



Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Moderately elevated temperatures increase macroalgal food consumption in two sea urchin species from coastal waters of Madeira

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ARTICLE INFO

Keywords:

Invertebrate macro-grazer
Ocean warming
Arbacia lixula
Paracentrotus lividus
Consumption rates
Gonad index
Sea urchins
Madeira

ABSTRACT

Rising sea surface temperatures affect the feeding behaviour and reproductive success of many coastal benthic invertebrates. This experimental study investigated the effects of ocean warming on macroalgal food consumption rates in the sea urchins *Arbacia lixula* and *Paracentrotus lividus* from Madeira Island to assess how the feeding pressure they exert may change under warmer ocean conditions. Additionally, in *A. lixula*, the relationship between temperature and the gonad index was examined to estimate potential future reproductive output. Over the course of 25 days, 180 individuals of each species were exposed to temperatures between 22 °C and 31 °C. After 20 days, consumption rates were assessed in 48-h feeding trials. Gonad indices of *A. lixula* were determined on the last day of the experiment. In the thermal range investigated, both traits were found to be unimodal functions of temperature. In addition to temperature, consumption rates in both species and the gonad indices in *A. lixula* were influenced by body size, while feeding in *A. lixula* also varied between sexes. Maximum food consumption rates (*A. lixula* and *P. lividus*) as well as maximum gonad indices (only *A. lixula*) were observed between 25 °C and 26 °C. These values are at the upper end of the temperature range that currently prevails around Madeira, and may become average summer temperatures by the year 2100. Consequently, both sea urchin species may thrive during future warm summers and may enhance their top-down control on local macroalgal populations.

1. Introduction

Temperature influences the metabolism of marine ectotherm species and can, by this, control their performance and reproduction success (Gillooly et al., 2001; Byrne et al., 2009; Tagliarolo et al., 2018). Many biological processes that are directly related to metabolic rates exhibit a unimodal performance curve when they are assessed across the suitable temperature range (Huey and Stevenson, 1979; Dell et al., 2011; Lemoine and Burkepile, 2012). Within the temperature range from 0 °C

to 40 °C, metabolic rates in ectotherms first increase almost proportionally with temperature, while above a critical temperature they decrease again rapidly (Gillooly et al., 2001; Dell et al., 2011; Lemoine and Burkepile, 2012). The decrease in performance beyond a certain temperature is caused by the fact that the energetic costs of cellular maintenance increase faster with warming temperatures than the rate at which energy can be supplied to the cells via, e.g., consumption – an imbalance that becomes more evident with larger body mass (Lemoine and Burkepile, 2012). Furthermore, at higher water temperatures,

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<https://doi.org/10.1016/j.jembe.2021.151603>

Received 13 November 2020; Received in revised form 10 March 2021; Accepted 3 June 2021

Available online 15 June 2021

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marine ectotherms additionally face reduced oxygen concentrations (IPCC, 2014), and this limits their capacity to feed or reproduce (Lemoine and Burkepile, 2012).

While increasing global mean temperatures alter ecosystems and their functioning gradually (IPCC, 2014; Hobday et al., 2016), extreme events (e.g. marine heat waves), which are predicted to increase in frequency and intensity (Smale et al., 2019; Stillman, 2019), can have sudden and strong impacts on the structure of populations and the composition of communities in marine benthic systems (Gianguzza and Bonaviri, 2013). This is because temperature anomalies can abruptly alter metabolic rates and resource demands in marine ectotherms (Rall et al., 2010; Lemoine and Burkepile, 2012). Several experimental studies demonstrated that a non-lethal temperature increase can significantly enhance grazing rates in aquatic herbivores and the extent to which they exert a top-down control (e.g. Barton et al., 2009; O'Connor et al., 2009; Yvon-Durocher et al., 2010; Kratina et al., 2012).

Sea urchins are ubiquitous omnivores that can have a severe impact on macroalgal populations, either by affecting benthic systems directly, e.g. by removing kelp, or indirectly, e.g. by restraining macroalgae from overgrowing coral reefs (Steneck, 2013). In extreme cases, fluctuations in sea urchin population density can cause shifts from one stable state to another, for instance, when the animals create and maintain 'barren grounds' (Gianguzza et al., 2011; Agnetta et al., 2013; Alves et al., 2019).

Altered food consumption rates, as an immediate response to changes in the ambient temperature, have been reported for many sea urchin species (reviewed in Lawrence et al., 2013). Furthermore, water temperature has also been found to influence gonadal tissue production and thus the reproductive output of these animals (Azad et al., 2011; Gianguzza and Bonaviri, 2013; Wangensteen et al., 2013). In the Mediterranean Sea, several strong increases in local densities of *Arbacia lixula* were presumably caused by a higher recruitment success that was mediated by elevated water temperatures (Francour et al., 1994; Wangensteen et al., 2012; Gianguzza and Bonaviri, 2013).

On the predominantly rocky shores of Madeira Island, the presence of sea urchins and the impact of waves are the main factors that control the abundance and distribution of macroalgae (Alves et al., 2001; Ferreira and Kaufmann, 2004; Alves et al., 2019). Alves et al. (2001) found *A. lixula* and *Paracentrotus lividus* to be among the most dominant local sea urchin species. They occur in intertidal rock pools and in the shallow subtidal if rocky substrates are present (Wirtz, 1995; Alves et al., 2001). Because of their impact on benthic ecosystems, both sea urchin species are considered ecosystem engineers (Wangensteen et al., 2012).

Arbacia lixula commonly inhabits the upper infralittoral (Wangensteen et al., 2012; Gianguzza and Bonaviri, 2013). Like other sea urchins, it is an omnivorous species (Privitera et al., 2008) but shows a tendency towards carnivory (Wangensteen et al., 2011; Trenzado et al., 2012; Agnetta et al., 2015). It usually forages on encrusting coralline algae and the associated endolithic fauna (Bulleri et al., 1999; Agnetta et al., 2013; Gianguzza and Bonaviri, 2013). This species does not exhibit external sexual dimorphism (Wirtz, 1995; Gianguzza and Bonaviri, 2013), while gonad colour differs between female (red) and male (yellow) individuals (Gianguzza and Bonaviri, 2013). The test diameter, for which sexual maturity has been reported, ranges from 6 to 14 mm (Gianguzza and Bonaviri, 2013).

Paracentrotus lividus can be found on natural and artificial hard substrates where it commonly hides in crevices or burrows (Alves et al., 2001; Ramalhosa et al., 2017). This species consumes opportunistically whatever organic matter is available (Hernández et al., 2007; Lawrence et al., 2013; Steneck, 2013), but seems to prefer algal material (e.g. Neill and Larkum, 1966; Neill and Pastor, 1973; Verlaque and Nédélec, 1983). While this species is gonochoric, sex can only be determined microscopically.

We examined food consumption rates in *A. lixula* and *P. lividus* as a function of water temperature to assess potential changes in the feeding pressure that these species might exert in a warming ocean. In the case of

A. lixula, we additionally investigated the gonad index as a function of temperature to assess potential changes in the reproductive output of this species.

2. Material and methods

2.1. Study site

This study was conducted on Madeira Island, a volcanic island with 153 km of rocky coastline that is located southwest of continental Europe and about 700 km off the Moroccan coast (Alves et al., 2003) (Fig. 1A). The experiments were conducted from June to August 2017 in the laboratory facilities of MARE – Marine and Environmental Research Centre, located at Quinta do Lorde Marina, Madeira, Portugal (32°44'31"N 16°42'45"W, Fig. 1B–D). From 2014 to 2018, the monthly average sea surface temperatures derived from NOAA satellite observations ranged from 17.5 °C (March) to 23.5 °C (September) with an overall mean of 20.7 °C (Schäfer et al., 2019).

2.2. Sea urchin collection

We collected sea urchin individuals first on June 26 and again on August 11, 2017. Both times, we picked 180 individuals of *Arbacia lixula* from several large rocks at a water depth of 3–5 m by free diving, while 120 individuals of *Paracentrotus lividus* were collected from several intertidal rock pools at low tide (Fig. 1D). The collection sites were 250 m away from the laboratory facilities of MARE in Quinta do Lorde. The ambient sea surface temperature (SST) during collection was 22 °C in June and 23 °C in August. All individuals were immediately placed into containers filled with seawater and transported to the laboratory. Upon arrival, they were moved to aerated 10 L aquaria in monospecific groups of 10–15 individuals. For the next two days, they were acclimatised to laboratory conditions at the temperature that prevailed during collection. On the third day of each experiment, we selected 90 individuals of each species that exhibited high mobility, rigid and upward-facing spines, and ambulacral feet that could adhere strongly to surfaces, thereby not showing any common signs of stress or disease (Wang et al., 2013). Individuals that did not fulfil these criteria were returned to the sea. We recorded each animal's test diameter (size) and wet weight. The size of individuals ranged from 3.7 cm to 5.1 cm, with a mean of 4.5 ± 0.3 cm in *A. lixula* (± standard deviation (SD); n = 180), and from 2.0 cm to 4.5 cm in *P. lividus*, with a mean of 3.2 ± 0.4 cm (± SD; n = 180). The mean wet weight of *A. lixula* individuals was 38.1 ± 7.7 g (± SD; n = 180), while it was 8.3 ± 2.8 g (± SD; n = 180) for *P. lividus*.

2.3. Experimental design and set-up

We exposed the sea urchins to temperatures between 22 °C, which was the SST during collection in June, and 31 °C. The latter temperature was expected to be above or close to the sea urchins' upper thermal tolerance limit. In the first experiment during June/July 2017, the following temperature levels were applied: 22 ± 0.3 °C (SST at the time), 23 ± 0.3 °C, 25 ± 0.1 °C, 27 ± 0.0 °C, 29 ± 0.1 °C, and 31 ± 0.1 °C (mean ± SD; monitored every 10 min by HOBO Pendant temperature loggers). In the second experiment, in August 2017, we focused, with a resolution of 1 °C, on the range around the temperature at which the sea urchins showed the highest consumption rates during the first experiment (i.e. 26 °C). Hence, we had the new temperature levels 24 ± 0.1 °C, 26 ± 0.1 °C, and 28 ± 0.1 °C (mean ± SD; monitored every 10 min by HOBO Pendant temperature loggers) and kept the levels 23 ± 0.3 °C (SST at the time of the second experiment), 25 ± 0.1 °C, and 27 ± 0.0 °C, which had already been realized in the first experiment.

We manipulated water temperature in six water baths (80 cm × 90 cm × 15 cm, 108 l), of which each represented one temperature level and contained a total of 35 partially submerged experimental units (EU). All EUs were independent, i.e. the water bodies inside the EUs were

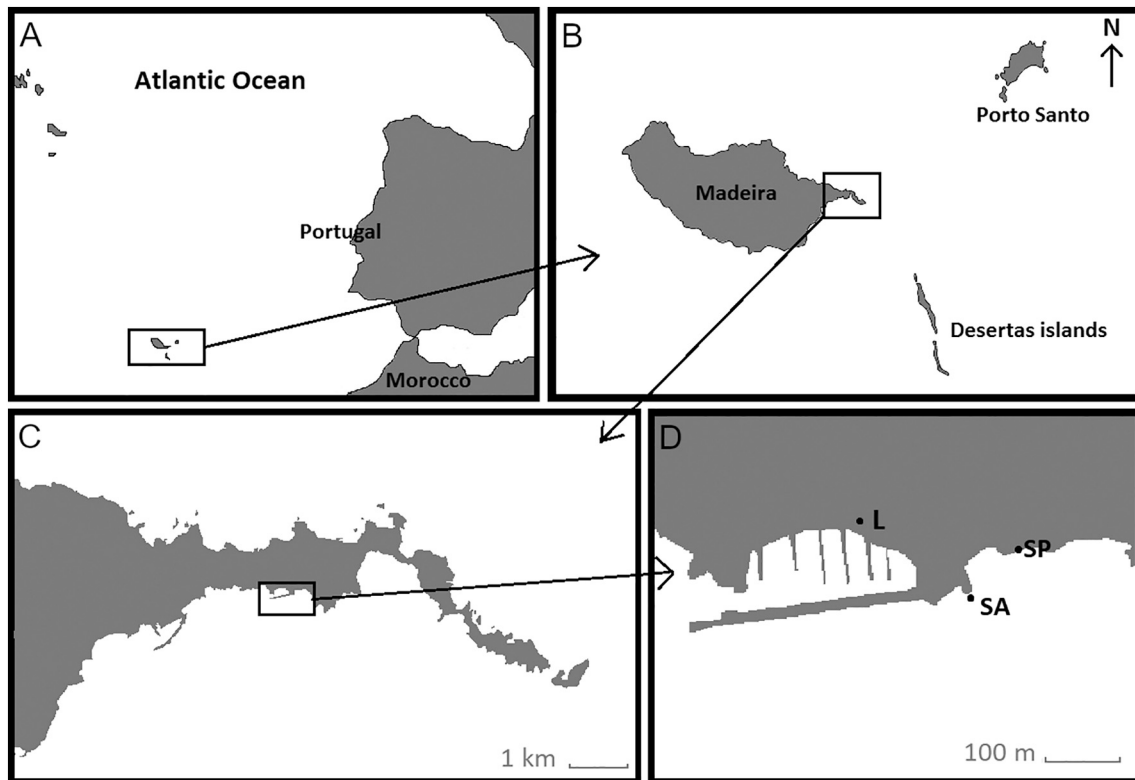


Fig. 1. Location of Madeira Island in the northeast Atlantic (A) and of the marina of Quinta do Lorde (D) in Caniçal at the southeast coast of the island (B - D). “L” indicates the laboratory facilities, “SA” the site where *Arbacia lixula* and “SP” where *Paracentrotus lividus* were collected.

isolated from each other and from the water baths. Light conditions and levels of disturbance (i.e. regular maintenance) were the same for all EUs.

To adjust the water temperature inside the EUs, each water bath was equipped with up to two titanium electric heaters (Scheço 300 W; Schemel & Goetz GmbH and Co KG), which were controlled by digital twin-circuit temperature regulators (HOBBY, Biotherm). Immersible pumps (Hailea IPX8 BT-200B; 3 W) ensured homogenous mixing of the water inside the water baths to avoid the formation of temperature gradients. For achieving the lower temperatures, we additionally installed electric cooling units (HOBBY, Aqua Cooler V4), which were also controlled by the HOBBY regulators. The regulators allowed to adjust the temperature at minimum intervals of 0.2 °C. In each water bath, we randomly chose two EUs to contain a temperature logger (HOBO Pendant) for continuous monitoring. One of these two EUs also contained the sensor of the respective temperature regulator for automated temperature control. Fig. 2 illustrates the laboratory set-up.

The sea urchins were randomly assigned to the EUs, which each held one individual sea urchin, and each water bath contained 15 EUs per species. The EUs were small buckets made of transparent propylene (13 cm top diameter x 14 cm height) with outflow holes at the top to maintain a constant volume of 1.1 l. Each EU was almost fully submersed in its water bath (Fig. 2). To maintain a good water quality, every morning and evening, one litre of sea water from pre-heated storage tanks was allowed to drip into each EU at a rate of 20 ml min⁻¹. Hence, the volume of water in each EU was renewed twice a day. The storage tanks were refilled every second day with seawater from the nearby ocean. Electric pumps (Hailea Hi-Blow HAP-120) provided air to each EU to keep the oxygen level above 80% saturation. Biofilms were removed from the walls of the EUs every fifth to sixth day using paper towels. Once a week, we inspected four randomly chosen aquaria for ammonia levels (consistently <0.15 ml l⁻¹) and oxygen concentrations (consistently >80% saturation). No additional light was provided, but large windows allowed the animals to experience a natural day/night cycle.

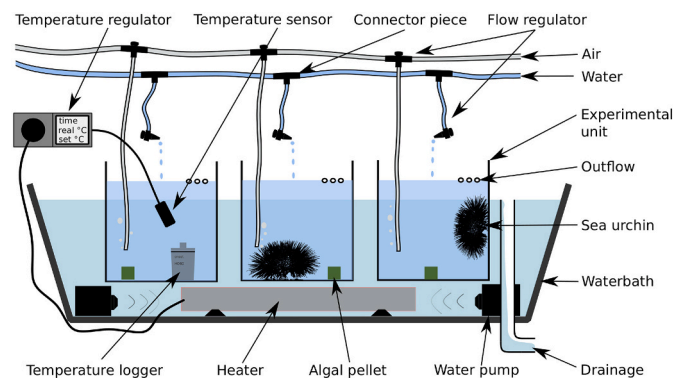


Fig. 2. Laboratory set-up with three experimental units (EUs), two of which contain a sea urchin and a food pellet (green), while the third EU contains a pellet control, a temperature logger and the sensor of a temperature regulating unit.

Prior to the start of temperature manipulation, the sea urchins were acclimatised to laboratory conditions for a total of five days. During this time, they were kept at the SST that prevailed during collection. Then, the water temperature was gradually increased at a rate of 1 °C per day (0.4 °C in the morning and 0.6 °C in the evening) over a maximum period of 10 days. We started with the replicates that were destined to experience the highest target temperature, while the other groups followed in a consecutive order. This ensured that all test individuals reached their respective target temperature on the same day. After this, the animals were kept under target thermal conditions for another 10 days. A 48-h feeding assay, which served to assess the sea urchin's consumption rates (cf. 2.5), was conducted after the animals spent 5.5 days at the respective target temperature. Gonads were extracted on the last day of the experiment.

Until target temperatures were reached, the sea urchins were fed with algal pellets *ad libitum* (mean dry weight per pellet: 0.52 ± 0.01 g (\pm SD)) every second day. However, prior to the feeding assays, we starved the animals for 4 days to increase the probability that they would feed.

2.4. Mortality

While only very few *P. lividus* died during the experiments, ten *A. lixula* individuals were found dead in the first experiment before the target temperature was reached on day 15. Seven of these died before warming even started. In the second experiment, 38 *A. lixula* died before the target temperature was reached, of which 34 were again dead before warming started. We assume that the differences in mortality rates between species can be explained by differences in their capacities to tolerate disturbance. While *P. lividus* individuals were collected from intertidal rock pools, which can experience extreme environmental fluctuations during the course of one tide, *A. lixula* individuals were collected from the shallow subtidal (cf. 2.2). Furthermore, we observed loss of spines and skin lesions on some dead sea urchins, which may be indications of disease (Wang et al. 2013). However, we have no confirmation on whether disease was involved in the observed mortality rates or not. Nevertheless, we carefully selected individuals that appeared to be in good physical condition at the beginning of the experiment (cf. 2.2) and data were collected only from surviving animals, which all appeared to be in good condition.

Survival rates at the target temperatures were also higher in *P. lividus* than in *A. lixula*. At 31 °C, the highest temperature that we applied, *A. lixula* individuals reached T_{L50} after four days and all individuals died before the end of the experiment. However, 60% of the *P. lividus* individuals survived at 31 °C during the same time span. At 29 °C, 56% of the *A. lixula* individuals survived the full 10 days at target temperature, while survival rates at the lower temperatures were always higher than 80% in this species. In contrast to this, *P. lividus* specimens showed an average survival rate of more than 90% across all temperatures lower than 31 °C.

2.5. Feeding assays and consumption rates

Sea urchins were fed with an artificial mono-diet, which consisted of pellets made from agar-agar and *Ulva rigida* powder. Since the objective of this study was to measure feeding rates across a range of temperatures in two sea urchin species, we decided to use hand-made pellets instead of live macroalgae. This allowed us to provide a standardised diet consisting of known ingredients, and we avoided changes in the texture or nutritional value of the food because of the temperature increase. *Ulva* spp. is usually well received as a food source by sea urchins (Schlosser et al., 2005; Souza et al., 2008; Cyrus et al., 2015), presumably because *Ulva* spp. contains large amounts of dimethylsulfoniopropionate (DMPS) that is known to be a strong feeding stimulant for sea urchins (Cyrus et al., 2015). Furthermore, according to studies that used pellets as food for sea urchins, the soft, agar-based, negatively buoyant, block-like material is generally well accepted and easily ingested by these animals (e.g. Klinger, 1982; Lawrence et al., 2013; Watts et al., 2013).

For each feeding event, we produced fresh algal pellets from agar-agar (SPECIAL INGREDIENTS LTD) and *U. rigida* powder (Banco Español de Algas, www.marinebiotechnology.org). For approximately 100 pellets, a suspension made of 10.8 g agar-agar and 150 ml of distilled water was heated (Microwave 800 W) until it scummed. It was then immediately mixed with a premade suspension of 30 g algae powder in 120 ml of distilled water. The hot mixture was poured into a silicon mould with cubic indentations. After 30 min, the mixture had solidified and the pellets (1.5 cm \times 1.5 cm \times 1.2 cm; dry weight: 0.523 ± 0.014 g, mean \pm SD) were removed from the mould, air dried for three hours and fed to the sea urchins.

During the feeding assays, which lasted for 48 h each, every sea

urchin was supplied with algal pellets *ad libitum*. Additionally, at each temperature level, five pellets were kept as controls in the absence of sea urchins to assess a possible autogenic change in pellet weight due to degradation or water soaking. At the end of the assays, we retrieved the pellet remains, dried them at 80 °C until their weight remained constant and recorded the dry weight. By subtracting the dry weight of the pellet leftovers from the mean dry weight of the pellets that were kept in absence of animals we determined the amount of pellet material consumed by the sea urchins. We averaged the autogenic change across all temperature levels as we did not find any difference between pellets that were kept at different temperatures. We then standardised the consumed pellet dry weight by sea urchin wet weight and by the duration of the feeding assay following this equation:

$$\text{consumption rate} = \frac{\text{dry weight}_{\text{pellet consumed}}}{\text{wet weight}_{\text{sea urchin}} * \text{time}} = \frac{[\text{mg}]}{[\text{g}] * [\text{d}]}$$

2.6. Assessing the gonad index

On the last day of the experiment, we recorded the total wet weight of each animal. Then we dissected *A. lixula* individuals by removing their feeding apparatus and breaking the test open to extract the gonads and to measure their wet weight. We were unable to identify the sex in individuals of *P. lividus* due to logistic constraints at the time, so we refrained from removing their gonads.

Gonad indices of *A. lixula* were calculated by dividing gonad wet weight by whole animal wet weight and multiplying the result by 100 (Fuji, 1967; Vadas Sr. et al., 2000; Azad et al., 2011):

$$\text{gonad index} = \frac{\text{wet weight}_{\text{gonads}}}{\text{wet weight}_{\text{sea urchin}}} * 100 = [\%]$$

A comparison with nine individuals that were collected at the beginning of the experiment revealed that gonad indices did not differ between specimens collected in the field and those that were kept at ambient SST during the experiment.

The sex of the *A. lixula* individuals was determined by gonad colour. Ova in female ovaries are dark red, while the spermatozoa in male testes are yellow (Gianguzza and Bonaviri, 2013).

Gonad weights were only determined in individuals from which gonads could be extracted undamaged and clearly separated from other sea urchin material. Sampling damage and release of gonad material during dissection reduced the number of suitable samples.

2.7. Data analysis

Sea urchin individuals that died or spawned before the end of the experiment were excluded from the analysis. Hence, consumption rates were measured in 82 individuals of *A. lixula* (31 females and 51 males) with at least five replicates per temperature level, while gonads were extracted from four to seven replicates per temperature level (16 females and 26 males). As for *P. lividus*, we measured consumption rates in 163 individuals with a minimum of 11 replicates per temperature level.

For the analysis, we pooled the data that were collected during the two parts of the experiment. This was decided because the ambient SST at our collection site differed only by approximately 1 °C between June and August 2017, and pooling allowed us to examine performance over a larger temperature range (nine instead of six temperature levels). Furthermore, there are no reports of seasonality, e.g. distinct reproductive cycles, in *A. lixula* or *P. lividus* from the waters around Madeira that would indicate autogenic change in the sea urchins over the time period between the experiments.

After the targeted temperatures were reached in the experiment, the survival of the sea urchins was monitored to determine median lethal time (T_{L50}) as a function of temperature. To analyse the influence of temperature on consumption rates in *A. lixula*, we applied an Analysis of Covariance (ANCOVA), in which we tested for the influence of sex as a

categorical covariate, test diameter as a continuous covariate, and temperature as a continuous predictor. We included temperature as a quadratic term in the model because we expected a unimodal thermal performance curve. The first, most complex model included all possible interactions between the independent variables. We then applied model simplification to find the minimal adequate model. When we found a significant interaction between sex and diameter, we used simple regressions to check how the effect of diameter differed between male and female sea urchins. In the same way as consumption rates, gonad indices of *A. lixula* were modelled as a function of sex, diameter and as a quadratic function of temperature. Correlations were used to assess a possible relationship between gonad index and consumption rates. We verified whether the assumptions of ANCOVA and simple regression were met by plotting histograms of the residuals and applying Shapiro-Wilk's-W tests ($10 < n < 70$) or Lilliefors corrected Kolmogorov-Smirnov normality tests ($n > 70$; R package 'nortest' [Gross and Ligges, 2015]) to test for normal distribution. We plotted the residuals as a function of the fitted values and applied Fligner-Killeen tests to confirm homogeneity of variances. To test for autocorrelation in the residuals, we used the Durbin-Watson test, and checked for influential data points using Cook's distance.

For analysing the consumption rates measured in *P. lividus*, we used a multiple regression that included test diameter and the effect of temperature as a quadratic term. Similar to the analysis of data from *A. lixula*, we first included all possible interactions and then simplified the maximal model to the minimal adequate model. Model assumptions were verified as described above. Furthermore, we checked for multicollinearity between predictors using the variance inflation factor.

All statistical analyses were conducted using the free statistical computing programme R (RStudio Team, 2020; R Core Team, 2020). Data were visualized using the package 'ggplot2' (Wickham, 2009).

3. Results

3.1. Food consumption rates

Arbacia lixula.

Food consumption rates in *A. lixula* varied with water temperature but were additionally influenced by the animal's sex. However, the amount of variation that was explained by the model was low (Table 1). Food consumption was a unimodal function of temperature (Fig. 3A), and, according to the modelling, peaked at 25 °C. No data were obtained for the group that was kept at 31 °C, since all individuals died before the end of the experiment.

When pooled across all temperature levels, female sea urchins consumed $1.9 \text{ mg} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ (difference between medians), i.e. about 1.5 times more pellet mass than male individuals. We additionally observed a significant interaction between sex and body size as well as between body size and temperature (Table 1). These interactions emerged because consumption rates decreased with increasing size in female *A. lixula* (linear regression, $R^2 = 0.11$, $F = 4.67$, $p = 0.04$), while there was a (non-significant) trend for consumption rates to increase with increasing size in males (Fig. 3B).

Table 1

Influence of sex, test diameter and temperature on the consumption of food pellets by the sea urchin *Arbacia lixula*. Results from ANCOVA: $R^2 = 0.16$, $F = 4.14$, $p < 0.01^*$. Asterisks indicate significant p -values.

Predictor	Estimate	Standard error	t	p
Sex (M)	-18.70	7.70	-2.43	0.02*
Test diameter	4.19	3.98	1.06	0.30
Temperature (quadratic)	0.05	0.03	2.08	0.04*
Sex (M): Test diameter	3.83	1.70	2.25	0.03*
Test diameter: Temperature (quadratic)	-0.01	0.01	-2.13	0.04*

Additionally, the relationship between body size and consumption rates varied with temperature: At 22 °C and 23 °C, consumption rates clearly increased with test diameter, while at 24 °C, 27 °C and 28 °C the opposite was the case (Supplement Fig. S1). At the remaining temperature levels, no clear relationship between body size and consumption rates emerged.

Paracentrotus lividus.

Temperature influenced food consumption in *P. lividus* significantly (Table 2), and the relationship followed a typical thermal performance curve, which peaked just above 25 °C (Fig. 3C), like in *A. lixula*. Another part of the variance in food consumption rates was explained by the body size (test diameter) of the sea urchins, since individuals at the lower end of the sampled size range consumed more food per unit biomass than larger individuals (Fig. 3D).

3.2. Gonad index

Gonad indices in *A. lixula* were significantly influenced by temperature and body size (Table 3). In contrast to the sea urchins' consumption rates, the sex of the animals had no influence on the gonad indices and was therefore eliminated from the model. The overall amount of explained variation was again low (Table 3). Similar to the consumption rates, gonad indices were a unimodal function of temperature (Fig. 4A), and reached a maximum between 25 °C and 26 °C. The larger the *A. lixula* individuals were, the higher was their gonad index when averaged across all temperature levels (Fig. 4B). However, for this variable we also observed a significant interaction between temperature and body size (Table 3), which emerged because the gonad index was a negative function of size at all temperature levels except at 22 °C and 26 °C (Supplement Fig. S2). Gonad indices correlated positively with the sea urchin's consumption rates (Pearson's $r = 0.31$, $t = 2.08$, $p \leq 0.05$; $n = 42$).

4. Discussion

We investigated the potential influence of ocean warming on food consumption rates in the sea urchin species *Arbacia lixula* and *Paracentrotus lividus* from the coastal waters of Madeira Island. Additionally, the relationship between temperature and the gonad index was assessed in *A. lixula*. In the thermal range that we investigated (22 °C to 31 °C), both traits were found to be a unimodal function of temperature. Such a relationship has already been reported for several sea urchin species from other environments (Lawrence, 2013), but our study is the first to demonstrate this for *A. lixula* and *P. lividus*. Maximum food consumption rates (*A. lixula* and *P. lividus*) as well as maximum gonad indices (*A. lixula* only) were observed at 25 °C to 26 °C, and these values are at the upper end of the temperature range that currently prevails in the sea urchins' natural habitat around Madeira.

4.1. Consumption rates as a function of temperature

In *A. lixula*, the main effects of temperature and the sex of the animals explained a part of the variation in food consumption rates, while significant interactions between body size and sex, and between temperature and body size emerged. In *P. lividus*, consumption rates were a function of body size and temperature without any interacting effects. Hence, our results indicate that elevated temperatures may affect different size classes within a sea urchin population to different degrees.

We found that food consumption rates were generally higher in female *A. lixula* than in male specimens and this was independent of the surrounding water temperature. The fact that female *A. lixula* consumed more food than males of the same size suggests that the females may have higher nutrient and/or energy requirements than the males. Indeed, a greater reproductive effort in females and differences in the composition of gonad fatty acids between female and male specimens have already been reported for *A. lixula* and other sea urchin species

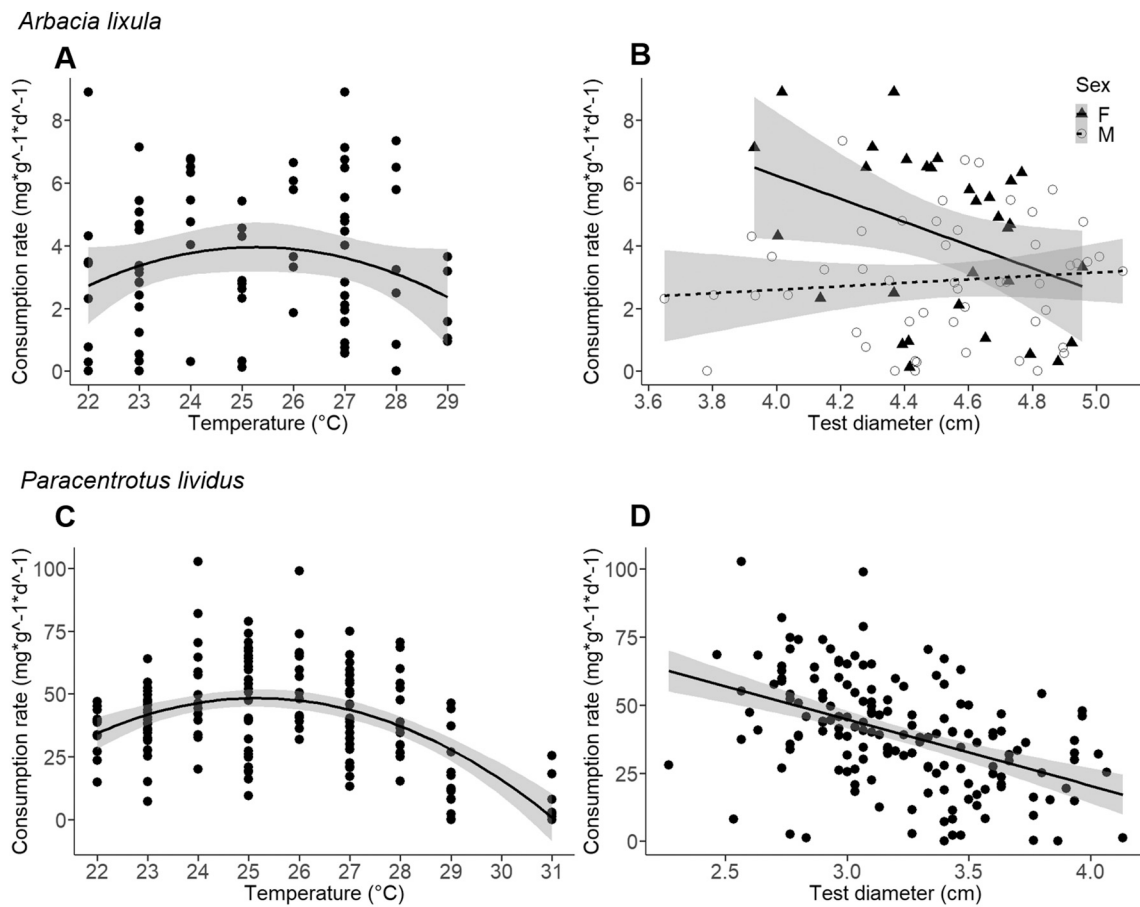


Fig. 3. Consumption of food pellets by the sea urchin *Arbacia lixula* as a function of temperature (A). Effects of test diameter on the consumption of food pellets by female (triangles, solid regression line) and male (circles, dashed regression line) *A. lixula* (B). Consumption of food pellets by the sea urchin *Paracentrotus lividus* as a function of temperature (C) and of test diameter (D). Polynomial (A, C) or linear (B, D) regression lines with 95% confidence intervals (grey shaded areas).

Table 2

Effects of water temperature and test diameter on the consumption of food pellets by the sea urchin *Paracentrotus lividus*. Results from multiple regression: $R^2 = 0.33$, $F = 40.41$, $p < 0.001^*$. Asterisks indicate significant p -values.

Predictor	Estimate	Standard error	t	p
Test diameter	-2.51e-02	3.44e-03	-7.29	$\leq 0.001^*$
Temperature (quadratic)	-5.61e-05	1.01e-05	-5.54	$\leq 0.001^*$

Table 3

Effects of test diameter and temperature on gonad indices in *Arbacia lixula*. Results from ANCOVA: $R^2 = 0.20$, $F = 4.48$, $p < 0.01^*$. Asterisks indicate significant p -values.

Predictor	Estimate	Standard error	t	p
Test diameter	17.81	4.98	3.58	$< 0.001^*$
Temperature (quadratic)	0.12	0.04	3.40	$< 0.01^*$
Test diameter: Temperature (quadratic)	-0.03	0.01	-3.39	$< 0.01^*$

(Martínez-Pita et al., 2010 and references therein). It therefore seems that energetic requirements during gametogenesis can differ largely between male and female individuals of this group of marine invertebrates (Martínez-Pita et al., 2010).

Marsh et al. (2013) reported that glycogen is the main carbohydrate for storing energy in sea urchin gonads and the filling of this storage requires high rates of food intake: in their study with *Strongylocentrotus intermedius* the glycogen content was found to be higher in female than

in male gonads. In the sea star *Asterias vulgaris*, Raymond et al. (2007) found that the energy content in ovaries prior to spawning was three times higher than in testes and that spawning was more costly for females than for males. All this could explain the higher food consumption rates that we observed in female *A. lixula*.

However, in the female but not in the male individuals of *A. lixula*, food consumption rates decreased with increasing body size (i.e. test diameter). Furthermore, similar to female *A. lixula*, small-sized *P. lividus* individuals, which were not distinguished by sex, consumed significantly more pellet material per unit biomass than larger ones. Other studies found a similar pattern in sea urchin species such as *Strongylocentrotus droebachiensis* and *Heliocidaris erythrogramma*, but they did not compare consumption rates between sexes (Siikavuopio et al., 2008; Carey et al., 2016). Carey et al. (2016) exposed sea urchins to increased water temperatures, which increased their metabolic demands and, thus, their respiration rates. The authors found that only small-sized individuals increased their feeding rates under elevated temperatures, presumably to satisfy their higher energy needs, while larger specimens, for an unknown reason, did not show this behaviour. Perhaps compensatory feeding is generally more common in female sea urchins due to their higher energy requirements during gametogenesis as outlined above. Our results suggest that, in warming coastal waters around Madeira, sea urchins of a smaller body size will likely show increased feeding rates that, in turn, will increase the top-down control they exert on the present macroalgae.

The influence of body size on food consumption rates in *A. lixula* varied not only with the sex of the animals, but also with the temperature regime: it seems that the larger the individuals of *A. lixula* were, the greater they were affected by the warming that we simulated. At the two

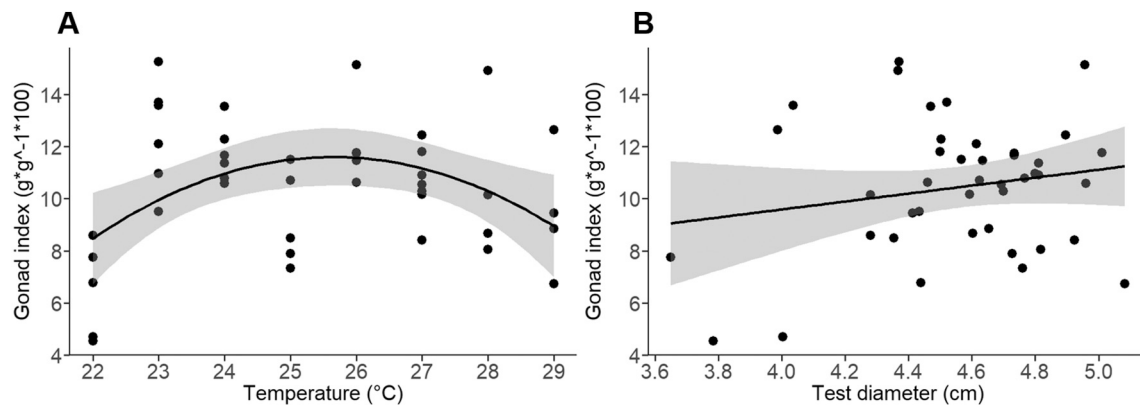


Fig. 4. Gonad indices as a function of temperature (A) and test diameter (B) in the sea urchin *Arbacia lixula*. Polynomial (A) or linear (B) regression lines with 95% confidence intervals (grey shaded areas).

lowest temperature levels that we applied, i.e. 22 °C and 23 °C, positive relationships between consumption rates and test diameter were observed, while at the higher temperature levels, consumption rates decreased with increasing test diameter. This aligns with the assumption that metabolic energy demands scale faster with body size than the amount of energy that can be supplied via consumption, and that this mismatch is enhanced under elevated temperatures (Lemoine and Burkepile, 2012). This could be explained by the fact that the body volume increases exponentially with the test diameter in the almost spherical sea urchins, i.e. the space for metabolically active tissue and coelomic fluid becomes disproportionately larger when the animals grow. It has also been shown that elevated temperatures reduce the pH of the coelomic fluid and impair fluidic features, e.g. protein concentrations, in the related sea urchin species *A. punctulata* (Johnstone et al., 2019). This process is accompanied by a considerable increase in free radicals, i.e. reactive oxygen and nitrogen species (Johnstone et al., 2019). Hence, the larger the sea urchins, the higher is the physiological stress that they experience under elevated temperatures and the more pronounced is their decrease in performance.

Our findings indicate that body size may modulate biological responses to ocean warming in ectotherms. While metabolic scaling has been intensely studied and debated (Agutter and Wheatley, 2004), the relationship between body size and the capacity to adapt to warming still requires research (Killen et al., 2010; Carey and Sigwart, 2014; Carey et al., 2016).

4.2. Gonad index as a function of temperature

In our study, individuals of *A. lixula* were exposed to elevated temperatures for up to 20 days (i.e. ten days of temperature acclimation plus ten days at the target temperature), and this time was presumably long enough to allow changes in gonad biomass (Johnstone et al., 2019). Unlike the food consumption rates that we assessed, the gonad index was not influenced by the sex of the sea urchins. This was, for the same species, also observed by Wangensteen et al. (2013). In our study, gonad indices generally increased with body size, confirming the findings of Fuji (1967), who observed that sea urchins increase their energy investments in gonadal tissue with increasing age, and thus, size. However, the significant interaction between the effects of temperature and test diameter again suggests, as discussed for the effects on consumption rates, that larger animals are impacted to a greater extent by warming, as increasing temperatures magnify the mismatch between metabolic demands and energy intake rates (Lemoine and Burkepile, 2012).

Our results show that gonad indices in sea urchins can be a unimodal function of temperature, indicating that this trait follows a thermal performance curve. We found the highest gonad indices at 25–26 °C and this conforms with Gianguzza et al. (2011), who monitored gonad

indices over a five-year period in the Mediterranean *A. lixula* and reported the highest values at 26 °C. Wangensteen et al. (2013) observed in *A. lixula* individuals from northeast Spain that an increase of 1 °C in the average water temperature during the gonad growth periods in 2011 and 2012 led to a 2.5-fold increase of the maximum gonad index compared to colder gonad growth periods in the years 2009 and 2010. In the closely related species *A. punctulata*, gonad indices were higher at 24 °C than at 28 °C and 32 °C (Johnstone et al., 2019).

Azad et al. (2011), who also found a significant influence of temperature on gonad mass in sea urchins, suggested that the temperature at which gonad growth rates and the resulting gonad indices peak varies with season. This is plausible, since gonad growth is more pronounced in some phases of the animals' reproductive cycle than in others, and, hence, each phase may exhibit a different optimum temperature for gonad development. Wangensteen et al. (2013) found evidence that the gonad index is most influenced by water temperature during the gonadal recovery and growth phase. The authors studied the reproductive cycle of *A. lixula* from the northwest Mediterranean by collecting monthly data about the gonad index, the length of the photoperiod and the water temperature over a five-year period. Hence, studies on this subject should only be compared when the reproductive cycle is well-understood and comparisons between temperature regimes at which gonad growth peaked are made between identical phases. While the reproductive cycle of *A. lixula* on Madeira has not been described yet, it can range from annual to semi-annual to spawning throughout the year in other *A. lixula* populations (Gianguzza and Bonaviri, 2013).

4.3. Ecological relevance in the light of current climate predictions

We examined the performance of the sea urchins *A. lixula* (food consumption, gonad index) and *P. lividus* (food consumption) as a function of temperature to assess potential changes in the feeding pressure that is exerted by these species and, in the case of *A. lixula*, in the reproductive output under warming SST. Both species are abundant in Madeira's coastal habitats (Alves et al., 2001) and they are ecosystem engineers, since they control the growth of macroalgae on rocky substrates (Wangensteen et al., 2012). Therefore, changes in their abundance or in their performance are relevant for the local marine ecosystem.

There are clear indications from previous studies on sea urchins that in a warmer ocean the strength of their top-down control on macroalgae will be enhanced (Kratina et al., 2012). This can have severe consequences for the integrity of these systems, e.g. if a massive loss in algal biomass leads to changes in the structure of associated trophic levels (e.g. Barton et al., 2009; O'Connor et al., 2009; Yvon-Durocher et al., 2010; Kratina et al., 2012). According to Schäfer et al. (2019), the warmest monthly mean temperature in the waters around Madeira during recent

years (2014–2018) was 23.5 °C, while annual mean water temperatures have continuously increased for the last 40 years. It can be expected that future summer and autumn mean temperatures will reach the temperature that we found to trigger maximum consumption rates in local individuals of *P. lividus* and *A. lixula* (25 °C). The same applies to the temperature at which the gonad index in *A. lixula* peaked (25–26 °C). Consequently, if the annual mean temperature continues to increase, the feeding pressure exerted by *A. lixula* and *P. lividus* will be more intense throughout the entire year.

Similarly, if warmer water temperatures prevail during the gonad growth seasons, this may enhance the reproductive output, and potentially reproductive success and recruitment in these sea urchins. This was seen in *P. lividus* from the eastern Mediterranean (Spirlet et al., 2000) and also matches with what is predicted for populations of *A. lixula* in the Mediterranean Sea (Gianguzza et al., 2011; Privitera et al., 2011; Wangenstein et al., 2012). Consequently, population densities may increase, which would further increase the feeding pressure exerted by the sea urchins.

In the Mediterranean as well as along the south coast of Madeira, *A. lixula* commonly co-occurs with *P. lividus*, and both species have been associated with the occurrence and expansion of barren grounds (Alves et al., 2001; Privitera et al., 2008, 2011; Bonaviri et al., 2011; Bulleri, 2013; Agnetta et al., 2013, 2015), while the exact roles that both species play for this process are still the subject of discussions (Bonaviri et al., 2011; Bulleri, 2013; Agnetta et al., 2013, 2015). According to Agnetta et al. (2013), *P. lividus* induces barren patch formation by deforesting entire macroalgal canopies, and thereby opens space for encrusting algae and animals, which are usually associated with the algal crusts and turf communities. *Arbacia lixula* is assumed to subsequently colonise the newly formed habitat and to maintain it by preventing fleshy macroalgae from re-colonising the bare rocks (Bulleri et al., 1999; Bonaviri et al., 2011). If, as a consequence of ocean warming, *P. lividus* will transform more macroalgal habitats into barren grounds in the future, more habitat and feeding grounds will be available for *A. lixula*, which may indirectly facilitate an increase in the abundance of this species (Privitera et al., 2011). In summary, sea urchin species around Madeira may thrive in a warmer ocean and, as a consequence of increased feeding rates and a higher reproductive output, are likely to enhance their top-down control on local macroalgal populations.

Ethics approval

National guidelines for sampling, care and experimental use of invertebrates for the study were followed and the permit for collection was provided by the “Instituto de Florestas and Conservação da Natureza” through the “Licença de Colheita/captura n°07/IFCN/2017-FAU MAD”.

Availability of data and material

All data used in this study will be made publicly available on PANGAEA (<https://www.pangaea.de>) following acceptance.

Code availability

The R code generated during the current study for data analysis is available from the corresponding author upon reasonable request.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgements

This study was conducted in the framework of the international research and student training programme GAME (Global Approach by Modular Experiments), which is coordinated by GEOMAR Helmholtz

Centre for Ocean Research Kiel, Germany. The participation of J. Roma in GAME was funded by the Lighthouse Foundation, while K. Schertenleib was supported by a scholarship programme of the Stiftung der Deutschen Wirtschaft, promoted by the Federal Ministry of Education and Research (“BMBWF”). P. Ramalhosa was partially funded by the Project Observatório Oceânico da Madeira-OOM (M1420-01-0145-FEDER-000001), co-financed by the Madeira Regional Operational Programme (Madeira 14-20), under the Portugal 2020 strategy, through the European Regional Development Fund (ERDF). I. Gestoso was supported by a postdoctoral grant in the framework of the 2015 ARDITI Grant Programme Madeira 14-20 (Project M1420-09-5369-FSE-000002). J. Canning-Clode is funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, Portugal, I.P., under the Scientific Employment Stimulus - Institutional Call – (CEECINST/00098/2018). This study also had the support of Fundação para a Ciência e Tecnologia (FCT) through the strategic project [UIDB/04292/2020] granted to MARE UI&I. The authors would also like to thank to the “Fundação para a Ciência e Tecnologia (FCT)” for funding the research in the Marine and Environmental Sciences Centre (MARE) throughout the project UID/MAR/04292/2013 and lastly thank the project PORTWIMS (Portugal Twinning for Innovation and Excellence in Marine Science and Earth Observation), which was co-funded by the European Union’s Horizon 2020 research and innovation programme under grant agreement No 810139.

Appendix A. Supplementary data

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.jembe.2021.151603>.

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