INTRODUCTION

Predicting organismal responses to environmental shifts is one of the main priorities of contemporary ecology (Calosi et al., 2017; Donelston et al., 2019; King, McKeown, Smale, & Moore, 2018). During the last few decades, scientific studies have linked global warming, characterized by both an increase in mean temperatures and frequency of heat waves (Jordà, Marbà, & Duarte, 2012; Oliver et al., 2018), to detrimental impacts on marine systems at different biological levels. These impacts can involve disruption of the whole ecosystem functioning, resulting from changes in food-web dynamics and in ecosystem productivity, together with biodiversity loss (see...
Smale et al., 2019; Stillman, 2019), to more specific effects. On the other hand, mean temperature increases and heat waves also result in a number of lethal and sublethal effects on particular species and/or populations, including coral reef bleaching (Hughes et al., 2017), alteration of animal migration behaviour, and shifts of marine taxa distribution patterns, among many others (e.g., Deutsch, Ferrel, Seibel, Pörtner, & Huey, 2015; Hoegh-Guldberg & Bruno, 2010; King et al., 2018).

Under sublethal extreme temperature, stress is likely to occur (e.g., Buckley & Huey, 2016), and organisms have developed different molecular and cellular mechanisms to maintain physiological performance and cell homeostasis (Buckley & Huey, 2016; Pörtner, 2002). Thermal responses include changes in expression patterns of stress-responsive genes, including genes that regulate metabolism (e.g., Xu, Zhou, & Sun, 2018; Zheng, Cao, Mao, Su, & Wang, 2019), oxidation-reduction processes (e.g., Gleason & Burton, 2015; Xu et al., 2018; Zheng et al., 2019), protein folding repair systems (Gleason & Burton, 2015; Kim, Kim, Choi, & Rhee, 2017; Xu et al., 2018; Zheng et al., 2019), and cell cycle (Xu et al., 2018; Zheng et al., 2019; Zhu, Zhang, Li, Que, & Zhang, 2016). Among the best-characterized molecules involved in stress response are the heat shock proteins (HSPs) (e.g., Feder & Hofmann, 1999; Kim et al., 2017; Tomanek, 2010). HSPs are highly conserved molecular chaperones that help proteins’ folding and transport across cell membranes during nonstressful conditions. They also refold and stabilize denatured proteins under different conditions of stress (e.g., Di Natale et al., 2019; Matranga, Toia, Bonaventura, & Müller, 2000).

Although the function of HSPs is well-known in some marine invertebrates, additional knowledge on the involvement of other molecular pathways, such as antioxidant genes, apoptosis-associated and immune-associated genes, is needed to uncover other relevant mechanisms involved in thermal stress responses in ecologically relevant species (Gleason & Burton, 2015; Zhu et al., 2016). One relatively recent approach to investigate rapid organismal responses to environmental perturbations, to identify potential physiological networks, and to discover candidate genes and isoform variants involved in their responses, is to explore the whole transcriptional profiles using RNA-seq techniques (e.g., Evans, Pespeni, Hofmann, Palumbi, & Sanford, 2017; Xu et al., 2018; Zhu et al., 2016). Although the relationship between mRNA transcript abundance and protein abundance is still not clear (Feder & Walser, 2005), some studies have shown a correlation between these two variables (Maier, Güell, & Serrano, 2009). Changes in gene expression are considered to be sensitive indicators of stress and potential predictors of organismal physiology under experimental conditions (Buckley, Gracey, & Somero, 2006; Feder & Walser, 2005; Schoville, Barreto, Moy, Wolff, & Burton, 2012).

Among marine ecosystems, one of the most impacted seas in the world is the Mediterranean (Coll et al., 2010; Lejeusne, Chevaldonné, Pergent-Martini, Boudouresque, & Pérez, 2010). This sea holds high levels of biodiversity while at the same time it is subjected to intense anthropogenic pressures (Claudet & Fraschetti, 2010; Templado, 2014), which interact with the ongoing global warming (Francour, Boudouresque, Harmelin, Harmelin-Vivien, & Quignard, 1994; Jordà et al., 2012). During the last three decades, sea surface temperature (SST) has risen in the Mediterranean at a rate of 0.25°C–0.65°C decade⁻¹ in the western and eastern basins, respectively (Marbà, Jordà, Agustí, Girard, & Duarte, 2015).

High-resolution ocean models, considering a diversity of potential climate change scenarios, have projected in all cases a significant increase in SST by the end of the century (see Parry, Canziani, Palutikof, Van Der Linden, & Hanson, 2007; Shaltout & Omstedt, 2014; Somot, Sevault, & Déqué, 2006; Somot, Sevault, Déqué, & Crépon, 2008). A warmer Mediterranean represents a challenge for most Mediterranean taxa, reflected in sublethal effects linked to behavioural and physiological responses (e.g., Anestis, Lazou, Pörtner, & Michaelidis, 2007; Prusina et al., 2014), lethal outcomes, including mass mortality events associated to heat waves (e.g., Cerrano et al., 2000; Coma et al., 2009; Garrabou et al., 2009), and the collapse of whole ecosystems along the warmest areas of the Mediterranean (Rilov, 2016).

This warming also brings about other indirect effects, accelerating the entrance of warm-water alien species (Raitos et al., 2010) and promoting the expansion of subtropical species that naturally colonized the Mediterranean during different geological periods (Briand, 2008; and examples of echinoderms in Garcia-Cisneros et al., 2018; Pérez-Portela et al., 2019; Wangensteen, Turon, Pérez-Portela, & Palacin, 2012).

The black sea urchin Arbacia lixula (Linnaeus 1758) has tropical affinities (Tortonese, 1965) and an amphi-Atlantic distribution across shallow rocky ecosystems, being the Moroccan coast its northern-most distribution limit in the east Atlantic. This sea urchin entered the Mediterranean basin during the last Pleistocene interglacial period (Pérez-Portela et al., 2019; Wangensteen et al., 2012), and it is now a common species across the whole Mediterranean (Palacin, Turon, Ballesteros, Giribet, & López, 1998; Tortonese, 1965). Densities of this species significantly increased in some Mediterranean areas during the recent decades (Francour et al., 1994; Harmelin et al., 1995; Hereu et al., 2012), and it is among the key drivers structuring littoral communities due to its grazing activity (Bonaviri, Fernández, Fanelli, Badalamenti, & Gianguzzia, 2011). The species is capable of shifting complex littoral macroalgal beds into “barren grounds”- areas of high densities of sea urchins deprived of erect seaweeds and dominated by crustose coralline algae - (Bonaviri et al., 2011; Gianguzzia et al., 2011). Several authors have predicted that the foreseen global warming might have a positive effect on its reproduction output and larval survival (Francour et al., 1994; Gianguzzia et al., 2014; Visconti et al., 2017; Wangensteen, Dupont, Castles, Turon, & Palacin, 2013; Wangensteen, Turon, Casso, & Palacin, 2013). This potential effect, if real, will represent a worrisome increase of the impact of this sea urchin on littoral ecosystems in a near future (Gianguzzia et al., 2011; Wangensteen, Dupont, et al., 2013; Wangensteen, Turon, et al., 2013). On the other hand, it seems that the distribution of A. lixula is constrained by low temperatures, like the low sea surface temperature provoked by the southward Portugal Current (Martins, Hamann, & Fiúza, 2002), which might be the cause of its absence...
along the Atlantic coast of Europe (Wangensteen et al., 2012). In this sense, experiments to investigate the potential of *A. lixula* to invade deep waters, analysing the combined effect of pressure (from 1 to 250 atm) and temperature (from 5 to 15°C) on the survival of embryos and larvae, showed that the combination of high temperatures and pressures, rather than temperature per se, might be the major factor limiting the distribution of the species at depth (Young, Tyler, & Fenaux, 1997). In contrast, more recent studies have demonstrated higher mortality rates, larval growth abnormalities and significant delays in settlement at the lowest experimental temperatures tested on this species (experimental temperatures from 18 to 22°C: Privitera, Noli, Falugi, & Chiantore, 2011; and from 16 to 19°C: Wangensteen, Dupont, et al., 2013). According to these studies, the abundance of *A. lixula* in the Mediterranean might be constrained by the low winter temperature of colder years, when mean temperatures can drop to 11°C, because gonad maturation is then considerably impaired (Lejeusne et al., 2010; Wangensteen, Dupont, et al., 2013). However, whereas the mentioned studies provided insights on the effects of thermal variation on the early development stages of *A. lixula*, almost nothing is known about its effects on the general performance of adult individuals, which can have different thermal sensitivity (Buckley & Huey, 2016). The capability of adult individuals to acclimatize and endure thermal changes is highly relevant from an evolutionary perspective. It not only affects their own physiological performance and/or the quality of their gametes, but it can also result in negative transgenerational carry-over effects on hatchability and larval size of the next generation, which have been shown after prolonged periods of parental exposure to elevated temperatures in some sea urchins (Zhao et al., 2018). In sea urchins, transcriptomes from different tissue types and larval thermal stress responses have been characterized (e.g., Clark et al., 2019; Gaitán-Espitia, Sánchez, Bruning, & Cárdenas, 2016; Gillard, Garama, & Brown, 2014; Jia et al., 2017; Pérez-Portela, Turon, & Riesgo, 2016; Runcie et al., 2012). But, to our knowledge, transcriptome-wide screenings have never been used for measuring responses to thermal variation in adult individuals of this animal group.

The aim of this study was to explore the short-term transcriptional response to thermal changes among individuals of the subtropical sea urchin *A. lixula*. We set three specific objectives for our study: (a) To quantify and compare transcriptional responses to both high and low temperature treatments in *A. lixula* under experimental conditions; (b) To identify some of the most important candidate genes involved in rapid thermal responses in sea urchins; and (c) To determine the existence of common genes involved in responses to increasing and decreasing temperatures.

Many studies on global warming focus on the negative effect of rising temperatures, but in this study, we worked under the hypothesis that *A. lixula* will experience higher stress when subjected to low rather than to high temperatures. Based on previous transcriptional information from marine invertebrates under thermal stress (e.g., Gleason & Burton, 2015; Zhu et al., 2016), we also expect changes of expression patterns in different gene pathways during our temperature treatments, including genes encoding HSPs, apoptosis and antiapoptosis mechanisms, ATP-associated genes due to an increase of energy demand to maintain cell homeostasis, antioxidant genes since extreme temperatures can increase cells’ oxidative stress, and immune-associated genes (Xu et al., 2018). The information obtained here will be relevant to understand the ecophysiological patterns of sea urchins exposed to thermal changes. We also discuss the significance of our findings for the foreseeable ecological spread of this keystone species in the Mediterranean.

## 2 | MATERIALS AND METHODS

### 2.1 | Sea urchin collection

Adult specimens of *A. lixula* were collected by SCUBA diving in December 2012 from the shallow subtidal population (5–8 m depth) of Punta Santa Anna, in the locality of Blanes (41°40′22.47″N, 2°48′10.81″E, North-western Mediterranean; Figure 1). Specimens were quickly transported to the laboratory (<2 km away) in a cooler with seawater and oxygen tablets to keep stress induced by land transportation to a minimum. Experiments were performed in the LEOV (Laboratory of Experimentation with Living Organisms) facility of the Centre for Advanced Studies of Blanes (CEAB), equipped with an open system of running seawater coming directly from a sea intake. Once in the laboratory, sea urchins were measured with calipers and left to adjust for 48 hr in a common chamber with airflow and flow-through running seawater at 13°C, which was the sea temperature in Blanes at the collection time. During these 48 hr animals had rocky surfaces available for grazing.

### 2.2 | Experimental design

To quantify rapid transcriptomic responses of *A. lixula* under thermal assays, we exposed adult sea urchins (test diameter 40–50 mm) to three different treatments under controlled laboratory conditions for 20 hr: control (CT) with sea water at 13 ± 1°C, sea water temperature at 7 ± 0.5°C (T7), and sea water temperature at 22 ± 0.5°C (T22). We set the temperature exposure time to 20 hr because previous experiments of thermal stress responses in other marine invertebrates demonstrated maximum peaks of expression between the first 6–24 hr, depending on the genes (e.g., Kim et al., 2017; Zhu et al., 2016).

It is important to note that our goal was to submit the test organisms to an acute thermal change to measure their responses, not to mimic highest or lowest seasonal temperatures in the area. The treatment temperatures were chosen to represent an important shift with respect to the controls (13°C, the surface water temperature at this location when sea urchins were collected in wintertime) while remaining within realistic values for our area of study, the NW Mediterranean. Thermal sensitivity and resistance of organisms are not constant over time and often shift in response to seasonal
conditions (Buckley & Huey, 2016). The temperatures chosen, therefore, would have been different had we performed the trials at other seasons. The average sea-surface temperatures during summer in the Mediterranean range from 22 to 28°C, with the lowest values at the north Aegean, Alboran Sea, and NW Mediterranean (Marbà et al., 2015; Pastor, 2012). The global average for the coldest month of the year (February) in the Mediterranean is 14.5°C, with a lower average value (12°–13°C) found at the NW Mediterranean (Pastor, 2012) (see Appendix S1, Figure S1). Since the species’ thermal history can determine the thresholds of stress response (Osovitz & Hofmann, 2005) and thermal sensitivity can change over the seasons, we made a preliminary assessment of the tolerance limits of our NW Mediterranean population at that time of the year (so-called here “trials”), with several temperatures assayed over a 20 hr period and visual inspection of the state and activity level of 10 sea urchins per temperature treatment. Specimens used for the trials were not used for further experiments and were returned to the sea after experimentation. For sex determination we used histological techniques. One gonad per individual was obtained and preserved in 4% formaldehyde. Gonad samples were washed in distilled water, dehydrated, quickly dissected under RNAase free conditions, and coelomocyte fluid collected and processed as explained in the next section. Animals were not fed during the 20 hr of the experimental time, and seawater pH (8.1) was monitored during the experiments. Eight different replicates (specimens) per treatment were included, although for gene expression analyses only six of them were processed. A sample size of eight was used to ensure an even proportion of sexes in the specimens analysed (since sex determination can be only performed a posteriori after dissection), and indeed we processed for transcriptomic analyses three males and three females per treatment. After the acclimation period of 48 hr, each sea urchin was placed in an independent aquarium to avoid interactions among specimens. Each aquarium had constant airflow and the seawater temperature was set at the required temperature (13° ± 1°C and experimental condition at 13° ± 1°C, hereafter named as “Control versus T7”, and a “High temperature” experiment comparing the control condition at 13° ± 1°C and experimental condition at 22° ± 0.5°C, hereafter named as “Control versus T22” (see Figure 1). Samples used as control condition at 13° ± 1°C and experimental condition at 22° ± 0.5°C for sex determination were the same for both experiments, since all treatments were run at the same time and laboratory. After the acclimation period of 48 hr, each sea urchin was placed in an independent aquarium to avoid interactions among specimens. Each aquarium had constant airflow and the seawater temperature was set at the required temperature (13° ± 1°C and experimental condition at 22° ± 0.5°C) prior to adding the sea urchins. Temperature of the aquaria was controlled with HOBO loggers (one per aquarium). Aquaria with different treatments were randomly allocated across the wet-lab space to avoid any bias related to their spatial distribution. Animals were not fed during the 20 hr of the experimental time, and seawater pH (8.1) was monitored during the experiments. Eight different replicates (specimens) per treatment were included, although for gene expression analyses only six of them were processed. A sample size of eight was used to ensure an even proportion of sexes in the specimens analysed (since sex determination can be only performed a posteriori after dissection), and indeed we processed for transcriptomic analyses three males and three females per treatment. After 20 hr of treatment, sea urchins were removed from the aquaria, quickly dissected under RNAase free conditions, and coelomocyte fluid collected and processed as explained in the next section.

For sex determination we used histological techniques. One gonad per individual was obtained and preserved in 4% formaldehyde. Gonad samples were washed in distilled water, dehydrated,
embedded in paraffin, cut in 5 μm sections using a Microm HM325
Microtome, and stained in haematoxylin-eosin as described in
Wangensteen, Turon, et al. (2013) and García-Cisneros et al. (2018).
Sex was then determined under the optical microscope.

2.3 | Coelomocytes collection and RNA sequencing

Coelomocytes consist of several cell types contained in the coelomic fluid and are immune effectors in echinoderms (Matranga et al., 2000; Smith et al., 2018). They have been used as biomarkers of stress due to their prompt response to changing environmental conditions (Matranga, Bonaventura, & Di, 2002; Matranga et al., 2000, 2005; Pinsino et al., 2008) that can reduce the protective capacity of these cells and rapidly induce activation of the heat shock proteins expression (Matranga et al., 2000; Pinsino et al., 2008). Additionally, these cells showed higher thermal response capacity than other tissues in sea urchins (e.g., digestive tissues, González, Gaitán-Espitia, Font, Cárdenas, & González-Aravena, 2016), and protocols for extraction of high quality RNA and high throughput sequencing have been developed for this tissue type in A. lixula (Pérez-Portela & Riesgo, 2013; Pérez-Portela et al., 2016).

Five millilitres of the coelomic fluid of each specimen (a total of 18 specimens; six per treatment) were collected using a sterile syringe inserted through the peristomic membrane, taking care not to puncture the gut. The fluid was then centrifuged (50 × g), and all fresh cellular components (coelomocytes) gathered and quickly embedded in TRizol reagent (Invitrogen, www.invitrogen.com). Total RNA was directly extracted from coelomocytes following a protocol previously optimized for this species (Pérez-Portela & Riesgo, 2013; Pérez-Portela et al., 2016). Integrity of total RNA and potential DNA contaminations were initially evaluated by visualizing the 28S rRNA and 18S rRNA bands into a 1% agarose gel in 1× TAE buffer.

Concentration of the RNA extracts was assessed in a Hellma spectrophotometer (Hellma Analytics), and total RNA extracts were also run in an Agilent 2100 Bioanalyzer (Agilent Technologies) at the Scientific and Technical Services of the University of Barcelona for quality measurements. High quality RNA (RINs over 8.5) samples were sent to the National Centre of Genomic Analyses of Barcelona (CNAG) for mRNA isolation, cDNA library construction, normalization and sequencing.

Isolation of mRNA and cDNA library preparation for each of the 18 specimens were performed using the Illumina TruSeq RNA Sample Prep Kit (Illumina Inc.) following the manufacturer’s recommendations, with an input of 800–900 ng of mRNA, and average insert size of the libraries of 300 bp. Quality and concentration of the 18 cDNA libraries was controlled with Ribogreen Assays in a NanoDrop 3300 Fluorospectrometer (Thermo Fisher Scientific, www.thermofisher.com). The 18 libraries (six per treatment) were multiplexed with Illumina barcodes, and five libraries per lane were sequenced on an Illumina HiSeq2000 Sequencer, generating 101 base paired-end reads. The 18 libraries from different treatments were randomized across Illumina lanes.

2.4 | Sequence processing and de novo assembly

The software FASTQC v. 0.10.0 (www.bioinformatics.babraham.ac.uk) was used to visualize and measure the quality of the raw reads generated in the HiSeq2000. Adapters and bases with low quality (phred scores < 33) were trimmed off, and a length filter was applied to keep only sequences of > 25 bases using TrimGalore v. 0.2.6 (www.bioinformatics.babraham.ac.uk). High-quality reads were re-screened in FASTQC to ensure a good quality of the samples after trimming. A basic scheme of the most important steps of our pipeline is presented in Figure 2.

Two de novo assemblies, hereafter named as “CT + T7” and “CT + T22”, one per experiment (“Control versus T7” and “Control versus T22”, respectively), were separately built up as reference for gene expression analyses. Due to technical difficulties and the low quality of two libraries, for gene expression analyses only five samples could be used for each of the T7 and T22 treatments (see details in Results section and Figure 1). Nevertheless, these two libraries discarded for gene expression could be used for the assembly of the respective references. The de novo assemblies were performed with the software Trinity (Grabherr et al., 2011), which allows detecting differentially spliced isoforms, with default parameters for this software. Only contigs with a minimum length for reported transcripts of 200 bp and at least 10x coverage were retained for the assemblies.

The two de novo assemblies were separately blasted against a selection of the nr database of NCBI containing only proteins from Metazoa (blastx) using BLAST (Altschul et al., 1997) with a cutoff E value of 1e−5. The highest scoring blast hit was used to assign a gene name to each contig. De novo assemblies were also blasted against both a database containing proteins of bacteria (blastx), and a database of ribosomal DNA of bacteria (blastn) obtained from NCBI to remove bacterial contaminations. Sequences with blast hit exclusively against proteins and nucleotides of bacteria were eliminated from the data sets.

Blast results against Metazoa served as a database for annotation of transcripts differentially expressed between treatments (see below). Moreover, Blast results of the assemblies were used to retrieve gene ontology (GO) terms with BLAST2GO (Conesa et al., 2005) under different categories: biological processes, cellular component and molecular function, which are hierarchically organized into different levels (see Figure 2). The completeness of the reference transcriptomes was assessed with BUSCO (Benchmarking Universal Single-Copy Orthologue) against the eukaryotic and metazoan databases (Simão, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015).

2.5 | Differential expression analyses and annotation

 Reads from all replicates in each experiment were aligned against the corresponding “reference” transcriptome as per experiment (see Figure 2). Paired reads after trimming were mapped using
Bowtie2 v. 2.2.1 (Langmead & Salzberg, 2012) as implemented in Trinity (Grabherr et al., 2011). RSEM v. 1.2.11 (Li & Dewey, 2011) was then run to generate a table with read counts, and unmapped reads were discarded. In the “reference” transcriptomes, transcripts of the same trinity component were treated as different isoforms. We retained information of differential expression of all isoforms detected for a given gene (or component) because they may have different functions. In order to identify common genes and/or isoforms differentially expressed under temperature treatments, the de novo assemblies of both experiments, that assigned different transcript names to all isoforms, were blasted against each other using BLASTn.

Differential expression (DE) analyses of the two experiments were performed with the package DESeq2 (Love, Huber, & Anders, 2014) in R v 3.2.1 (R Development Core Team, 2008). Before performing the analyses, preliminary tests to investigate differences in gene expression between sexes and treatments were performed in DESeq2. No significant differences in response to treatments were observed between males and females (p-adjusted > .01), and “sex” was not considered as a variable in further analyses.

For differential gene expression analyses, read counts were first normalized in DESeq2, and then a negative binomial model was fit to accurately estimate differential expression. The significance value for multiple comparisons was adjusted to 0.01 with the function “padj” (Benjamini-Hochberg adjustment) as implemented in DESeq2. Transcripts with significantly different expression values relative to the controls will be hereafter called “DE” transcripts. Principal component analyses (PCAs) were performed and plotted with the same package to visualize variation of expression levels among samples and treatments. Visualization of the significant outcomes of isoforms differentially expressed (up- and downregulated) between treatments of each experiment was obtained with a heatmap performed with the “gplots” package of R (Warnes, Bolker, Bonebakker, & Gentleman, 2016).
Using the GO annotation results from the de novo assemblies of the two experiments, we obtained the GO terms associated to the differentially expressed isoforms, which were then input (together with their associated log₂-fold-change) to the REVIGO web server (Supek, Bošnjak, Škunca, & Šmuc, 2011) to obtain summaries of GO terms. Results were graphically represented with the “treemap” R package. Size of the rectangles was adjusted to reflect the log₂-fold-change in REVIGO. Differentially expressed isoforms without blast hit, unknown function and/or without annotation for each experiment were further assessed with the InterProScan 5 software (Jones et al., 2014), which predicts protein family membership and the presence of functional domains and sites, at the Superfamily level (de Lima Morais et al., 2011). The InterproScan was run as implemented in the Blast2GO software with default parameters. We finally merged the results of the associated GO terms and those from InterProScan with the purpose of increasing our knowledge of oomocyte gene functions and GO annotations.

3 | RESULTS

3.1 | Data filtering and de novo assembly

A total of 18 RNA-seq data sets were generated in this study. For de novo assembling of each reference (see Figure 2) we used 12 RNA-seq data sets. For quantifying transcriptomic responses, we used 11 data sets per experiment since one sample from treatment T7 and another from T22 were discarded for gene expression analyses because of their low quality (see Figure 1). Data sets have been deposited in Mendeley Data (https://doi.org/10.17632/5673n552y.1) and the NCBI (BioProject n° PRJNA642021). The number of trimmed reads used for de novo assembly, as per sample replicate and treatment, are detailed in Appendix S1 (Tables S1 and S2). All replicates had over 26 million reads.

The de novo assembly “CT + T7”, used as a reference for the “Control versus T7” experiment, included 141.5 Megabases that rendered 211,650 transcripts (including both genes and their different isoforms), and 19.6% of them had blast hit with known proteins of metazoans (see species blast hit distribution in Appendix S1, Figure S2). The reference assembly “CT + T22” for the “Control versus T22” experiment included 147.4 Megabases, and rendered 219,655 different transcripts, from which 17.9% had blast hit (see species blast hit distribution in Appendix S1, Figure S2). Both de novo assemblies were very comparable (and had 99.5% transcripts in common), presenting relatively high N50 values, between 1,102 and 1,114, meaning that over 50% of the transcripts were longer than 1,100 bases. Details of the de novo assemblies for the two different experiments are presented in Appendix S1 (Table S1). Both, “CT + T7” and “CT + T22”, showed high completeness when compared with BUSCO conserved orthologue databases of eukaryotes and metazoans (see Appendix S1, Table S3). For the reference assemblies, “CT + T7” and “CT + T22”, 194 and 4,293 transcripts, respectively, had blast hits against proteins and/ or nucleotides of bacteria and were removed from subsequent analyses. In fact, most differences between the reference assemblies “CT + T7” and “CT + T22” were due to the amount of bacterial transcripts.

3.2 | General results of differential expression analyses

The differential expression analyses revealed changes in gene expression between controls and temperature treatments in both experiments, “Control versus T7” and “Control versus T22”. Additionally, we detected a remarkable difference in the magnitude of the transcriptomic responses between experiments, which was over six-fold greater in number of DE transcripts in the “Control versus T7” experiment, as explained below. We also observed differences in gene expression among different isoforms of the same genes.

In the “Control versus T7” experiment, we detected 1,181 DE transcripts between CT and T7, with 720 transcripts upregulated at T7 (61% of the total DE transcripts) and 461 transcripts downregulated at T7 (49% of the total DE transcripts) (see Figure 3). A total of 445 transcripts (37.7% of the total DE transcripts) had blast hit and known function (see Appendix S1, Table S4), including 28 transcripts within the top 50 most significant DE (see Appendix S2). Regarding isoforms, over all DE transcripts (potential genes), 176 presented different isoforms (see Appendix S2). Ten genes presented all isoforms DE between CT and T7, whereas the other 166 genes only showed some of their isoforms differentially expressed between treatments.

The number of DE transcripts in the “Control versus T22” experiment was much lower than in the “Control versus T7” experiment, with only 179 DE transcripts, being 57 transcripts upregulated (32% of the total DE transcripts) and 122 transcripts downregulated (68% of the total DE transcripts) in the T22 treatment (see Figure 2). Only 35 transcripts (19.7% of the total DE transcripts) were assigned annotation and known function (Appendix S1, Table S4), 10 of them within the top 50 most significant DE. Of these 35 transcripts, 27 had different isoforms, and in all cases only one of their isoforms was DE between CT and T22 (see Appendix S2). A complete list of differentially expressed, annotated transcripts for both experiments is presented as (Appendix S2), including transcript identification code (id), logarithm of the fold change, adjusted p-value with FDR correction obtained from the expression analyses, gene description, number of isoforms found and transcripts with known function within the top 50 most significant DE (‘Top 50 DE’). A total of 14 DE transcripts were common between experiments (see Figure 3) and most of them featured opposite responses between treatments. Only four of these transcripts had annotations; three of them showed opposite responses between the two experiments (fam-55cc with log₂-fold-change = –6.01 and 5.47, tripartite motif-containing protein 3 with log₂-fold-change = 4.51 and –6.36, and wsc domain-containing protein 1 with log₂-fold-change = –1.19 and 1.84 at “Control versus T7” and “Control versus T22” experiments, respectively), whereas the histone-lysine n-methyltransferase prdm 9 was downregulated in both temperature treatments, T7 and T22, of the two experiments.
Figures 4 and 5 showed, in general, little differentiation between replicates of the same treatment, and large differences in transcript expression between treatments. Only one of the control replicates from the “Control versus T22” experiment had a mixed pattern of expression between control and treatment samples (Control replicate 1, see Figure 4), and clustered with the treatment samples on the PCA (see Figure 5). However, this same control sample did not follow the same trend in the other experiment (“Control versus T7”) and grouped neatly with the other control samples (Figures 4 and 5).

A total of 84 and three GO terms were found associated to DE genes in the “Control versus T7” and “Control versus T22” experiments, respectively (Appendix S1, Table S4). The InterProScan could only predict information of protein domains in six uncharacterized transcripts of the “Control versus T22” experiment. In Figures 6 and 7 the up- and downregulated GO categories associated to DE
transcripts from the two experiments are depicted. These GO terms were not equally represented among categories between up- and downregulated DE transcripts, or between experiments. For the experiment “Control versus T7” the most important upregulated GO term categories (Figure 6) were: for Biological Process (BP), “tyrosine metabolism” (including “positive regulation of apoptotic process”), “peptidyl-tyrosine dephosphorylation”, “protein folding” and “ATP hydrolysis coupled proton transport”. For Cellular Component (CC), “proton-transporting V-type ATPase-V0 domain” and “sarcoplasmic reticulum”). For Molecular Function (MF), “GTP binding”, “protein tyrosine phosphatase activity”, “protein tyrosine phosphatase activity”, “sulfo-transferase activity”, “hydrogen ion transmembrane transporter activity” and “lipid binding” (among others). Among the most important downregulated GO categories (Figure 6) we found: for BP, “neurotransmitter transport”, “intracellular signal transduction”, “positive regulation of DNA metabolic process”, “integral component of membrane”, “cell”, “intracellular” and “integral component of membrane”. For MF, “protein-N-acetylglucosaminyltransferase activity”, “sequence-specific DNA binding”, NAD-dependent histone deacetylase activity” and “zinc ion binding” (among others). For the experiment “Control versus T22” only GO information for down regulated transcripts could be obtained and, among them, the most important DE categories were “notch signalling pathway”, “multicellular organismal development” for BP, “integral component of membrane”, “membrane”, and “SAGA-type complex” for CC, and “calcium ion” and “protein binding” for MF.

3.3 | Differentially expressed genes involved in thermal stress, apoptotic processes and immune responses in Arbacia lixula

At 7°C, the expression of genes encoding different heat shock proteins was upregulated, including transcripts for the Heat Shock family proteins: an inducible Hsp70 (Appendix S2, Figure S3), and Hsp71, Hsp90 and the Dnaj homolog subfamily c member 21(DNAJC21), which encodes a member of the DNAJ heat shock protein 40 family (Hsp40), which acts as a co-chaperone of Hsp70 (Appendix S2, Figure S3). See Appendix S2 for Hsp40 transcripts and foldchanges: c256938_g1_i3, log2fold-change = 2.98; c260821_g2_i1, log2fold-change = 1.35; c260821_g1_i2, log2fold-change = 3.05; c264479_g1_i1, log2fold-change = 1.19; c261252_g1_i1, log2fold-change = 3.69. In addition, the receptor of stress Wsc domain-containing protein 1 was found downregulated at 7°C and upregulated at 22°C (Appendix S2: c266025_g2_i1, log2fold-change = -1.84, respectively).

Several transcripts from the apoptotic gene complements were differentially expressed between controls and T7. They included the Bcl2 (upregulated in T7, Appendix S2: c263429_g1_i1, log2fold-change = 2.17; and c271119_g2_i1, log2fold-change = 1.73), sequestosome 1 (upregulated in T7, Appendix S2: c257995_g1_i1, log2fold-change = 3.72) and fas-associating death domain-containing protein and death ligand signal enhancer (down- and upregulated in T7, respectively; Appendix S2: c268119_g1_i3, log2foldchange = -1.48 and c270362_g1_i1, log2fold-change = 1.99). In T22, we found upregulation of immediate early response 3-interacting protein 1-like (Appendix S2: c276658_g1_i2, log2fold-change = 1.55).

At 7°C, there was an upregulation of genes involved in the innate immune response identified as echinoidin, senescence associated-gene and Tripartite motif-containing protein 3 (TRIM) (Appendix S2: c258741_g1_i1, log2fold-change = 5.35; c150071_g1_i1, log2fold-change = 6.22; c271252_g1_i1, log2fold-change = 2.90). In addition, the genes interleukin-17 and cytohesin-like were also upregulated in T7 (Appendix S2: c239836_g1_i1, log2fold-change = 6.22; and c263807_g1_i1, log2fold-change = 1.77, respectively).

4 | DISCUSSION

The response of marine organisms to thermal shifts is probably different across the species’ range of distribution (Donelson et al., 2019).
In our study, we investigated transcriptional responses of a keystone species, the black sea urchin, in the northern part of its range of distribution (NW Mediterranean). We found contrasting responses to low (7°C) and high (22°C) temperatures, with the former eliciting a much stronger reaction. Such differences were related to both the magnitude of the transcriptional response (e.g., number of up- and downregulated transcripts and gene expression fold-change) and the diversity of genes and pathways involved in these responses.

The capacity of ectotherm species to thrive across wide temperature ranges is, in part, based on their ability to modulate the expression of genes encoding proteins involved in the physiological, metabolic and cellular stress responses (Kim et al., 2017; Runcie et al., 2012; Stillman, 2003; Tomanek, 2010). Resistance to acute sublethal temperatures is an adaptive trait that varies among species of the same genus from different latitudes and habitats (Stillman, 2003; Yao & Somero, 2010). In general, marine tropical species are more heat tolerant than their temperate and cold counterparts (Somero, 2010). Paradoxically, analyses of both marine and terrestrial ectotherms suggest that tropical, or the warm-adapted species, may be more threatened by global warming because they live closer to their upper physiological thermal limit, and have higher metabolic rates that accelerate quicker than in colder species under rising thermal conditions (e.g., Somero, 2010; Stillman, 2003). According to this expectation, *A. lixula*, a heat tolerant species with subtropical affinities (Tortonese, 1965; Wangensteen et al., 2012), could be threatened by global warming across the warmest areas of its geographical distribution (Elmasry, Razek, El-Sayed, Omar, & El Sayed, 2015; Rilov, 2016), where it might be closer to its thermal physiological limits. However, in the northwestern Mediterranean this species is in the coldest part of its range of distribution, which encompasses both sides of the tropical and subtropical Atlantic (Wangensteen et al., 2012), and thus it could be more limited by cold temperatures. Current Mediterranean sea warming may be removing thermal limitations for this species (Francour et al., 1994; Gianguzza et al., 2014; Visconti et al., 2017; Wangensteen, Dupont, et al., 2013; Wangensteen, Turon, et al., 2013) allowing an increase in its abundance in the Mediterranean.

In general, it is difficult to determine whether changes of expression in particular genes have important functional consequences, because for each gene the threshold for metabolic and physiological downstream effects can be different, and relatively small changes in gene expression of only a few genes can be as functionally important.

![Gene ontology treemaps for annotated differentially expressed genes in Control versus temperature 7°C.](image-url)
as larger changes in other genes (Oleksiak, Roach, & Crawford, 2005). However, the overall changes of gene expression patterns found in *A. lixula*, the number of genes differentially expressed, and the clustering of one control individual with the 22°C experimental individuals at the PCA and heatmap, indicates a lower transcriptional clustering of one control individual with the 22°C experimental in -

ter responses in coelomocytes of *A. lixula*. Among the upregulated genes related to the stress response at 7°C, we detected the *Hsp71*, *Hsp90*, an inducible *Hsp70*, and *Hsp40*; with the last one being a cochaperone of the *Hsp70*. The protein *Hsp40* s stimulate the ATPase activity of *Hsp70* s and targets unfolded proteins to *Hsp70* s (Ngosuwan, Wang, Fung, & Chirico, 2003). In general, these HSP chaperones are involved in the strong and mild thermal stress responses, including protein-folding reactions to avoid protein denaturation of adults, early development stages and eggs of sea urchins (e.g., González et al., 2016; Matranga et al., 2002; Matranga et al., 2000; Runcie et al., 2012). Their presence might be involved in the wide thermal distribution of some particular marine species (see Zhu et al., 2016, and references herein), and the HSP family seems to be a mechanism to cope with the stress associated with cold in *A. lixula* (e.g., NW Mediterranean). On the other hand, no upregulation of genes encoding HSPs was detected at 22°C relative to the control condition in *A. lixula*.

Under conditions of thermal stress, protein refolding by HSPs may not be efficient enough, and misfolded protein degradation can be necessary to restore cell homeostasis (Mosser et al., 2000). Therefore, other mechanisms such as proteolysis to eliminate dis- functional proteins via the Ubiquitin proteasome pathway and, fi- nally, apoptosis to eliminate damaged cells, can be activated (Logan & Somero, 2011; Somero, 2010; Zhu et al., 2016). We only detected signs of Ubiquitin proteasome pathway activation (upregulation of some genes involved in this pathway) in the 7°C treatment, with the upregulation of the gene sequestosome 1 (Appendix S2), which is an autophagosome cargo that detects proteins for autophagy and has been previously identified in echinoderms (Bitto et al., 2014), and the e3 ubiquitin-protein ligase, which targets damaged proteins for trans- port and degradation by the proteasome (Ardley & Robinson, 2005).

In addition, we observed differential expression of several apop- tosis-associated genes in both treatments, 7 and 22°C. Several stud- ies demonstrated that sea urchins hold a complex apoptotic system (Agnello & Roccheri, 2010; Lesser, 2012). We found transcriptional changes at 7°C in apoptosis suppressor genes such as the Bcl2 (upregulated, Appendix S2), widely distributed in different marine invertebrates (see Lesser, 2012), and in genes containing death do- mains (downregulated: fas-associating death domain-containing pro- tein and death ligand signal enhancer, Appendix S2) that induce cell apoptosis through the regulation of caspase activation (Agnello & Roccheri, 2010; Zhu et al., 2016). These findings suggest the activation of some particular pathways to control the programmed cell death at low temperatures. The upregulation at 22°C of the gene im- mediately early response 3-interacting protein 1-like (Appendix S2), which is a molecule involved in protein transport between the sarcoplasmic reticulum and Golgi apparatus and that mediates apoptosis in human cells (https://www.uniprot.org), suggests that apoptosis is also oc- curring as a response to increased experimental temperatures.

Additionally, a Serine threonine- protein kinase pim3, an enzyme involved in the regulation of cell transport and survival, which pre- vents apoptosis by inducing the release of the anti-apoptotic Bcl2 mentioned before (Cross et al., 2000) was also overexpressed at 7°C, whereas a Serine threonine-protein phosphatase 6, with opposite function to the kinase enzyme (Cross et al., 2000), was downreg- ulated at 22°C. Another interesting finding is the opposite pattern of gene expression found between experiments for the Wsc do- main-containing protein 1 (downregulated at 7°C and upregulated at 22°C) (Appendix S2). Different members of the Wsc family are identi- fied as putative receptors of stress and required for the heat shock response and the maintenance of cell wall integrity in yeasts (Lodder, Lee, & Ballester, 1999). The Wsc members are upstream regulators of other serine-threonine kinases, the protein kinase C1 (PKC1) and mitogen-activated protein kinase (MAPK), which can promote apoptosis (Cross et al., 2000; Lodder et al., 1999). The differential

**FIGURE 7** Gene ontology treemaps for annotated differentially expressed genes in Control versus temperature 22°C. Only the function of downregulated genes at 22°C was obtained for Biological processes, Cellular components, and Molecular functions. The size of the rectangles reflects the log2 fold-change associated to the differentially regulated categories [Colour figure can be viewed at wileyonlinelibrary.com]
expression of these molecules between control conditions, 7°C, and 22°C, evidences the different regulation systems of apoptosis and control of cell damage at different temperatures in *A. lixula*.

Previous experiments on echinoderms demonstrated the effect of thermal stress on the immune capacity of coelomocytes; this effect is greater at higher rather than lower temperatures in the sea cucumber *Apostichopus japonicas* (Wang, Yang, Gao, & Liu, 2008). However, in *A. lixula*, it was the lowest temperature the one that triggered a higher immune response in terms of gene expression. The *echinodin, senescence associated-gene, cytohesin-like* and *tripartite motif-containing protein 3* (TRIM) (Appendix S2) involved in the infection response and/or pathogen-recognition process against bacteria, fungi and viruses (Ozato, Shin, Chang, & Morse, 2008; Smith et al., 2006) were upregulated at 7°C. In addition, the gene *interleukin-17* (Appendix S2) which is a cytokine inducing and mediates pro-inflammatory responses in metazoans and stimulates phagocytosis in echinoderms (Beck et al., 1993), was also upregulated at 7°C. None of these immune genes were, however, upregulated (or, when detected, were downregulated) at the highest experimental temperature (e.g., TRIM), suggesting no immune response at 22°C.

The differentially expressed genes for the low and high temperature experiment were associated to different GO categories that provide additional information. These GO categories summarize the most significant biological processes, cellular components, and molecular functions that were up- and downregulated during the experimental response in *A. lixula*. For the high temperature experiment, we could only recover GO terms of three transcripts, and therefore, there is limited information to reach conclusions on the GO categories for this experiment. However, we detected the down-regulation of two interesting GO terms, the “notch signaling pathway” with the associated gene *neurogenic locus notch* (Notch1), and the “integral component of membrane” with the associated gene encoding a notch ligand, the *delta protein*. Notch is a calcium-dependent cell signalling system involved in different functions, including cell differentiation, proliferation and apoptosis. In general, notch inhibits apoptosis and induces cell proliferation but, in vitro studies using different cell lineages, it was shown that hyperthermia reduced Notch1 expression and apoptosis in some cell lineages, whereas an opposite pattern was obtained in other cell lineages (Basile, Biziato, Sherbet, Comi, & Cajone, 2008). Although the effect of the notch downregulation at high temperatures in coelomocytes is not completely clear, it suggests the existence of an alternative pathway of apoptosis under thermal stress.

Among the GO terms upregulated during cold exposure that add further information was the “tyrosine metabolism” term, which is related to cell protection against stress, including the upregulation of HSPs, cytoskeletal stabilization and apoptosis decrease (Baird, Niederlechner, Beck, Kallweit, & Wirschmeyer, 2013). This major GO term also includes the subordinate “Positive regulation of apoptotic process”, which can induce apoptosis when protein refolding by HSPs is not efficient enough. The induction of HSPs during thermal stress can considerably increase the energy demand in cells (Dong, Yu, Wang, & Dong, 2011; Tomanek, 2010). This increased energy demand is reflected in the overrepresentation of the GO category “ATP hydrolysis”, a catalytic process that releases energy previously stored in the form of ATP, and the upregulation of the *V*-type proton ATPase gene (see Appendix S2), a proton pump found within the “proton-transporting V-type ATPase, V0 domain” term. Likewise, the terms “protein folding” and “protein transport”, the last one subordinate to the “ATP hydrolysis” category, are linked to protein transport to the Sarcoplasmic reticulum for folding reaction to avoid protein denaturation by HSPs. Hence, the “Sarcoplasmic Reticulum” category, corresponding to a key organelle involved in the thermal stress response that ensures that misfolded proteins are directed towards a degradative pathway to the central cytoplasmic proteolytic machinery (Malhotra & Kaufman, 2007), was also overrepresented at 7°C. Actually, the induction of expression of Hsp70s has been directly associated to the accumulation of unfolded proteins in the sarcoplasmic reticulum (Rao et al., 2002), which are later eliminated if refolding fails by retrograde transport across the reticulum membrane (Kostova & Wolf, 2003). Other minor upregulated GO terms, at the biological process and molecular function, were “oxidation-reduction processes” (1 GO term) and “oxidoreductase activity” (2 GO terms). These terms suggest that low temperature affects the intracellular redox state in coelomocytes.

Among the downregulated GO terms at the 7°C treatment we found “Neurotransmitter transport” with the associated differentially expressed genes *Creatine transporter* and *Trafficking protein particle complex subunit 2* protein. The *Creatine transporter* is essential for normal brain function in humans and tissues with high energy demands because, together with other molecules, maintains ATP levels (Christie, 2007). The downregulation of these genes and pathways could be a potential response to energy competition with the induction of HSPs during thermal stress. The 7°C treatment also seemed to inhibit nuclear replication, as represented by the downregulation of the “Nuclear origin of replication recognition complex” and “DNA replication” terms, among others. The origin recognition complex is an ATP-dependent system that, together with other factors, enables the initiation of DNA replication in eukaryotic cells (Li & Stillman, 2012). Cells under stressful conditions must prevent cell division in favour of protective functions (Jonas, Liu, Chien, & Laub, 2013), as well as to avoid entering in a new DNA replication cycle if there is DNA damage (Lee et al., 2009). We also found downregulation of the “intracellular signal transduction” term, with the subordinate “cell redox homeostasis” and “smoothed signalling pathway” terms, and the “protein O-linked glycosylation” term. Smoothed is a key transmembrane protein involved in a critical cell-to-cell communication system for tissue homeostasis. Glycosylation, on the other hand, is one of the most common post-transcriptional modifications during protein biosynthesis, which contributes to increase protein solubility and stability against proteolysis, and can also be involved in their correct folding (Shental-Bechor & Levy, 2008). Hence, the downregulation of these last two terms may reflect a negative effect of low temperatures on protein biosynthesis and stabilization, and homeostasis control in coelomocyte cells.
Another remarkable result from our work is the large number of gene isoforms found in *A. lixula* transcriptome. Different gene isoforms are mostly generated by alternative splicing, whose function is to increase the diversity of mRNA expressed by the genome (e.g., Kelemen et al., 2013; Stamm et al., 2005). It has been demonstrated that alternative splicing can promote from neutral or subtle effects on transcripts, and finally proteins functioning, to drastic physiological changes (see a review in Kelemen et al., 2013). Therefore, the presence of different gene isoforms is relevant when studying gene expression and physiological pathways. Although our objective was not to analyse differential splicing in *A. lixula*, our results show different levels of expression of some gene isoforms under thermal treatments.

In summary, and despite the limited proportion of annotated transcripts obtained, our results based on RNA-seq analyses of the whole transcriptome of coelomocytes in *A. lixula* show that this NW Mediterranean population in the coldest part of its distribution range (Pérez-Portela et al., 2019; Wangensteen et al., 2012), displays strong gene expression changes in response to the cold treatment, with activation of many genes whose functions could be related to stress responses in the form of chaperone production, apoptosis regulation, ATP-associated genes, enhancement of the immune system and redox processes, and downregulation of gene pathways related to protein biosynthesis and DNA replication. Nevertheless, contrary to that found in other studies (e.g., Gleason & Burton, 2015; Zhu et al., 2016) no activation of genes encoding antioxidant enzymes was detected in our experiments. As we initially expected, a markedly lower response is found in the warm treatment, with no activation or deactivation of the previously mentioned pathways, with the exception of the apoptosis regulation. Although some caution is needed, as we have characterized transcriptional changes and not protein levels, the differential patterns found in these genes strongly indicated that sea urchins are more stressed under lowered experimental temperatures.

We acknowledge that we have tested only acute thermal conditions, without any progressive acclimation. This is an unrealistic scenario but was chosen to elicit a short-term measurable response. This response was much more marked against lower than higher temperatures, which indicates potential to compensate for cold stress. However, our results indicate that *A. lixula* might require energy expenditure to withstand the stress associated with low temperatures, while it does not undergo relevant transcriptional changes when exposed to warm temperatures. This is coherent with the notion of a thermophilous species living near the colder limit of its physiological tolerance, as found also when analysing reproductive and larval features (Wangensteen, Dupont, et al., 2013; Wangensteen, Turon, et al., 2013). Future research should consider a wider panoply of temperature regimes and populations, combined with acclimation periods, to explore the potential effect of global warming and heat waves across warmer areas where the species inhabits. Additionally, considering different reproductive seasons would be also interesting since organisms may display different thermal sensitivity depending on their gonad maturation stage, and may experience changes in energy allocation. Increasing gene annotation quantity and quality for this species is also desirable to obtain meaningful physiological conclusions in further studies.

It has been suggested that the tropicalization of NW Mediterranean can lead to a shift in dominance between the temperate common sea urchin *Paracentrotus lividus*, which will suffer from warming temperatures, and the thermophilous black sea urchin *A. lixula* (Carreras et al., 2020; Gianguzza et al., 2011; Wangensteen, Dupont, et al., 2013; Wangensteen, Turon, et al., 2013). Such a shift can have drastic ecological impacts, as both species are conspicuous engineer species shaping benthic communities (Bulleri, Benedetti-Cecchi, & Cinelli, 1999; Bonaviri et al., 2011). Specific biological and genomic studies are needed to understand the adaptive capabilities of *A. lixula* to ongoing warming, but our results add to the available evidence that colder rather than warmer temperatures may be a limiting factor for *A. lixula*. The absence of clear signs of stress at warm temperatures in adults of *A. lixula*, together with information on larva development and gonad maturation (Wangensteen, Dupont, et al., 2013; Wangensteen, Turon, et al., 2013), support the hypothesis of a positive effect of winter warming on the species’ reproduction output and larval survival. The ongoing expansion of the species across the littoral coast of the Mediterranean, with the concomitant impacts of its grazing activity on littoral communities, may be exacerbated in the near future by rising winter temperatures in the NW Mediterranean.

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**AUTHOR CONTRIBUTIONS**

All authors contributed to the design of this study and were involved in the aquarium experiments. R.P.P., A.R., X.T., and O.S.W. analysed the data. R.P.P. wrote the first draft of the manuscript and created figures and tables. X.T., A.R., and C.P. contributed to involved in the aquarium experiments. R.P.P., A.R., X.T., and O.S.W. analysed the data. R.P.P. wrote the first draft of the manuscript and created figures and tables. X.T., A.R., and C.P. contributed to involved in the aquarium experiments. R.P.P., A.R., X.T., and O.S.W. analysed the data. R.P.P. wrote the first draft of the manuscript and created figures and tables. X.T., A.R., and C.P. contributed to improved the first draft, and all authors revised the final version of the manuscript.

**DATA ACCESSIBILITY**

The *de novo* assemblies, RSEM, annotation and DEseq files are available at Mendeley Data [https://doi.org/10.17632/5673n552yj.1](https://doi.org/10.17632/5673n552yj.1). Raw reads are available at the Sequence Read Archive under the BioProject number PRJNA642021 and BioSamples SAMN15375803-SAMN15375820.

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REFERENCES

Agnello, M., & Roccheri, M. C. (2010). Apoptosis: Focus on sea urchin development. *Apoptosis*, 15(3), 322–330. https://doi.org/10.1007/s10495-009-0420-0

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*, 25, 3389–3402. https://doi.org/10.1093/nar/25.17.3389

Anestis, A., Lazou, A., Pörtner, H. O., & Michaelidis, B. (2007). Behavioral, metabolic, and molecular stress responses of marine bivalve *Mytilus galloprovincialis* during long-term acclimation at increasing ambient temperature. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 293, R911–R921.

Arle, H. C., & Robinson, P. A. (2005). E3 ubiquitin ligases. *Essays in Biochemistry*, 41, 15–30. https://doi.org/10.1042/E0410015

Baird, C. H., Niederlechner, S., Beck, R., Kallweit, A. R., & Witschmeyer, P. E. (2013). L-Threonine induces heat shock protein expression and decreases apoptosis in heat-stressed intestinal epithelial cells. *Nutrition*, 29, 1404–1411. https://doi.org/10.1016/j.nut.2013.05.017

Basile, A., Biazi, D., Sherbet, G. V., Comi, P., & Cajone, F. (2008). Hyperthermia inhibits cell proliferation and induces apoptosis: Relative signaling status of P53, S10A4, and Notch in heat sensitive and resistant cell lines. *Journal of Cellular Biochemistry*, 103, 212–220. https://doi.org/10.1002/jcb.21401

Beck, G., O’Brien, R. F., Habicht, G. S., Stillman, D. L., Cooper, E. L., & Raftos, D. A. (1993). Invertebrate cytokines III: Invertebrate interleukin-1-like molecules stimulate phagocytosis by tunicate and echinoderm cells. *Cellular Immunology*, 146, 284–299. https://doi.org/10.1016/cimm.1993.1027

Bito, A., Lerner, C. A., Nacarelli, T., Crowe, E., Torres, C., & Sell, C. (2014). P62/SQSTM1 at the interface of aging, autophagy, and disease. *Nature Communications*, 5, 3674. https://doi.org/10.1038/ncomms4674

Buckley, B. A., Gracey, A. Y., & Somero, G. N. (2006). The cellular response to heat stress in the goby *Giliichthys mirabilis*: A cDNA microarray and protein-level analysis. *Journal of Experimental Biology*, 209, 2660–2677. https://doi.org/10.1242/jeb.02292

Buckley, L. B., & Huey, R. B. (2016). How extreme temperatures impact organisms and the evolution of their thermal tolerance. *Integrative and Comparative Biology*, 56, 98–109. https://doi.org/10.1093/icb/icw004

Bulleri, F., Benedetti-Cecchi, L., & Cinelli, F. (1999). Grazing by the sea urchin *Arbacia lixula* and the sponge *Paracrinus lividus* in the Northwest Mediterranean. *Journal of Experimental Marine Biology and Ecology*, 241(1), 81–95.

Calosi, P., Melatunian, S., Turner, L. M., Artioli, Y., Davidson, R. L., Byrne, J. J., ... Rundle, S. D. (2017). Regional adaptation defines sensitivity to future ocean acidification. *Nature Communications*, 8, 13994. https://doi.org/10.1038/ncomms13994

Carreras, C., García-Cisneros, A., Wangensteen, O. S., Ordóñez, V., Palacin, C., Pascual, M., & Turon, X. (2020). East is East and West is West: Population genomics and hierarchical analyses reveal genetic structure and adaptation footprints in the keystone species *Paracrinus lividus* (Echinodera). *Diversity and Distributions*, 26, 382–398.

Cerrano, C., Bavestrello, G., Bianchi, C. N., Caitaneo-vietti, R., Bava, S., Morganti, C., ... Sponga, F. (2000). A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999. *Ecology Letters*, 3, 284–293. https://doi.org/10.1046/j.1461-0248.2000.00152.x

Christie, D. L. (2007). Functional insights into the creatine transporter. In G. S. Salomons & M. Wyss (Eds.), *Creatine and Creatine kinase in health and disease*. *Subcellular Biochemistry* (vol. 46, pp. 99–118). Dordrecht, Netherlands: Springer.

Clark, M. S., Suckling, C. C., Cavolo, A., Mackenzie, C. L., Thorne, M. A., Davies, A. J., & Peck, L. S. (2019). Molecular mechanisms underpinning transgenerational plasticity in the green sea urchin *Psammechinus miliaris*. *Scientific Reports*, 9, 952. https://doi.org/10.1038/s41598-018-37255-6

Claudet, J., & Fraschetti, S. (2010). Human-driven impacts on marine habitats: A regional meta-analysis in the Mediterranean Sea. *Biological Conservation*, 143, 2195–2206. https://doi.org/10.1016/j.biocon.2010.06.004

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., Aguzzi, J., ... Voultsiadou, E. (2010). The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. *PLoS One*, 5, e11842. https://doi.org/10.1371/journal.pone.0011842

Donelson, J. M., Sunday, J. M., Figueira, W. F., Gaitán-Espitia, J. D., Hobday, J. A., ... Palumbi, S. R., & Sanford, E. (2015). Aberrant gene expression profiles in Mediterranean Sea urchin reproductive tissues after metal exposure. *Molecular Ecology*, 35), CIESM, Monaco.

Evans, T. G., Pespeni, M. H., Hofmann, G. E., Palumbi, S. R., & Sanford, E. (2017). Transcriptomic responses to seawater acidification among sea urchin populations inhabiting a natural pH mosaic. *Molecular Ecology*, 26, 2257–2275. https://doi.org/10.1111/mec.14038
Feder, M. E., & Hofmann, G. E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology*, 61, 243–282. https://doi.org/10.1146/annurev.physiol.61.1.243

Feder, M. E., & Walser, J. C. (2005). The biological limitations of transcriptomics in elucidating stress and stress responses. *Journal of Evolutionary Biology*, 18, 901–910. https://doi.org/10.1111/j.1420-9101.2005.00921.x

Francour, P., Boudouresque, C. F., Harmelin, J. G., Harmelin-Vivien, M. L., & Quignard, J. P. (1994). Are the Mediterranean waters becoming warmer? Information from biological indicators. *Marine Pollution Bulletin*, 28, 523–526. https://doi.org/10.1016/0025-326X(94)90071-X

Gaitán-Espitia, J. D., Sánchez, R., Bruning, P., & Cárdenas, L. (2016). Fluctuations temporelles des peuplements d’échiquiers et d’échinodermes. *Fluctuations and ecotones of the NZ endemic sea urchin Kina* (*Evechinus chloroticus*). *Molecular Ecology*, 25, 493–511. https://doi.org/10.1111/1365-294X.12686

Kelemen, O., Convertini, P., Zhang, Z., Wen, Y., Shen, M., Falalvee, M., & Stamm, S. (2013). Function of alternative splicing. *Gene*, 514(1), 1–30. https://doi.org/10.1016/j.gene.2012.07.083

Kim, B. M., Kim, K., Choi, I. Y., & Rhee, J. S. (2017). Transcriptome response of the Pacific oyster, *Crassostrea gigas* susceptible to thermal stress: A comparison with the response of tolerant oyster. *Molecular & Cellular Toxicology*, 13(1), 105–113. https://doi.org/10.1007/s13273-017-0011-z

King, N. G., McKeown, N. J., Smale, D. A., & Moore, P. J. (2018). The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. *Ecography*, 41, 1469–1484. https://doi.org/10.1111/ecog.03186

Kostova, Z., & Wolf, D. H. (2003). For whom the bell tolls: Protein quality control of the endoplasmic reticulum and the ubiquitin–proteasome connection. *The EMBO Journal*, 22, 2309–2317.

Logan, C. A., & Somero, G. N. (2011). Effects of thermal acclimation on transcriptional responses to acute heat stress in the eurythermal fish *Gillichthys mirabilis* (Cooper). *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 300, R1373–R1383. https://doi.org/10.1152/ajpregu.00689.2010

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., ... Wilson, S. K. (2017). Global warming and recurrent mass bleaching of corals. *Nature*, 543, 373. https://doi.org/10.1038/nature21707

Jia, Z., Wang, Q., Wu, K., Wei, Z., Zhou, Z., & Liu, X. (2017). De novo transcriptome sequencing and comparative analysis to discover genes involved in ovoarial maturity in *Strongylocentrotus nudus*. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 23, 27–38. https://doi.org/10.1016/j.cbd.2017.05.002

Jonas, K., Liu, J., Chien, P., & Laub, M. T. (2013). Proteotoxic stress induces a cell cycle arrest by stimulating Lon to degrade the replication initiator DnaA. *Cell*, 154, 623–636. https://doi.org/10.1016/j.cell.2013.06.034

Jones, P., Binns, D., Chang, H.-Y., Fraser, M., Li, W., McAnulla, C., ... Hunter, S. (2014). InterProScan 5: Genome-scale protein function classification. *Bioinformatics*, 30, 1236–1240. https://doi.org/10.1093/bioinformatics/btu031

Feder, M. E., & Hofmann, G. E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology*, 61, 243–282. https://doi.org/10.1146/annurev.physiol.61.1.243

Feder, M. E., & Walser, J. C. (2005). The biological limitations of transcriptomics in elucidating stress and stress responses. *Journal of Evolutionary Biology*, 18, 901–910. https://doi.org/10.1111/j.1420-9101.2005.00921.x

Francour, P., Boudouresque, C. F., Harmelin, J. G., Harmelin-Vivien, M. L., & Quignard, J. P. (1994). Are the Mediterranean waters becoming warmer? Information from biological indicators. *Marine Pollution Bulletin*, 28, 523–526. https://doi.org/10.1016/0025-326X(94)90071-X

Gaitán-Espitia, J. D., Sánchez, R., Bruning, P., & Cárdenas, L. (2016). Functional insights into the tests transcriptome of the edible sea urchin *Loxechinus albus*. *Scientific Reports*, 6, 36516. https://doi.org/10.1038/srep36516

Garcia-Cisneros, A., Palacin, C., Ventura, C. R. R., Feitäl, B., Paiva, P. C., & Pérez-Portela, R. (2018). Intraspecific genetic structure, divergence and high rates of clonality in an amphi-Atlantic starfish. *Molecular Ecology*, 27, 752–772. https://doi.org/10.1111/mec.14454

Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., ... Cerrano, C. (2009). Mass mortality in Northwestern Mediterranean rocky benthic communities: Effects of the 2003 heat wave. *Global Change Biology*, 15, 1090–1103. https://doi.org/10.1111/j.1365-2486.2008.01823.x

Gianguzza, P., Atena, D., Bonaviri, C., Di Tranapi, F., Visconti, G., Gianguzza, F., & Riggio, S. (2011). The rise of thermophilic sea urchins and the expansion of barren grounds in the Mediterranean Sea. *Chemistry and Ecology*, 27, 129–134. https://doi.org/10.1007/10275 540.2010.547484

Gianguzza, P., Visconti, G., Gianguzza, F., Vizzini, S., Sartá, G., & Dupont, S. (2014). Temperature modulates the response of the thermophilous sea urchin * Arbacia lixula* early life stages to CO2-driven acidification. *Marine Environmental Research*, 93, 70–77. https://doi.org/10.1016/j.marenres.2013.07.008

Gillard, G. B., Garama, D. J., & Brown, C. M. (2014). The transcriptome of the NZ endemic sea urchin Kina (*Evechinus chloroticus*). *BMC Genomics*, 15, 45. https://doi.org/10.1186/1471-2164-15-45

Gleason, L. U., & Burton, R. S. (2015). RNA-seq reveals regional differences in transcriptome response to heat stress in the snail *C. hlorostoma* funeralis. *Molecular Ecology*, 24, 610–627.

González, K., Gaitán-Espitia, J., Font, A., Cárdenas, C. A., & González-Aravena, M. (2016). Expression pattern of heat shock proteins during acute thermal stress in the Antarctic sea urchin, *Strechiches neumayeri*. *Revista Chilena De Historia Natural*, 89, 2. https://doi.org/10.40693/016-0052-z

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., ... Regev, A. (2011). Trinity: Reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nature Biotechnology*, 29, 644–652.

Harmelin, J. G., Hereu, B., De Masionnave, L. M., Teixidor, N., Domínguez, L., & Zabala, M. (1995). *Indicateurs de biodiversité en milieu marin: les échinodermes*. Fluctuations temporelles des peuplements d’échinodermes à Port-Cros. Comparaison entre les années 1982–84 et 1993–95. Internal Report. Port Cros National Park.

Hereu, B., Linares, C., Sala, E., Garrabou, J., Garcia-Rubies, A., Diaz, D., & Zabala, M. (2012). Multiple processes regulate long-term population dynamics of sea urchins on Mediterranean rocky reefs. *PLoS One*, 7, e36901. https://doi.org/10.1371/journal.pone.0036901

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world’s marine ecosystems. *Science*, 328, 1523–1528. https://doi.org/10.1126/science.1189930
Malhotra, J. D., & Kaufman, R. J. (2007). Endoplasmic reticulum stress and oxidative stress: A vicious cycle or a double-edged sword? Antioxidants & Redox Signaling, 9, 2277-2294. https://doi.org/10.1089/ars.2007.1782

Marbà, N., Jordà, G., Agustí, S., Girard, C., & Duarte, C. M. (2015). Footprints of climate change on Mediterranean Sea biota. Frontiers in Marine Science, 2, 56. https://doi.org/10.3389/fmars.2015.00056

Martins, C. S., Hamann, M., & Fiúza, A. F. (2002). Surface circulation in the eastern North Atlantic, from drifters and altimetry. Journal of Geophysical Research: Oceans, 107, 10-11. https://doi.org/10.1029/2000JC000345

Matranga, V., Bonaventura, R., & Di G. B. (2002). Hsp70 as a stress marker of sea urchin coelomocytes in short term cultures. Cellular and Molecular Biology (Noisy-le-Grand, France), 48, 345–349.

Matranga, V., Pinsino, A., Celli, M., Natoli, A., Bonaventura, R., Schröder, H. C., & Müller, W. E. G. (2005). Monitoring chemical and physical stress using sea urchin immune cells. In V. Matranga (Ed.), Echinodermata (vol. 39, pp. 85–110). Heidelberg, Berlin: Springer.

Moncada, S., Ferrer, J. M., & Palacios, J. M. (1998). Endothelial nitric oxide synthase. Physiology & Biophysics, 15, 1–10. https://doi.org/10.1016/S0962-8924(97)01079-9

Morimoto, R. I., & Muñoz, R. A. (1999). The chaperone function of cytosolic Hsp70 is required for protection against stress-induced apoptosis. Molecular and Cellular Biology, 20, 71467159. https://doi.org/10.1128/MCB.20.19.7146-7159.2000

Mosser, D. D., Caron, A. W., Bourget, A., Meriin, A. B., Sherman, M. Y., Morimoto, R. I., & Massie, B. (2000). The chaperone function of hsp70 is required for protection against stress-induced apoptosis. Molecular and Cellular Biology, 20, 71467159. https://doi.org/10.1128/MCB.20.19.7146-7159.2000

Nicosia, A., Delia Torre, C., Sammarini, V., Bonaventura, R., Amato, E., & Matranga, V. (2008). Sea urchin coelomocytes as a novel cellular biosensor of environmental stress: A field study in the Tremiti Island Marine Protected Area, Southern Adriatic Sea, Italy. Cell Biology and Toxicology, 24, 541-552. https://doi.org/10.1007/s10565-008-9055-0

Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Oleksiak, M. F., Roach, J. L., & Crawford, D. L. (2005). Natural variation of the hsp70 gene in purple sea urchin: Biogeographic pattern and the effect of temperature acclimation. Journal of Experimental Marine Biology and Ecology, 327, 134–143.

Ozato, K., Shin, D. M., Chang, T. H., & Morse, H. C. III (2008). TRIM family proteins and their emerging roles in innate immunity. Nature Reviews Immunology, 8, 849. https://doi.org/10.1038/nri2413

Paracentrotus lividus and Arbacia lixula larvae and settlement. Journal of Experimental Marine Biology and Ecology, 407(1), 6–11.

Parry, M. L., Canziani, O. F., Palutikof, J. P., Van Der Linden, P. J., & Hanson, C. E. (2007). IPCCC: 2007: climate change 2007: impacts, adaptation and vulnerability. In Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. Cambridge, UK: Cambridge University Press.

Pastor, F. J. (2012). Ciclogénesis intensas en la cuenca occidental del Mediterráneo y temperatura superficial del mar: Modelización y evaluación de las áreas de recarga. PhD dissertation. Retrieved from https://www.tdx.cat/handle/10803/83620

Pérez-Portela, R., & Riesgo, A. (2013). Optimizing preservation protocols to extract high-quality RNA from different tissues of echinoderms for next generation sequencing. Molecular Ecology Resources, 13, 884–889. https://doi.org/10.1111/j.1755-0998.12122

Pérez-Portela, R., Turon, X., & Riesgo, A. (2016). Characterization of the transcriptome and gene expression of four different tissues in the ecologically relevant sea urchin Arbacia lixula using RNA-seq. Molecular Ecology Resources, 16, 794–808.

Pérez-Portela, R., Wangensteen, O. S., García-Cisneros, A., ValeroJiménez, C., Palacin, C., & Turon, X. (2019). Spatio-temporal patterns of genetic variation in Arbacia lixula, a thermophilous sea urchin in expansion in the Mediterranean. Heredity, 122, 244. https://doi.org/10.1038/s41437-018-0098-6

Pérez-Portela, R., Turon, X., & Amato, E., & Matranga, V. (2008). Sea urchin coelomocytes as a novel cellular biosensor of environmental stress: A field study in the Tremiti Island Marine Protected Area, Southern Adriatic Sea, Italy. Cell Biology and Toxicology, 24, 541-552. https://doi.org/10.1007/s10565-008-9055-0

Pörtner, H. O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 132, 739–761. https://doi.org/10.1016/S0962-8436(02)00045-4

Privitera, D., Noli, M., Falugi, C., & Chiantore, M. (2011). Benthic assemblages and temperature effects on Paracentrotus lividus and Arbacia lixula larvae and settlement. Journal of Experimental Marine Biology and Ecology, 407(1), 6–11.

Prusina, I., Sará, G., De Pirro, M., Dong, Y. W., Han, G. D., Glumuzina, B., & Williams, G. A. (2014). Variations in physiological responses to thermal stress in congenic limpets in the Mediterranean Sea. Journal of Experimental Marine Biology and Ecology, 456, 34–40. https://doi.org/10.1016/j.jembe.2014.03.011

Rachel, A., Tyson, J. R., & Stirling, C. J. (1997). A novel subfamily of Hsp70s in the endoplasmic reticulum. Trends in Cell Biology, 7, 277–282. https://doi.org/10.1016/S0962-8924(97)01079-9

Raitos, D. E., Beaugrand, G., Georgopoulos, D., Zenetos, A., Panucci-Papadopoulou, A. M., Theocharis, A., & Papathanassiou, E. (2010). Global climate change amplifies the entry of tropical species into the Eastern Mediterranean Sea. Limnology and Oceanography, 55, 1478–1484. https://doi.org/10.4319/lo.2010.55.5.1478

Rao, R. V., Peel, A., Logvinova, A., del Rio, G., Hermes, E., Yokota, T., & Bredesen, D. E. (2002). Coupling endoplasmic reticulum stress to the cell death program: Role of the ER chaperone GRP78. FEBS Letters, 514, 122–128. https://doi.org/10.1016/S0014-5793(02)02289-5

R Development Core Team (2008). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0. Retrieved from http://www.R-project.org

Rilov, G. (2016). Multi-species collapses at the warm edge of the warming sea. Scientific Reports, 6, 36897.

Runcie, D. E., Garfield, D. A., Babbitt, C. C., Wygoda, J. A., Mukherjee, S., & Wray, G. A. (2012). Genetics of gene expression responses to temperature stress in a sea urchin gene network. Molecular Ecology, 21, 4547-4562. https://doi.org/10.1111/j.1365-294X.2012.05717.x
Schoville, S. D., Barreto, F. S., Moy, G. W., Wolff, A., & Burton, R. S. (2012). Investigating the molecular basis of local adaptation to thermal stress: Population differences in gene expression across the transcriptome of the copepod Tigriopus californicus. BMC Evolutionary Biology, 12, 170. https://doi.org/10.1186/1471-2148-12-170

Shaltout, M., & Omstedt, A. (2014). Recent sea surface temperature trends and future scenarios for the Mediterranean Sea. Oceanologia, 56, 411–443. https://doi.org/10.5697/oc.56-3.411

Shental-Behor, D., & Levy, Y. (2008). Effect of glycosylation on protein folding: A close look at thermodynamic stabilization. Proceedings of the National Academy of Sciences, 105, 8256–8261. https://doi.org/10.1073/pnas.0801340105

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics, 31(19), 3210–3212. https://doi.org/10.1093/bioinformatics/btv351

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., ... Moore, P. J. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nature Climate Change, 9, 306. https://doi.org/10.1038/s41558-019-0412-1

Smith, L. C., Arriza, V., Hudgell, M. A. B., Barone, G., Bodnar, A. G., Buckley, K. M., ... Furukawa, R. (2018). Echinodermata: The complex immune system in echinoderms. In E. L. Cooper (Ed.), Advances in Invertebrate Survival Journal, 35, 25–39.

Somoero, G. N. (2010). The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine ‘winners’ and ‘losers’. Journal of Experimental Biology, 213, 912–920. https://doi.org/10.1242/jeb.037473

Somot, S., Sevault, F., & Déqué, M. (2012). Transient climate change scenario simulation of the Mediterranean Sea for the twenty-first century using a high-resolution ocean circulation model. Climate Dynamics, 27, 851–879. https://doi.org/10.1007/s00382-006-0167-z

Somot, S., Sevault, F., Déqué, M., & Crépon, M. (2008). 21st century climate change scenario for the Mediterranean using a coupled atmosphere–ocean regional climate model. Global and Planetary Change, 63, 112–126. https://doi.org/10.1016/j.gloplacha.2007.10.003

Stamm, S., Ben-Ari, S., Rafalska, I., Tang, Y., Zhang, Z., Toiber, D., ... Soreq, H. (2005). Function of alternative splicing. Gene, 344, 1–20. https://doi.org/10.1016/j.gene.2004.10.022

Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to stress response of Marsupenaeus japonicus. Marine Biology and Ecology, 12, 151–165. https://doi.org/10.1016/j.molpec.2003.02.002

Stillman, J. H. (2019). Heat waves, the new normal: Summertime temperature extremes will impact animals, ecosystems, and human communities. Physiology, 34, 86–100. https://doi.org/10.1152/physiol.00040.2018

Supek, F., Bošnjak, M., Škunca, N., & Šmuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. PLoS One, 6, e21800. https://doi.org/10.1371/journal.pone.0021800

Templado, J. (2014). Future trends of Mediterranean biodiversity. In S. Goffredo & Z. Dubinsky (Eds.), The Mediterranean Sea (pp. 479–498). Dordrecht: Springer.

Temanek, L. (2010). Variation in the heat shock response and its implication for predicting the effect of global climate change on species’ biogeographical distribution ranges and metabolic costs. Journal of Experimental Biology, 213, 971–979. https://doi.org/10.1242/jeb.038034

Tortone, E. (Ed.). (1965). Fauna d'Italia: Echinodermata. Calderini.

Visconti, G., Gianguzzo, F., Butera, E., Costa, V., Vizzini, S., Byrne, M., & Gianguzzo, P. (2017). Morphological response of the larvae of Arbacia lixula to near-future ocean warming and acidification. ICES Journal of Marine Science, 74, 1180–1190. https://doi.org/10.1093/icesjms/fsx037

Wang, F., Yang, H., Gao, F., & Liu, G. (2008). Effects of acute temperature or salinity stress on the immune response in sea cucumber, Apostichopus japonicus. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 151, 491–498. https://doi.org/10.1016/j.cbpa.2008.06.024

Wangensteen, O. S., Dupont, S., Casties, I., Turon, X., & Palacin, C. (2013). Some like it hot: Temperature and pH modulate larval development and settlement of the sea urchin Arbacia lixula. Journal of Experimental Marine Biology and Ecology, 449, 304–311. https://doi.org/10.1016/j.jembe.2013.10.007

Wangensteen, O. S., Turon, X., Casso, M., & Palacin, C. (2013). The reproductive cycle of the sea urchin Arbacia lixula in northwest Mediterranean: Potential influence of temperature and photoperiod. Marine Biology, 160, 3157–3168. https://doi.org/10.1007/s00227-013-2303-8

Wangensteen, O. S., Turon, X., Pérez-Portela, R., & Palacin, C. (2012). Natural or naturalized? Phylogeography suggests that the abundant sea urchin Arbacia lixula is a recent colonizer of the Mediterranean. PLoS One, 7, e45067. https://doi.org/10.1371/journal.pone.0045067

Warnes, M. G. R., Bolker, B., Bonebakker, L., & Gentleman, R. (2016). Package ‘gplots’. Various R Programming Tools for Plotting Data.

Xu, D., Zhou, S., & Sun, L. (2018). RNA-seq based transcriptional analysis reveals dynamic genes expression profiles and immune-associated regulation under heat stress in Apostichopus japonicus. Fish & Shellfish Immunology, 78, 169–176. https://doi.org/10.1016/j.fsi.2018.04.037

Yao, C. L., & Somoero, G. N. (2012). The impact of acute temperature stress on hemocytes of invasive and native mussels (Mytilus galloprovincialis and Mytilus californianus): DNA damage, membrane integrity, apoptosis and signaling pathways. Journal of Experimental Biology, 215, 4267–4277. https://doi.org/10.1242/jeb.073577

Young, C. M., Tyler, P. A., & Fenaux, L. (1997). Potential for deep sea invasion by Mediterranean shallow water echinoids: Pressure and temperature as stage-specific dispersal barriers. Marine Ecology Progress Series, 154, 197–209. https://doi.org/10.1354/meps154197

Zhao, C., Zhang, L., Shi, D., Ding, J., Yin, D., Sun, J., ... Chang, Y. (2018). Transgenerational effects of ocean warming on the sea urchin Strongylocentrotus intermedius. Ecotoxicology and Environmental Safety, 151, 212–219. https://doi.org/10.1016/j.ecoenv.2018.01.014

Zheng, J., Cao, J., Mao, Y., Su, Y., & Wang, J. (2019). Comparative transcriptome analysis provides comprehensive insights into the heat stress response of Marsupenaeus japonicus. Aquaculture, 502, 338–346. https://doi.org/10.1016/j.aquaculture.2018.11.023

Zhu, Q., Zhang, L., Li, L., Que, H., & Zhang, G. (2016). Expression characterization of stress genes under high and low temperature stresses in the Pacific oyster, Crassostrea gigas. Marine Biotechnology, 18, 176–188. https://doi.org/10.1007/s10126-015-9678-0

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.