cylinder I'.*

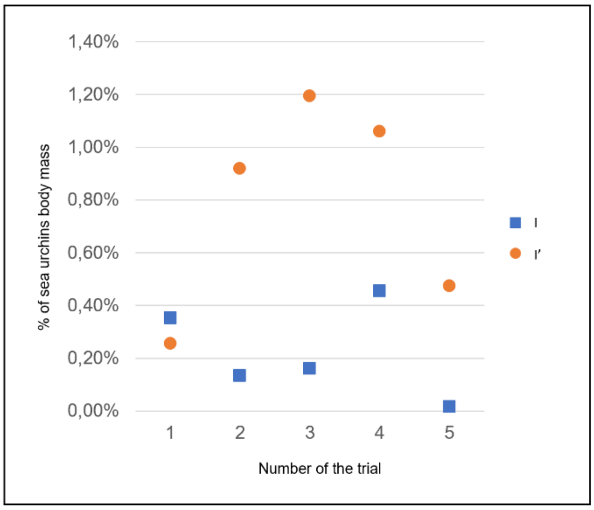

Figure 7 - Feed intake rate as a percentage of body mass of Paracentrotus lividus *at 26˚C, for trials 1, 2, 3, 4 and 5. Blue for cylinder I and orange for cylinder I'.*

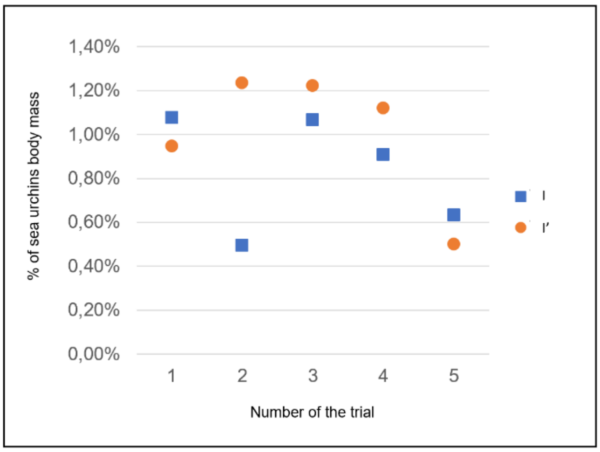

Figure 8 - Feed intake rate as a percentage of body mass of Paracentrotus lividus *at 28˚C, for trials 1, 2, 3, 4 and 5. Blue for cylinder I and orange for cylinder I'.*

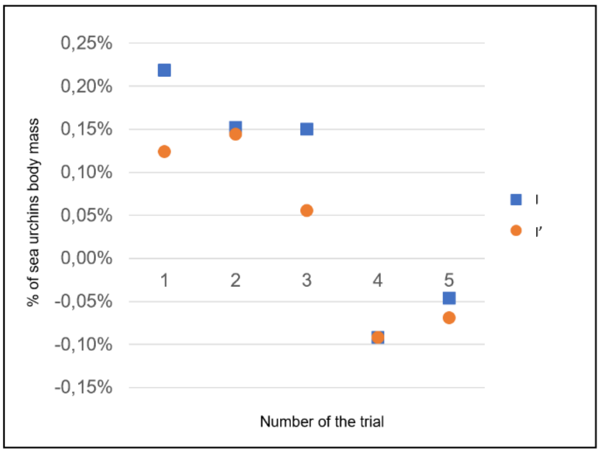

Figure 9 - Feed intake rate as a percentage of body mass of Paracentrotus lividus *at 30˚C, for trials 1, 2, 3, 4 and 5. Blue for cylinder I and orange for cylinder I'.*

Annex 2 – Growth, feed intake and respiration rates’ trend lines for the whole temperature levels

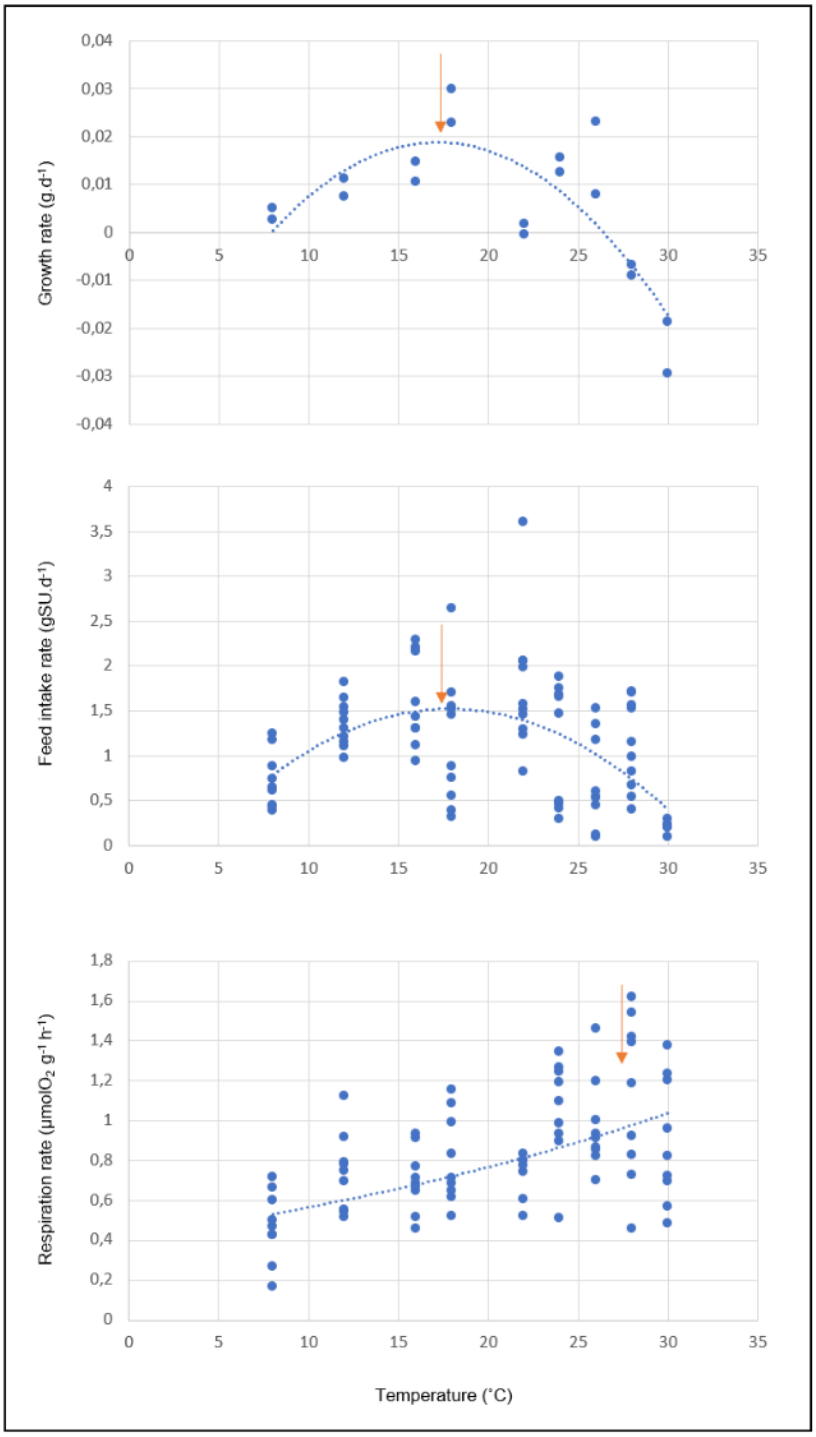

Figure 1 - Growth, feed intake and respiration rate for Paracentrotus lividus *fed ad libitum between 8 and 30˚C. Orange arrows point to the thermal peak of each rate.*

Annex 3 – Arrhenius plots for growth, feed intake and respiration rates within the whole range of temperatures

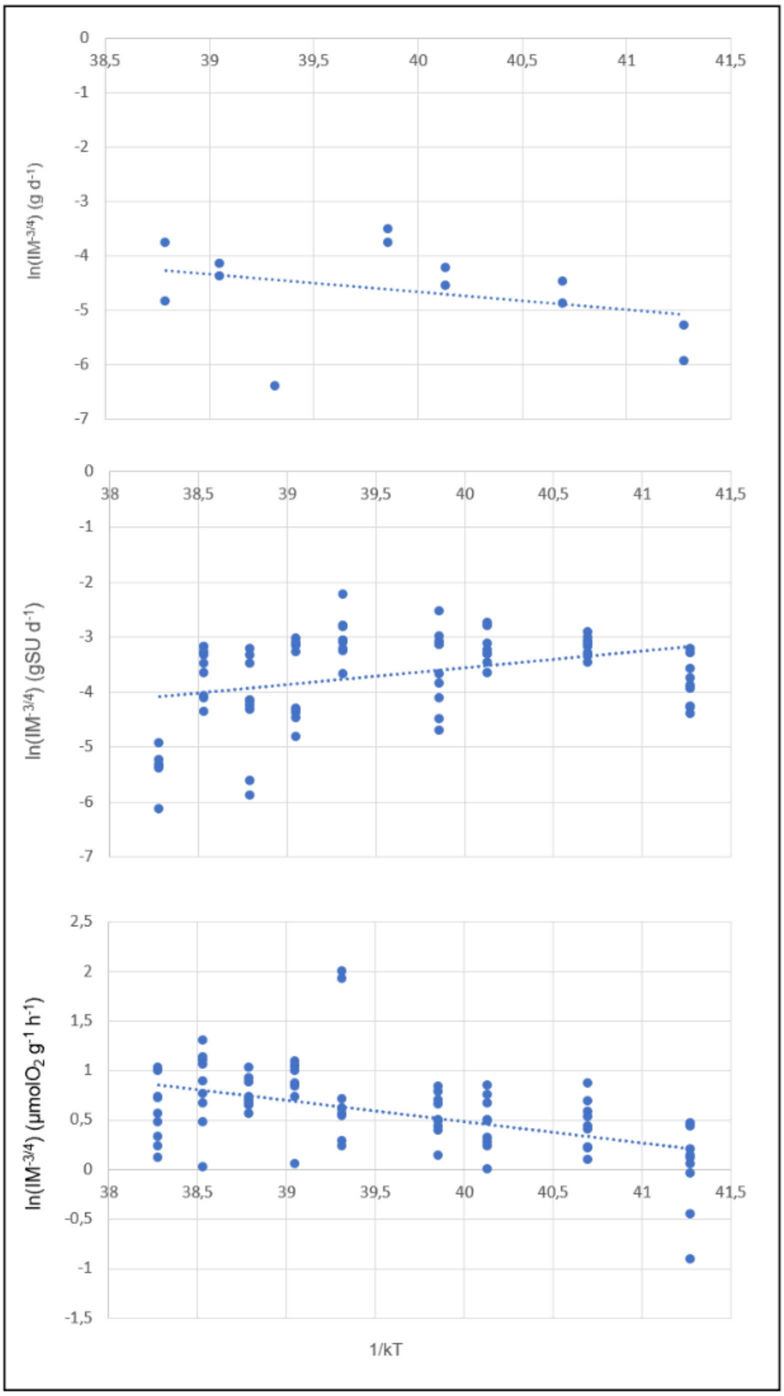

Figure 1 - Arrhenius plots for growth (Ea =0.29), feed intake (Ea = -0.30) and respiration rate (Ea = 0.22) of Paracentrotus lividus. *For the growth rate, it was not possible to draw the plot in the whole range of temperatures due to negative values for temperature levels above 26˚C.*

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