The short and long-term implications of warming and increased sea water pCO2 on the physiological response of a temperate neogastropod species

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Received: 29 January 2021 / Accepted: 29 October 2021 / Published online: 20 November 2021
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Abstract
Global average temperatures and seawater pCO2 have rapidly increased due to the oceanic uptake of atmospheric carbon dioxide producing severe consequences for a broad range of species. The impacts on marine ectotherms have been largely reported at short-term scales (i.e. from days to weeks); however, the prolonged effects on long-term processes such as reproduction have received little attention. The gastropod *Ocenebra erinaceus* is a key predator structuring communities on rocky shores of the French and UK coasts. Even though rocky shore species are regarded as being very tolerant to changes in temperature and pH, many of them are living near their upper tolerance limits, making them susceptible to rapid environmental changes. Here, we report that future mean seawater conditions (RCP8.5, +3 °C and ~900 μatm CO2) do not significantly affect the physiology and molecular response of *O. erinaceus* adults after 132 days. During the first 50 days, there was a slight impact on oxygen consumption rates and body weight; however, after 95 days of exposure, gastropods fully acclimated to the experimental condition. Despite this, reproduction in females exposed to these future seawater conditions ceased after long-term exposure (~10 months). Therefore, in the short-term, *O. erinaceus* appear to be capable of full compensation; however, in the long-term, they fail to invest in reproduction. We conclude studies should be based on combined results from both short- and long-term effects, to present realistic projections of the ecological consequences of climate warming.

Keywords Warming · pCO2 · Neogastropod · Short-term · Long-term

Introduction
Anthropogenic greenhouse gas emissions (i.e. carbon dioxide (CO2), methane (CH4), nitrous oxide (N2O)) have risen to unprecedented levels (Gattuso et al. 2015) producing changes in the weather, air and sea-surface temperatures, sea level, and seawater pCO2 (and hence pH), amongst others (IPCC 2013). Carbon dioxide is one of the most important greenhouse gases in terms of its effects on climate change (e.g. regulating Earth’s surface temperature) and in regulating global ocean chemistry. The CO2 emissions into Earth’s atmosphere have accelerated, ranging from preindustrial 280 to 410.27 ppm in 2019 (NOAA). The oceans have absorbed 30% of the atmospheric CO2 emissions, and, at the same time, release a high amount of hydrogen [H+] molecules causing ocean pH to drop, with an expected decrease by the end of 2100 of 0.33 (± 0.003) units (Bopp et al. 2013; Orr et al. 2005). It is well known that increases in seawater pCO2 produce negative effects in marine animals, especially in calcifying organisms, reducing growth, survival, reproduction, and shell mineralization, amongst others (Barton et al. 2012; Dupont et al. 2013; Kroeker et al. 2010; Parker et al. 2013). In addition, and due to the atmospheric CO2 level increase, global sea temperatures are also rising, with an expected increase of 2.73 (± 0.72) °C by the end of this century (Bopp et al. 2013; Meinshausen et al. 2011). It remains controversial, however, to what extent combined temperature and pCO2 affects ectotherms’ metabolism, as increased
temperatures produce an increase in metabolism and pH/hypercapnia can induce metabolic depression (Harvey et al. 2013; Michaelidis et al. 2005; Kroeker et al. 2013). To date, most experimental research has focused on single factor (stressor) effects (e.g. CO₂, Dupont et al. 2013, Hernroth et al. 2011) and over short time scales (Kroeker et al. 2013). The long-term survival of marine ectotherms largely depends on how fast they can acclimate or adapt to environmental changes (Hofmann and Todgham 2010). Phenotypic plasticity has been identified as one of the most powerful mechanisms to adjust to climate change (Nagelkerken and Munday 2016). During short-term exposure to stressful conditions, physiological plasticity can facilitate population persistence by acclimating to environmental conditions (Foo et al. 2012; Kelly et al. 2012). However, physiological compensatory mechanisms develop over prolonged periods, often resulting in differential trade-offs, because acclimation to one physiological function can result in a significant cost of another one. For example, at the organism level, Godbold and Solan (2013) did not detect any effect on the physiology of a non-calcifying marine polychaete, Alitta virens, when exposed to warming and acidification for a short period. However, after 18 months, the organism’s physiology revealed negative effects in growth and behaviour, potentially affecting ecosystem functioning. Dupont et al. (2013), showed that after a short-term exposure to high pCO₂ (~ 4 months), female fecundity decreased, and negative carry-over effects were observed on the survival of larvae and juveniles of the sea urchin Stringylocentrotus droebachiensis. However, when females were exposed for 16 months, fecundity was not affected, nor any trans-generational effects were observed. Similar results were found in the cold-water coral Lophelia pertusa, in which calcification rate was significantly affected after short-term exposure (~ 1 week). However, after 6 months, corals were able to fully acclimate to the acidified conditions, enhancing calcification rates (Form and Riebesell 2012). These studies suggest a gap exists in our understanding of acclimation and adaptation processes to climate warming. Because short-term responses can be ameliorated or exacerbated by prolonged exposure, it appears plausible that long-term responses need to be considered to make accurate ecological projections towards climate change (warming).

The aim of this study was to understand the importance of evaluating the short-term but also long-term effects of climate warming on the physiology of marine ectotherms. Rocky shore species are regarded as being particularly tolerant of impacts of future ocean conditions; however, many of them are living close to upper physiological limits, which makes them highly susceptible to rapid environmental changes (Somero 2002). Understanding how rocky shore organisms will compensate for the effects of climate change over extended periods of time will improve our projections of the ecological consequences of climate warming. In this study, we assessed the short and long-term effects of temperature and pCO₂ increases expected for the end of this century according to RCP8.5 IPPC 2013 (+ 3 °C, ~ 900 μatm CO₂) on the physiological and molecular response (at 135 days) and reproductive investment (at ~ 300 days) of the globally distributed intertidal gastropod Ocenebra erinaceus. To determine any physiological response, we measured the effects of these stressors on physiological energetics: i.e. energy gain (ingestion rate) and investment (growth, metabolic rate, cellular response).

Material and methods

Animal collection and maintenance

Ocenebra erinaceus (also known as “oyster drill”) adults (30–40 mm shell length) were collected by hand from the low intertidal in the Solent, UK (50° 51’ N, 001° 21’ W). After collection, specimens were transferred to the National Oceanography Centre, Southampton, UK (NOCS), fed ad libitum with mussels and maintained for thirty days prior to experimentation under laboratory conditions (14–15 °C; 33–34 psu). Then, gastropods were transferred to one of the two experimental conditions: control (14 °C—400 μatm CO₂) and future conditions (17 °C—900 μatm CO₂), representative of a future IPCC RCP8.5 scenario (IPCC 2013).

Temperature and CO₂ system

The ‘CO₂ system’ was designed following the methodology described by Hauton et al. (2009) and Riebesell et al. (2011). Two experimental systems were set up: two control tanks (14 °C and pH 8.0—400 ppm of CO₂) and two CO₂ injection tanks (+ 3 °C and pH 7.7—900 μatm CO₂). Each tank comprised of a ‘mixing tank’ (50 L) and separate ‘incubation tank’ (20 L) that were maintained constantly—using water bath—at 14 or 17 °C, respectively. Seawater was mixed between the mixing and incubation tanks with a peristaltic pump and was continually aerated. In the CO₂ injection tanks, pH was adjusted using AquaMedic™ pH computers with calibrated electrodes. Temperature and salinity were measured every 2 days with a EC170 meter and pH was measured three times per week using a three-decimal-place SevenMulti pH meter. Water samples were taken and fixed using mercuric chloride every fortnight in control and CO₂ system mixing tanks, according to Riebesell et al. (2011). Total alkalinity (A₇) and dissolved inorganic carbon (DIC) were determined to characterize the carbonate chemistry in each tank, using VINDTA 3C following standard protocols at the carbonate facility at NOCS. pCO₂ was calculated from measured pH, A₇, temperature and salinity using CO₂calc.
(Lewis and Wallace 1998). The seawater conditions for the adult physiology experiment are summarised in Table 1.

**Short-term exposure (0–135 days)**

Forty gastropods (i.e. 17–20 snails per experimental condition; ~10 snails per experimental tank) were exposed to control and climate change conditions and measured at regular intervals of 0, 15, 50, 95 and 135 days to observe the physiological impact on growth, weight, ingestion, routine metabolic rate, and *hsp*70 gene expression. Shell growth (in mm per day) and weight (in grammes per day) were monitored. Ingestion rate was determined sensu Navarro et al. (2002), with minor modifications. Ingestion rate was estimated every week in control and climate change tanks. Each tank was fed with 5 mussels of *Mytilus edulis* per week. At the end of each week, only empty valves of prey were collected and replaced by live mussels. The shell length of empty valves was measured to estimate food ingested by *O. erinaceus* snails. A regression curve was calculated to estimate dry mussel weight (dry meat (g) = 0.00006 * mussel length (mm); 3.4412; *n* = 23 mussels with sizes ranging between 20 and 55 mm). To estimate individual food intake, total dry mass of mussel meat ingested per week was divided by the number of snails in each aquarium.

Routine metabolic rate (RMR) was determined on fed ad libitum snails in hermetically sealed chambers with a pre-calibrated oxygen sensor attached to the inner wall (FIBOX, PreSens fiber optic equipment calibrated according to manufacturer’s protocols) filled with 365 ml of filtered seawater (0.5 μm and salinity 33–34), at 100% air saturation and at the same temperature and CO₂ concentration of experimental conditions. Shell growth, weight and RMR were evaluated in the same snails throughout the experiment (*n* = 10 stress; *n* = 5 control snails) on day 0, 15, 50, 95 and 135. Ingestion rate was evaluated on each tank (i.e. number of snails depending on the day, please see Fig. 1c), and *hsp*-70 was evaluated in 8–9 snails stress/control per experimental day (on day 95 only stress snails were evaluated).

**Hsp70 gene expression (short-term exposure)**

For the molecular analysis of changes in the expression of 70 kDa heat shock protein, degenerate primers were first designed to isolate *O. erinaceus*-specific genes sequences for *hsp*70 gene and two endogenous reference genes for quantitative real-time PCR: glyceraldehyde-3-phosphate dehydrogenase (*gapdh* gene) and β-actin (*β-act* gene). Degenerate primers were designed based on conserved regions that were identified from protein sequences alignments of using CLUSTAL Omega. Each alignment was examined, and any mismatches were corrected manually. For *hsp*70, degenerate primers (sense 5'-GAYATGAAARCAYTGGCCNT-3', antisense 5'-TANGCNANGCYTCTRCNG-3', *Tm* = 58 °C) were designed against an amino acid alignment of 7 moluscan heat shock sequences. For *gapdh* (sense 5'-CNCNATGTGYGTNGRTNGG-3', antisense 5'-ATNCCNGNYNOCRTCRRAA-3', *Tm* = 59 °C) and *β-act* (sense 5'-ATGGTNGGNGATGGNCARAA-3', antisense 5'-TCYT TTYTGCATNCKRTCNGC-3', *Tm* = 57 °C) gene, degenerate primers were designed against an amino acid sequence of 4, and 10 sequences, respectively (Table 2). Primers were synthesized by Eurofin MWG operon (Ebersberg, Germany).

Degenerate primers were used in a conventional PCR reaction to isolate fragments of each gene. PCR reactions were performed according to the manufacturer protocol using 0.25 μl of GoTaQ DNA polymerase (5u/μl; Promega Corporation), 2 μM of each of sense and antisense degenerate primers and 1 μl of template cDNA (whole-tissue snails) in a final volume of 25 μl with the following PCR cycle conditions: 1 cycle of 95 °C for 2 min, 35 cycles of [95 °C 1 min, ‘AT°C 1 min, 72 °C 30 s], followed by 72 °C for

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**Table 1** Environmental seawater parameters for control and high T°/pCO₂ in adult gastropods (*Ocenebra erinaceus*) exposed to short and long-term conditions

| Parameters          | Control          | High T°/pCO₂ | Control          | High T°/pCO₂ |
|---------------------|------------------|--------------|------------------|--------------|
| pH                  | 8.022 ± 0.2      | 7.678 ± 0.1  | 8.114 ± 0.04     | 7.646 ± 0.2  |
| Temperature (°C)    | 14.6 ± 0.4       | 17.2 ± 0.2   | 14.6 ± 0.3       | 17.3 ± 0.2   |
| Salinity            | 33.2 ± 0.8       | 33.4 ± 0.5   | 32.85 ± 0.1      | 33.65 ± 0.21 |
| pCO₂ (μatm)         | 499 ± 60         | 1013 ± 157   | 475 ± 9          | 1072 ± 120   |
| Total alkalinity (μmol Kg⁻¹) | 2314 ± 71 | 2221 ± 52    | 2104 ± 164      | 2232 ± 290   |
| DIC (μmol Kg⁻¹)     | 2140 ± 79        | 2146 ± 71    | 1936 ± 150       | 2136 ± 238   |
| [CO₃²⁻] (μmol kg⁻¹) | 128 ± 14         | 76 ± 11      | 114 ± 17         | 65 ± 30      |
| Ωḥca                | 3.1 ± 0.3        | 1.8 ± 0.2    | 1.7 ± 0.3        | 1.55 ± 0.7   |
| Ωḥar                | 2.0 ± 0.2        | 1.2 ± 0.2    | 2.7 ± 0.4        | 1.23 ± 0.7   |

All parameters were measured each fortnight. (DIC) means dissolved inorganic carbon. ‘Ωḥca’ means the saturation state of calcite minerals, ‘Ωḥar’ means the saturation state of aragonite minerals. Values are given as mean ± standard deviation.

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8 min (‘AT’ = annealing temperature of each pair of degenerate primers). PCR products obtained from degenerate PCR were gel-purified and potential positive gene fragments were cut from agarose gel (1% agarose). The DNA from the gel was extracted using QIAquick® Gel extraction kit (Qiagen Ltd. UK) following the manufacturer’s protocol. Extracted

Table 2 Sequences used for alignments to design degenerate primers

| Protein name | Species names and accession numbers |
|--------------|------------------------------------|
| HSP70 (GOI)  | Pomacea canaliculata (KF356182) 906 SL; Haliotis gigante (MF375339) 804 SL; Cellana toreuma (JX169849) 861 SL; Biomphalaria glabrata (AF025477) 1085 SL; Haliotis fulgens (MH220529) 827 SL; Pomacea canaliculata (KM405321) 871 SL; Mytilus galloprovincialis (AY861684) 793 SL; Crassostrea gigas (AB122063) 743 SL; Rattus norvegicus (L16764) 810 SL |
| β-Actin (ERG)| Cepaea nemoralis (MH035489) 472 SL; Haliotis diversicolor (EU244396) 499 bp; Hypriopsis cumingii (HM045420) 479 SL; Loligo pealei (AY701849) 718 SL; Crassostrea brasiliana (KY707329) 377 SL; Azunapecent farreri (KJ081194) 613 SL; Gecarcinus lateralis (L76943) 463 SL; Peneaus monodon (MG775230) 434 SL; Macaca mulatta (NM_001033084) 523 SL; Pan troglodytes (AB188274) 610 SL |
| GAPDH (ERG) | Homo sapiens (AF261085) 434 SL; Cryptocercus punctulatus (JQ686947) 439 SL; Bufo gargarizans (KX698056) 381 SL; Scophthalmus maximus (KU057925) 435 SL |

Listed are the sequences, species name, and accession numbers from the NCBI database. GOI gene of interest, ERG endogenous reference genes, SL aminoacidic sequence length.
PCR products were cloned using the pGEM®-T Easy Vector cloning kit (Promega Ltd.). Plasmid DNA was isolated from three bacterial colonies using the Qiaprep Spin Miniprep kit (QIAGEN) and sequenced using vector-specific (M13) primers by SourceBioscience LifeScience, Nottingham. The nucleotide sequences were used to deduce amino acid sequences for each gene fragment, and these were compared against the ENA database search engine using a BLAST sequence similarity search.

Total RNA was extracted from gills from control and climate change gastropods using TRI reagent (Sigma Aldrich) following the manufacturer’s instructions. RNA purity was confirmed by analysing the A260/A280 ratio (ND-1000 spectrophotometer, thermo Scientific) and RNA integrity and quantity were measured by an automated capillary electrophoresis system (Experion Bio-Rad laboratories) with Std-Sens chips and reagents. Genomic DNA contamination of total RNA samples was removed before cDNA synthesis; 4 μg total RNA was treated following the manufacturer’s protocol (Promega Corporation). DNASE-treated total RNA was then reverse transcribed using Superscript III reverse transcriptase (Invitrogen, UK) according to the manufacturer’s protocol.

For the quantitative real-time PCR analysis, qPCR primers were designed based on the nucleotide sequences for each gene obtained from cloning and sequencing. Each primer pair was designed using PrimerQuest Tool (Integrated DNA Technologies) according to the recommended criteria. The sequences of the primers are summarised in Table 3. All real-time qPCR reactions were performed in a LightCycler 96 (Roche, Switzerland); each 22 μl reaction contained: 12.5 μl of Precision Plus 2×qPCR Master mix with SYBR green (PrimerDesign, UK), 2 μl of each primer and 2 μl of template cDNA with the following PCR conditions: 1 cycle of 95 °C for 5 min, 40 cycles of [90 °C 10 s, 60 °C 1 min]. Each gene was run in duplicate in 96-plates and NTCs (“no template controls”) were included for every primer pair. Optimized primer concentrations per each gene are described in Table 3. After each run a melt curve analysis was performed to determine if a single product (i.e., the specific gene) was amplified. Standard curves of tenfold serial dilutions were conducted to estimate the amplification efficiency and the linearity ($r^2$), according to the MIQE guidelines (Bustin et al. 2009) with efficiency ranging between 90 and 105% and linearity greater than $r^2 = 0.98$ (Table 3). Gene expression was estimated with the relative quantification method, gadph and β-Act were used as references genes after assessing their stability as endogenous genes with qBase + software (Biogazelle, UK). geNorm analysis (software qBase +) was used to calibrate and normalize the relative quantities (CNRQs) of the gene of interest expression (hsp70).

### Long-term effects on reproduction (~ 300 days)

For the long-term exposure, a separate group of 35 gastropods (17–18 specimens per experimental conditions; ~ 10 snails per tank) were exposed to control and climate change conditions, respectively, to estimate the effects on reproduction (i.e. reproductive output: number of eggs per female, number of capsules and capsule size). The beginning of the experimental period started when females finished their reproductive cycle and started a new one. The sex proportion in the tanks was 40% males and 60% females. After one or two days of laying, female size, and number of capsules was recorded. Five capsules per egg mass were dissected under microscope (LEICA MZ16) to measure different reproductive traits: total eggs per egg mass, number of eggs per capsule, and capsule size.

### Statistical analysis

Two-way repeated-measures ANOVA were used to compare the impact of high temperature and pCO$_2$ on growth (shell length and wet weight) and routine metabolic rates at regular intervals (0, 15, 50, 95 and 135 days) followed by Holm-Sidak method post hoc analysis. Two-way

### Table 3  Summary of real-time PCR primers and validated optimal reactions conditions

| Gene assay | Sequence | Tm (°C) | AC (nM) | AS (nt) | RF | Regression relation | $R^2$ |
|------------|----------|---------|---------|---------|----|---------------------|------|
| hsp70      | F: GCTTCTGGATCTTAGGATAC | 59      | 50      | 101     | 1.7 | Cq = (− 4.315 × log dilution) + 21.04 | 0.99 |
|            | R: GCCAGTGGAGAAGTCTATG |         |         |         |     |                     |      |
| β-act      | F: CTTCGTCTCTGGATTG   | 59      | 50      | 92      | 1.8 | Cq = (− 3.6895 × log dilution) + 23.23 | 0.97 |
|            | R: GTACAGGTCTTACGGATGT |         |         |         |     |                     |      |
| gapdh      | F: GTGACGCTCAAACAGAAG | 58      | 50      | 95      | 1.9 | Cq = (− 3.4070 × log dilution) + 22.55 | 0.93 |
|            | R: TCTGTGTAGCCATGAATC |         |         |         |     |                     |      |

*F* forward, *R* reverse, *AC* assay concentration, *Nm* nanomolar, *AS* amplicon size, *nt* nucleotide, *RF* reaction efficiency, *Tm* melting temperatures, $R^2$ coefficient of determination.
ANOVA was used to analyse the ingestion rate and relative expression of hsp70 between control and stressed gastropods followed by Tukey post hoc analysis. Normality and homogeneity of variance were confirmed before respective analysis and statistical significance was identified at $p < 0.05$.

### Results and discussion

The physiological impacts of high temperature and $p$CO$_2$ on marine ectotherms are species-specific (Foo et al. 2012; Kroeker et al. 2013) and largely depend on the physiological plasticity that such organisms possess to acclimate to rapid environmental changes (e.g. climate change). The principal aim of this study was to determine the short and long-term effects on gastropods exposed to future ocean conditions (+3 °C above normal and 900 μatm CO$_2$). Our results showed that *O. erinaceus* adults were able to physiologically acclimate after 95 days of exposure. However, after prolonged exposure to modified conditions, snails suffered negative impacts on reproduction. After ~300 days, reproduction ceased (Table 5), which shows that *O. erinaceus* could be at risk of population decline and/or local extinction under future climate conditions.

In the short-term exposure (~135 days), the first fifty days of exposure to high T°/pCO$_2$ affected gastropod physiology, with a slight but none significant increase in routine metabolic rate and a significant decrease in growth rate (Fig. 1a, b, Table 4). Increases in metabolic rates have been reported for several marine ectotherms exposed to high pCO$_2$ with limited extracellular pH regulatory capacity, such as molluscs and echinoderms (Kroeker et al. 2013). For example, Uthicke et al. (2014) found, after ten weeks of exposure to high T°/pCO$_2$ (i.e. +3 °C and 963 μatm CO$_2$), an increase in metabolic demands and a decrease in growth rates in the Pacific sea urchin *Echinometra* sp. Similarly, adults of the temperate intertidal gastropod *Littorina littorea* exposed to elevated temperature and pCO$_2$ concentration for 30 days (+5 °C, 1000 μatm CO$_2$) showed lower shell growth rates, reduction in shell thickness and an increase in ATP production (Melatunan et al. 2013). Therefore, and in the present study, the slight increase in metabolic rate observed during the first fifty days could be the result of the maintenance of internal homeostasis. The observed increase in homeostasis is energetically demanding, and likely supported by tissue catabolism providing the necessary energy.

### Table 4

Summary of two-way repeated-measures ANOVA for growth (shell and wet weight), routine metabolic rate, ingestion rate and hsp70 expression in adult snails (*Ocenebra erinaceus*) exposed to control and high T°/pCO$_2$ within and between experimental intervals (0, 15, 50, 95, 135 days)

| Variable       | Source | df  | SS   | $F$   | $P$   |
|----------------|--------|-----|------|-------|-------|
| Shell length rate | Group  | 1   | 0.0000001 | 0.212 | 0.653 |
|                | Exp. day | 3   | 0.000003 | 1.116 | 0.354 |
|                | Interaction | 3   | 0.000003 | 0.130 | 0.942 |
|                | Residual | 39  | 0.0003  |       |       |
| Weight rate    | Group  | 1   | 0.00001  | 5.94  | 0.031 |
|                | Exp. day | 3   | 0.00001 | 1.074 | 0.371 |
|                | Interaction | 3   | 0.0001  | 3.554 | 0.02  |
|                | Residual | 39  | 0.002   |       |       |
| RMR            | Group  | 1   | 0.001   | 0.382 | 0.547 |
|                | Exp. day | 4   | 0.008   | 1.385 | 0.252 |
|                | Interaction | 4   | 0.001   | 1.229 | 0.310 |
|                | Residual | 52  | 0.002   |       |       |
| Ingestion rate | Group  | 1   | 111.257 | 2.611 | 0.112 |
|                | Exp. day | 3   | 785.074 | 6.141 | 0.001 |
|                | Interaction | 3   | 55.905  | 0.437 | 0.727 |
|                | Residual | 58  | 2471.4  |       |       |
| hsp70          | Group  | 2   | 3.667   | 0.111 | 0.741 |
|                | Exp. day | 1   | 0.024   | 8.400 | 0.001 |
|                | Interaction | 2   | 0.106   | 0.243 | 0.786 |
|                | Residual | 35  | 11.388  |       |       |

Factors: ‘Group’ (control or climate change conditions) and ‘Exp. Days’ (experimental days). Source of variation (source), degrees of freedom (df), variance ratios ($F$), Probability ($P$). $P < 0.05$ are in bold.

### Table 5

Reproductive output per female after ~300 days of exposure to control and high T°/pCO$_2$

| Treatment          | Female size (mm) | Fecundity | No. of capsules | Capsule size (mm) |
|--------------------|------------------|-----------|----------------|------------------|
| Control            | 31.4             | 1642      | 28             | 6.94             |
|                    | 32.3             | 1120      | 20             | 7.15             |
|                    | 38.9             | 1483      | 25             | 8.55             |
|                    | 37.5             | 1690      | 26             | 8.79             |
|                    | 37.5             | 1717      | 28             | 9.05             |
|                    | 35.7             | 1820      | 28             | 8.39             |
|                    | 37.2             | 1204      | 27             | 7.67             |
| High T°/pCO$_2$    | No laying process|           |                |                  |

Factors: ‘Treatment’ (control or climate change conditions) and ‘Female size (mm)’. Fecundity (No. of capsules), Capsule size (mm).
After 95 days of exposure to the short-term exposure experiment, *O. erinaceus* adults showed full acclimation to experimental conditions (Fig. 1a, b, \( p < 0.05 \)). Gastropods exposed to high \( T°/p\text{CO}_2 \) recovered, growing at similar rates and exhibiting similar metabolic rates compared to control gastropods (\( p > 0.05 \)). The ingestion rate increased in both experimental groups; however, a slight increase was observed in snails exposed to high \( T°/p\text{CO}_2 \) (Fig. 1c). This may suggest that an increase in food intake by snails aids acclimation. Indeed, a meta-analysis conducted on the role of food supply in marine calcifiers (i.e. corals, molluscs, and echinoderms) under acidified and warming conditions found that the negative effects of climate warming on growth and calcification rates decreased when there were sufficient food resources (Ramajo et al. 2016). For example, calcification rates in juveniles of the blue mussel *Mytilus edulis* were not affected by high \( p\text{CO}_2 \) (i.e. from 1000 to 3000 \( \mu\text{atm CO}_2 \)) when food supply was abundant (Thomsen et al. 2013). Conversely, food deprivation in the marine copepods *Calanus* sp. exposed to climate warming conditions (+2 °C and 1000 \( \mu\text{atm CO}_2 \)) exacerbated the impact of temperature and \( p\text{CO}_2 \) on their physiology (Mayor et al. 2015). Our study showed that *O. erinaceus* increased feeding rates to likely compensate for the increase in metabolic rate. This could indicate that, as long as food resources remain abundant, *O. erinaceus* adult can acclimate to future climate warming conditions.

Most coastal and estuarine ectothermal species are already experiencing natural variation in seawater chemistry with temperature, dissolved oxygen, and pH values, predicted for the end of this century (Duarte et al. 2013; Hofmann et al. 2011). *Ocenebra erinaceus* from the Solent population inhabit a flooded river-valley estuarine system experiencing natural short-term fluctuations in pH and temperature (e.g. from river discharges; Shi 2000). Full acclimation potential observed in stressed gastropods demonstrates that *O. erinaceus* adults have developed physiological responses to cope with fluctuating environments. At a molecular level, *hsp70* gene expression in gill tissue of snails was non-significant between control and when exposed to high \( T°/p\text{CO}_2 \) (Fig. 1d, \( p > 0.05 \)). *Hsp70* expression has been widely used to study the acute effects of temperature and acidification on organisms, because of its protective and restorative function against thermal damage or protein denaturation (Whiteley and Mackenzie 2016). For example, high temperatures increased *hsp70* expression after 5 days of exposure in gills of the blue mussel *Mytilus edulis* (Teden gren et al. 2000) as well as high \( p\text{CO}_2 \) (~1500 \( \mu\text{atm CO}_2 \)) increased the *hsp70* expression after 72 h in juveniles of the neogastropod *Concholepas concholepas* (Lardies et al. 2014). Few studies have attempted to study the combined effects of temperature and \( p\text{CO}_2 \) on *hsp70* expression. For example, Liu et al., (2012) conducted an acute exposure for 96 h to elevated temperature (+3 °C) and elevated \( p\text{CO}_2 \) (~1400 \( \mu\text{atm CO}_2 \)) in the pearl *Oyster Pinctada fucata*. They found that warming and acidified seawater conditions activated *hsp70* expression. After 24 h, the impacts of these two stressors were synergistic; \( p\text{CO}_2 \) exposure aggravated the sensitivity to temperature in *P. fucata*. After 96 h, the expression decreased as a result of the decrease in the energy budget, to meet the energy requirements for *hsp70* gene expression (Liu et al. 2012).

Two hypotheses can be suggested to explain why there were no effects on *hsp70* expression:

1. **Gill cells of gastropods exposed to high \( T°/p\text{CO}_2 \) could have activated the heat shock response (i.e. *hsp70*) during the first hours and days after acute stress; however, *hsp70* gene expression in this study was measured 15 days after initial exposure; thus, it was undetectable. Alternately,**

2. **Temperature and alkalinity stress (high \( T°/p\text{CO}_2 \)) on gill cells of *O. erinaceus* was insufficient to activate *hsp70* shock response. *Ocenebra erinaceus* adults were very tolerant to high \( T°/p\text{CO}_2 \) in all the physiological variables assessed in our study; therefore, to activate the heat shock response, one may suggest greater environmental stress be required.**

Previous reports have suggested that *hsp70* is a suitable stress biomarker because it is ubiquitous, highly conserved in almost all organisms, and sensitive to stress (Tomanek and Somero 1999). However, there are some limitations to consider, for example: the time after initial stress exposure, duration of the stress, ontogenetic stage, number of environmental stressors that are producing stress, and what form of *HSP70* is expressed, amongst others (Morris et al. 2013). Therefore, the expression of *hsp70* can be induced by a wide range of factors, which as a single stress marker, questions its suitability for estimating complex environmental stress in organisms (Morris et al. 2013).

After long-term exposure (i.e. 300 days) to future ocean conditions, females did not lay any eggs (Table 5). Under control conditions, six females laid 1525 ± 269 eggs each, which matches previous estimations (Mardones et al. 2014). An explanation could be that energetic demands at high \( T°/p\text{CO}_2 \) are as high in exposed females to only invest energy in maintenance rather than reproduction. By comparison, histological examination of female gonads of the Pacific sea urchin *Echinometra* sp. exposed to comparable experimental conditions, identified that females were unable to generate new cohorts of gametes, as eggs were degenerated and/or reabsorbed (Uthicke et al. 2014). It should be considered, however, that sea urchin females were exposed for 10 weeks only. The observations may rather be the result of experimental stress, and may help explaining patterns.
seen after 10 months in *O. erinaceus* females. *Ocenebra erinaceus* only exhibits one reproductive peak per year (Martel et al. 2004); thus, cessation of reproduction could directly impact the maintenance of the Solent population in the future. However, to determine the impact of future ocean conditions at species level, comparative studies among geographic populations are necessary.

Our study showed that adults of *O. erinaceus* are able to fully compensate for the effects of high temperature and pCO₂ (RCP8.5 climate change scenarios). After 95 days of exposure, any physiological variables measured in this study were no longer affected, which means that stressed snails can physiologically acclimate. However, full acclimation of *O. erinaceus* came at high energetic costs, resulting in high food ingestion rates. After ten months of exposure, reproduction ceased under climate change conditions demonstrating that adults invested energy toward survival and not in reproduction. Under future climate conditions of warming, *O. erinaceus* populations could decline, or even become locally extinct. Therefore, our study implies a need for science to assess the impact of climate warming on appropriate time scales, taking into account the combined results of both short- and long-term experiments as well as using multiple metrics of animal performance. This, in turn, helps to develop accurate projections to manage the ecological consequences of climate warming.

**Acknowledgements** The authors would like to thank Ms. Nicola Pratt for her insights on molecular analysis and Mr. R. Robinson for helping with the setup of experiments at the NOCS. This work was funded by the National Agency for Research and Development (ANID)/DOCTORADO BECAS CHILE/ 2015–72160099 and the University of Southampton. Most of the work presented in this short note is part of the PhD dissertation carried out by Mardones (2020) during her PhD.

**Data availability** The data generated and analysed during this study are available from the corresponding author upon request.

**Declarations**

**Conflict of interest** All authors declare that they have no conflict of interest on connection with this study.

**Ethical approval** Permission to conduct the fieldwork and collect samples was granted by the National Oceanographic Centre (NOCS), Southampton, UK. No special animal ethics approval was needed.

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