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Greatorex, R

http://hdl.handle.net/10026.1/20314

10.1016/j.marenvres.2023.105903
Marine Environmental Research
Elsevier

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Rebecca Greatorex, Antony M. Knights

PII: S0141-1136(23)00031-4
DOI: https://doi.org/10.1016/j.marenvres.2023.105903
Reference: MERE 105903

To appear in: Marine Environmental Research

Received Date: 21 July 2022
Revised Date: 19 January 2023
Accepted Date: 29 January 2023

Please cite this article as: Greatorex, R., Knights, A.M., Differential effects of ocean acidification and warming on biological functioning of a predator and prey species may alter future trophic interactions, Marine Environmental Research (2023), doi: https://doi.org/10.1016/j.marenvres.2023.105903.

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Title: Differential effects of ocean acidification and warming on biological functioning of a predator and prey species may alter future trophic interactions

Authors: Rebecca Greatorex, Antony M. Knights

Affiliations: School of Biological and Marine Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA. United Kingdom.

* Corresponding Author Email: aknights@plymouth.ac.uk

ABSTRACT

Independently, ocean warming (OW) and acidification (OA) from increased anthropogenic atmospheric carbon dioxide are argued to be two of the greatest threats to marine organisms. Increasingly, their interaction (ocean acidification and warming, OAW) is shown to have wide-ranging consequences to biological functioning, population and community structure, species interactions and ecosystem service provision. Here, using a multi-trophic experiment, we tested the effects of future OAW scenarios on two widespread intertidal species, the blue mussel Mytilus edulis and its predator Nucella lapillus. Results indicate negative consequences of OAW on the growth, feeding and metabolic rate of M. edulis and heightened predation risk. In contrast, Nucella growth and metabolism was unaffected and feeding increased under OAW but declined under OW suggesting OA may offset warming consequences. Should this differential response between the two species to OAW, and specifically greater physiological costs to the prey than its predator come to fruition in the nature, fundamental change in ecosystem structure and functioning could be expected as trophic interactions become disrupted.

Keywords – Climate Change; Predation; Stressor Interaction; Biotic Interaction; Physiology
1. INTRODUCTION

Ocean warming (OW) and acidification (OA) are arguably two of the greatest threats facing marine organisms as a result of increasing atmospheric carbon dioxide ($CO_2$) from anthropogenic sources (Shukla et al., 2019). Increasingly, their interaction (i.e. ocean acidification and warming (OAW)) has been shown to have wide-ranging consequences to the biological functioning of organisms including changes to physiology (Lemasson et al., 2018; Li et al., 2015), morphology (Knights et al., 2020), and behaviour (Manríquez et al., 2021) resulting in changes to population and community structure (Lemasson et al., 2018; Manríquez et al., 2021), inter- and intra-species interactions (Sadler et al., 2018), and the provision of ecosystem services (Listiawati and Kurihara, 2021).

Exposure to multiple stressors like OAW has been shown to be more biologically costly than a single stressor (e.g., temperature or pH; Gunderson et al., 2016); a scenario arguably more reflective of expected change in marine environments (Wernberg et al., 2012) than independent fluctuations in these metrics. While organisms can adapt to changes in the abiotic and biotic conditions where they occur (Alley, 1982; Jupe et al. 2020), this can come at a biological cost such as change in metabolic performance and fitness (e.g., Braby and Somero, 2006; Lemasson et al., 2018; Breitberg et al., 2015; Clements and Comeau, 2019). These costs may be detectable as an upregulation of metabolism (Lemasson et al., 2018; Matoo et al., 2013) or through increased $O_2$ consumption rates as individuals attempt to maintain homeostasis through physiological (e.g. cardio-circulation and the ‘oxygen and capacity dependent thermal tolerance’ concept, Pörtner 2012) or behavioural compensation (Giomi et al., 2016; Lemasson et al., 2018).

Increasing feeding may be one behavioural mechanism available to an organism to negate the negative effects of OAW (Clements and Darrow, 2018). But what remains unclear is the extent to which organisms can ‘upregulate’ feeding in response to associated increases in the metabolism, and whether this regulation can be maintained (Harvey and Moore, 2017; Lord et al., 2017). Indeed, in some cases, increasing energy intake may not be a viable option, such as when animals reduce feeding as an anti-predator response (Naddafi and Rudstam, 2013) which itself can indirectly result in modified biomineralization processes (Bibby et al., 2007), or changes in body size and reproductive output (Harvey and Moore, 2017; Lemasson and Knights, 2021). Predation is well known to be an essential driver of ecosystem dynamics (e.g., Sherker et al. 2017; Sadler et al. 2018) influencing prey population dynamics via both consumptive effects (CEs) and non-consumptive effects (NCEs)(Orrock et al., 2008). Under OAW, changes in the magnitude of NCEs (Bibby et al., 2007; Clements and Comeau, 2019) and CEs (Sadler et al., 2018) during predator-prey interactions are predicted (see reviews: Briffa et al., 2012; Clements and Hunt, 2015), affecting physiological, morphological and behavioural mechanisms,
as well as feeding strategies and induced defences (Lemasson and Knights, 2021; Manríquez et al., 2021; Sadler et al., 2018). However, the biological cost of reduced physiological performance in metrics like O₂ consumption and acid-base regulation may ultimately affect the extent to which organisms can respond to an external input and individuals may autonomously prioritise maintenance of internal homeostasis over a behavioural response (Bibby et al., 2007; Briffa et al., 2012; Harvey and Moore, 2017; Lord et al., 2017).

Susceptibility to OAW may be dependent on species and taxa (Briffa et al., 2012; Clements and Comeau, 2019) although calcifying species are shown to be particularly sensitive (Lemasson & Knights, 2021; Knights et al., 2020; Li et al., 2015; Sadler et al., 2018). *Mytilus edulis* (blue mussel) is a marine intertidal and subtidal bivalve most commonly distributed in the Atlantic Ocean in temperate regions (Knights, 2012), forming highly complex reef structures which support a multitude of other organisms. In the UK, *Mytilus* spp. is an integral part of UK aquaculture and the national fisheries economy. The species is resilient to environmental perturbations but stressors like OAW may reduce their nutritional quality and fitness (Lemasson et al., 2019; Li et al., 2015). A major predator of *M. edulis* is the gastropod whelk, *Nucella lapillus*, (Hunt and Scheibling, 1998). Unlike bivalves, gastropods have been shown to be physiologically more resilient to OAW (Clements and Comeau, 2019) and mis-match in response to environmental change could lead to predator-prey relationships becoming unbalanced (Harvey and Moore, 2017; Sadler et al., 2018).

Given ocean acidification and temperature conditions are predicted to drastically change by end-of-century (Shukla et al., 2019) with potential consequences for the physiology and morphology of species and trophic interactions, here we evaluate the effects of elevated temperature and acidification scenarios on the performance and trophic interaction of *Mytilus edulis* and *Nucella lapillus*. Specifically, we test the effect of future climate scenarios on (1) individual physiological responses of *M. edulis* and *N. lapillus* including standard metabolic rate, feeding rate, changes in shell and somatic growth, and condition index of mussels (CI); and (2) the strength of trophic interactions between *M. edulis* and *N. lapillus* to assess potential changes in ecosystem functioning under future climate scenarios.

### 2. MATERIALS AND METHODOLOGY

#### 2.1. Animal collection and husbandry

Adult blue mussels (*M. edulis*) and adult dog whelks (*Nucella lapillus*) of a similar size were collected from a mid-shore intertidal site in Sidmouth, UK (50°40'41.1" N, 3°14'05.1" W) in April 2021. All animals were cleaned of epibiota and individually marked using a water-based non-toxic nail varnish
Animals were acclimated for 2-weeks under standard laboratory conditions (12:12 h Light:Dark cycle, 15 °C, Salinity = 34 - 36, pH 8 (with natural variation due to fluctuations in atmospheric pressure; see Lemasson et al. 2018 and Knights et al. 2020 for a full description)). Throughout acclimation and treatment, not including experimental starvation periods, mussels and whelks were fed twice weekly. Full water changes were conducted post feeding to maintain water quality (NH$_3$ < 0.5 mg L$^{-1}$). Each N. lapillus was fed one opened mussel (M. edulis) (< 10 mm) and given 12 h to feed. Each M. edulis was given 1 h to feed on cultured Isochrysis galbana at a cell density of 24000 to 30000 cells mL$^{-1}$.

2.2. Experimental design


Temperature and pCO$_2$ treatments were chosen to simulate future predicted IPCC OAW scenarios (Shukla et al., 2019). There were 3 tanks per treatment (M. edulis; n = 3). N. lapillus (25 ± 3 mm in length) were exposed to the same conditions as the mussels but without a predator cue; ambient control, OA, OW, and OAW again with 3 tanks per treatment (N. lapillus, n = 3). N. lapillus were identifiable by number and kept fully submerged in perforated containers to control for treatment exposure. The size of M. edulis and N. lapillus did not differ between tanks (M. edulis: p = 0.981, F$_{23,48}$ = 0.445; N. lapillus: p = 0.872, F$_{11,22}$ = 0.517) or treatments (M. edulis: p = 0.95, F$_{3,72}$ = 0.117; N. lapillus: p = 0.225, F$_{3,30}$ = 1.54).

2.3. OA and Temperature Design

For the controls and OW treatments, air stones gently bubbling ambient air under atmospheric pressure were present in each tank. For OA, pure CO$_2$ was slowly released into a Buchner flask mixed with dry air (≈ 500 ppm pCO$_2$) using multistage CO$_2$ regulators (EN ISO 7291; GCE, Worksop, UK). pCO$_2$ levels were monitored using a CO$_2$ analyser (LI-820; LI-COR, Lincoln, NE, USA). pH was measured twice a week using a microelectrode (InLab® Expert Pro-ISM; Mettler- Toledo Ltd, Beaumont Leys, UK).
attached to a pH meter (S400 Seven Excellence; Mettler-Toledo Ltd, Beaumont Leys, UK), calibrated with Mettler Toledo buffers.

The experiment took place in a 15°C controlled temperature laboratory. Tanks under elevated temperature were kept in a water bath, with the temperature kept constant using aquarium heaters (thermocontrol®200, EHEIM Jager GmbH and Co. KG, Stuttgart, Germany).

For predator cue treatments, two *N. lapillus* were placed in an individual perforated polypropylene plastic container to prevent predation of mussels and submerged in each tank for the duration of the experiment. Whelk density per tank is representative of *Nucella lapillus* densities on U.K. intertidal shores (Knights, unpublished data) and similar to densities found elsewhere (e.g. Hunt and Scheibling, 1998).

### 2.4. Carbonate chemistry

Total alkalinity (TA) was measured weekly using a calibrated potentiometric titrator (TitraLab AT1000© series HACH Company, USA). Weekly, a 50 mL sample was taken from each tank and tested to calculate TA. Temperature and salinity were taken *in situ* using a temperature probe (HH806AU, Omega, U.K.) and a handheld refractometer (S/Mill, Atago, Tokyo, Japan) respectively. TA, salinity, and temperature data were recorded to calculate calcite and aragonite saturation, and $p$CO$_2$ concentration in each treatment tank using CO2SYS software (Lewis and Wallace, 1998) using Mehrbach solubility constants (Mehrbach et al., 1973), refitted by Dickson and Millero (1987).

Seawater chemistry data are shown in Appendices Tables 1 and 2.

### 2.5. Morphological and physiological metrics

#### 2.5.1. Body measurements and dry mass equation

Body metrics and mass were recorded at three time points: (1) prior to experimental treatment exposure; (2) week 4; and (3) week 8. For *M. edulis*, length, width, and height were recorded. Wet weight was recorded using an analytical balance (Mettler Toledo, ML, Germany) after placing animals on paper towel for 15 min. Dry mass of *M. edulis* was estimated for each time point using the equation (eq. 1) from Knights (2012) as follows:

\[
y = 0.0508e^{0.9441x}
\]

where $x$ is shell length and $y$ is total dry mass.

After 8-weeks, *M. edulis* were dissected and biometrics (length, width, height, total wet weight, wet tissue weight, shell weight, and dry tissue weight) were measured (see condition index).
For *N. lapillus*, wet weight was recorded by leaving animals out of water for 5 min and drying, then recording total weight to the nearest 1/100th g using an analytical balance (Mettler Toledo, ML, Germany). Length from apex to siphonal canal was recorded using callipers.

2.5.2. *M. edulis* condition index

Body condition index (CI) of *M. edulis* was calculated using the following equation (eq. 2) after Davenport and Chen (1987; BCI, eq. (1)):

\[
\text{eq. (2)} \quad \frac{\text{Dry tissue weight}}{\text{shell length}} \times 100
\]

The shell length of *M. edulis* was measured to the nearest 0.05 mm using callipers. Animals were dissected to remove all tissue from shell, which was placed into a pre-weighted plastic weighing boat to dry in an oven at 60°C. Tissue was weighted at 48 h and 72 h to ensure a constant mass (dry tissue) had been achieved and CI calculated from eq. 2.

2.5.3. O₂ consumption rate

Respiration rate was used as a proxy for Standard Metabolic Rate (SMR). Respiration rate was recorded using microfibre optic oxygen sensors (Fibox 4, PreSens Germany). Temperature and salinity were recorded prior to each set of data collection and barometric pressure was obtained from the Plymouth Live weather Station ([http://www.bearsbythesea.co.uk](http://www.bearsbythesea.co.uk)). Each was input into the PreSens to allow O₂ measurements to be corrected for fluctuations in temperature, salinity, and pressure.

All *M. edulis* (n = 72; 9 per treatment) and *N. lapillus* (n = 36, 9 per treatment) were placed in 250 mL and 120 mL sealed jars, respectively. For the first respiration data point, sea water (salinity = 34 - 36) was filtered to 2 μm and then autoclaved and aerated at 15°C. For time point 4 and 8, water was pre-equilibrated to the appropriate treatment conditions. To maintain stable temperature, during data collection, jars were kept in a water bath at 15°C or 20°C. All animals were starved for ~8 days prior to data collection to eliminate any change in respiration due to digestion and alter respiration rates (Sejr et al. 2004, Ansell & Sividas 1973). Within the jar, water was mixed using a magnetic stir bar for the duration of the experiment (400 rpm). Data collection started when jars were closed. For *M. edulis*, data points were only counted if the animal was visibly open. All data points before 15 min were discounted for both animals to allow for acclimation. O₂ (mg L⁻¹) was recorded every 5 min for 40 min or until O₂ saturation reached 75 % to avoid exposure to hypoxic conditions. O₂ measurements were corrected for background bacterial respiration or primary productivity by offsetting respiration rate with O₂ changes in jars without an animal in them. Respiration rate was also normalised to 1 g of calculated dry weight (Knights, 2012). SMR was calculated using the following equation (eq. 3).
where $v$ is volume of jar (L), $r$ is change in O$_2$ in jar (mg L$^{-1}$), $t$ is time (min), and $DM$ is dry mass (g) calculated using the relationship defined in Knights (2012).

2.5.4. *M. edulis* clearance rate

The same individuals used for respirometry were also used for clearance rate (CR). *M. edulis* were starved for 24 to 72 h. The CR assay followed methodology in Lemasson et al. (2018). Individuals were placed in 300 mL of UV treated and filter sea water (15 °C, 500 ppm $p$CO$_2$, salinity = 34 – 36) and subsequent data points were recorded in water pre-equilibrated to treatment conditions. A dilution of 1:100 mL shellfish diet (Shellfish diet 1800, Reed Mariculture, USA) was used as feed. *M. edulis* were given up to 20 min to open and algae added once opened. Any animals which closed during the assay were discounted and re-done the following day. Once open, 700 μL of stock solution was used per beaker at a concentration of 24,000 to 30,000 cell mL$^{-1}$. In each beaker, a magnetic stirrer bar (400 rpm) was used to keep the water well-mixed. A 20 mL sample ($t_0$) was taken 2 min after stock solution was added to allow for adequate mixing of algae. Another 20 mL sample ($t_1$) was taken after 20 min of filtering. Counts of the algae in the water were done in triplicate by a Coulter Counter (Beckman Coulter, Z2). CR was calculated using the following equation (eq. 4).

\[
CR = \left( \frac{v \times 60}{t} \right) ( \ln t_0 - \ln t_1 )
\]

where CR is clearance rate (L h$^{-1}$), $v$ is volume (L), $t_0$ is the initial sample (cell L$^{-1}$) and $t_1$ is the sample (cell L$^{-1}$) taken after 20 min. CR was then normalised to 1 g by dividing by calculated dry mass of individual (Knights, 2012).

2.6. Feeding behaviour

Feeding behaviour of *N. lapillus* was assessed under treatment conditions to look at both predator risk of *M. edulis* and feeding rate of *N. lapillus*. After 8-weeks of exposure to the experimental treatments, *N. lapillus* were starved for 7 to 9 d. *M. edulis* used in the experiment were pre-acclimated for 8 weeks in each of the experimental treatments. Five pre-acclimated *M. edulis* (20 to 45 mm length) were placed in each tank, with 11-12 tanks in each of the four treatments. There were control tanks ($n = 3$) included in each treatment which contained only *M. edulis*. *N. lapillus* were placed into tanks and mortality was measured every 24 h over 8 d. Mussels were considered dead when they gaped open and did not respond when physically disturbed (Lupo et al., 2021). In tanks without predators there was one mortality in the elevated temperature and $p$CO$_2$ treatment (6.7 % mortality).
2.7. Statistics

Data were tested for assumptions of normality, bias and homoscedasticity of residuals. Data were log-transformed or square-root transformed if data did not meet assumptions. All data were analysed using R (version 4.1.1, R Core Team, 2021) and all graphs were produced using the ‘ggplot2’ package (Wickham, 2016). Where significance was identified Tukey HSD post-hoc pairwise comparison was used to find differences between groups. ‘Tank’ was included as a random factor in all analyses.

2.7.1. Shell, body metrics and CI

Mussel shell length, width, height, wet weight, CI, and whelk shell length, and wet weight were all analysed using the same linear mixed effects model (Package ‘nlme’; Pinheiro et al., 2021). For everything except CI, change in metric from week 0 to week 8 was used in the analysis. ‘Temperature’ (two levels: 15°C; ambient, 20°C; elevated) and ‘pCO₂’ (two levels: 500 ppm; ambient, 1000 ppm; elevated) were considered fixed factors for all analyses. Mussel metrics had an additional factor, ‘cues’ (two levels: present, absent).

2.7.2. SMR and CR

SMR and CR were analysed with a linear mixed effects model with temporal autocorrelation. ‘Temperature’ (two levels: 15°C; ambient, 20°C; elevated) and ‘pCO₂’ (two levels: 500 ppm; ambient, 1000 ppm; elevated) were considered fixed factors for all SMR and CR analyses. Mussel SMR and CR had an additional factor, ‘cues’ (two levels: present, absent). ‘Time (in treatment)’ (3 time points: week 0, 4, and 8) was also included in the analysis.

2.7.3. Whelk feeding rate

A two-factor linear mixed effects model was used to analyse differences in proportional mortality (or whelk predation rate) of mussels at day 8 of the experiment. ‘Temperature’ (two levels: 15°C; ambient, 20°C; elevated) and ‘pCO₂’ (two levels: 500 ppm; ambient, 1000 ppm; elevated) were considered fixed factors.

3. RESULTS

3.1. Mortality and growth

3.1.1. M. edulis

Mortality was 23 % under OAW, 6 % under OW and control (ambient) conditions, and 0 % under OA. M. edulis mortality was significantly higher in the OAW treatment over all other treatments (p < 0.01, $F_{4,67} = 4.382$).
There was a significant interaction between OW, OA and predator presence on shell length (p < 0.05, F1,61 = 5.635) and a significant reduction in growth when predators were present (Fig 1). Shell length increased by 159 %, from an average increase of 0.24 mm, in the presence of predators, to 0.63 mm, when predator cues were absent (Tukey HSD; p < 0.001). There was no effect of OW alone (Tukey HSD; p = 0.438) or predator presence alone (Tukey HSD; p = 0.300) on growth in length.

There was no effect of OW (p = 0.176, F1,65 = 1.870), OA (p = 0.998, F1,65 = 0.000), or cues (p = 0.107, F1,65 = 2.664) on width change over the 8-week exposure (p = 0.051, F7,61 = 2.149).

There was a significant interaction between OW and OA on height (p < 0.01, F1,53 = 7.420). There was a 113 % increase in shell height compared to the control under OA increasing by 0.40 mm under OA, versus just 0.19 mm under ambient conditions (Tukey HSD; p < 0.01). There was no effect of OAW on height (Tukey HSD; p = 0.415). There was also an interaction between cue presence and OA (p < 0.05, F1,53 = 5.420) on mussel height with a 175 % increase in height under OA compared to the control (Tukey HSD; p < 0.01). There was no effect of OA on height when predators were present (Tukey HSD; p = 0.574).

Median wet weight decreased by 55% under OA from 0.59 g to 0.26 g after 8-week regardless of OW and cue presence (Fig. 4; p < 0.05, F1,61 = 4.988).

3.1.2. *Nucella lapillus*

There was 12 % mortality in the OW treatments and 0 % in all other treatments. However, there was no significant effect of OW (p = 0.167, F1,32 = 2.000) or OA (p = 1.000, F1,32 = 0.000) on survival in *N. lapillus*.

There was no effect of OW (p = 0.666, F1,30 = 0.190) or OA (p = 0.678, F1,30 = 0.176) on length change in *N. lapillus* after 8 weeks. There was no effect of OW (p = 0.849, F1,30 = 0.037) or OA (p = 0.738, F1,30 = 0.114) exposure on wet weight change in *N. lapillus* after 8 weeks exposure to treatments. There were no interactions.

3.2. Condition index

Cl of *M. edulis* increased by 8.1 % from 3.07 to 3.32 when predator cues were present (p < 0.01, F1,63 = 7.225). OW (p = 0.819, F1,63 = 0.053) and OA (p = 0.875, F1,63 = 0.025) had no effect on Cl.

3.3. Metabolic rate

3.3.1. *M. edulis*

There was a significant interaction between OW and OA on SMR in *M. edulis* (p < 0.05, F1,194 = 4.44)(Fig. 2). O2 consumption rates increased in an additive fashion by 18.8 % under OA. OW increased the SMR
of *M. edulis* by 33%. However, there was no effect of OA on SMR under OW. There was also an interaction between time in treatment and cue presence (*p* < 0.05, *F*$_{(2,194)}$ = 3.399). In the absence of cues, SMR decreased by 16% from week 0 to 4 and remained the same from week 4 to 8. SMR in response to predator presence was maintained until week 4, and from week 4 to week 8, SMR reduced by 31%.

### 3.3.2. *N. lapillus*

There was an effect of OW (figure 7; *p* < 0.001, *F*$_{1,88}$ = 18.631) but not OA (*p* = 0.808, *F*$_{1,88}$ = 0.059) on whelk O$_2$ consumption rates, with OW increasing whelk O$_2$ consumption rates by 42% (Fig. 2).

#### 3.4. Clearance rate

There was a four-way interaction between OW, OA, predator presence, and time in treatment (*p* < 0.01, *F*$_{2,194}$ = 6.184, Fig. 3). At 15°C, when predators were absent, CR stayed the same through time in the control (Tukey HSD; *p* = 0.929) and OA treatments (Tukey HSD; *p* = 0.939). When predators were present, CR reduced by 35% over the 8-week exposure for both the control (Tukey HSD; *p* < 0.05) and OA treatment (Tukey HSD; *p* < 0.01). When cues were absent, OW had no effect on CR (Tukey HSD; *p* = 0.450). OAW significantly reduced CR by week 8 (Tukey HSD; *p* < 0.001). OW with predator presence did not affect CR until after week 4 of exposure. There was no difference in CR of mussels by week 4 (Tukey HSD; *p* = 0.480), by week 8, CR in the presence of predators was 31% less than in the absence of predators (Tukey HSD; *p* < 0.01). Under OAW, when predators were absent, CR increased 21% by week 4 (Tukey HSD; *p* = 0.100) then decreased 44% from week 4 to week 8 (Tukey HSD; *p* < 0.001), with an 33% overall decrease in CR (Tukey HSD; *p* < 0.01). When predators were present, CR decreased 31% by week 4 (Tukey HSD; *p* < 0.05), then increased 48% from week 4 to week 8 (Tukey HSD; *p* < 0.05), with no overall change in CR (Tukey HSD; *p* = 0.942).

#### 3.5. Whelk feeding rate

There was a clear trend, if not significant interaction, between OW and OA on mussel mortality as a result of whelk predation (Fig. 4; *p* = 0.056, *F*$_{1,30}$ = 3.961). At 15°C, percentage mortality of mussels decreased 9% from 33% to 24% (Tukey HSD; *p* = 0.170) under OA. At 20°C (OW), mussel mortality increased by 10% from 48% to 58% under OAW (Tukey HSD; *p* = 0.146) (Fig. 4).

### 4. DISCUSSION

OAW impacts are being documented ubiquitously across marine taxa and marine ecosystems with wide ranging variable effects and complex interactions between pH and temperature stressors (e.g., Clements and Hunt, 2015; Knights et al., 2020; Kroeker et al., 2013). In this study, the impacts of future
predicted OAW on growth and physiology have been highlighted in two major marine invertebrate taxa, mussels and dog whelks. Further investigation elucidated the impacts to the predator response of *M. edulis* and effects of OAW on the predator-prey relationship between these species. Results indicate significant effects of OA, OW, OAW, and predator presence on growth, CR and SMR in *M. edulis*. Less pronounced effects on growth and SMR were seen in *N. lapillus*, alongside an increase in predation rate under OAW, indicating increased predation risk to *M. edulis*.

### 4.1. Growth and condition

The effect of OAW on shell and somatic growth in marine invertebrates appears highly species dependent (Gazeau et al., 2013; Kroeker et al., 2013; Lemasson et al., 2018; Lemasson and Knights, 2021). For *M. edulis*, OA was found to increase shell growth (length and height) alongside an increase in SMR. In *N. lapillus*, there was no effect of OAW or individual effects of OA or OW on growth in shell length or growth in wet weight (but see Mayk et al. 2022 where shell growth was shown to increase under OA). Increased shell growth in *M. edulis* may be explained in terms of carbonate chemistry. For example, *M. edulis* biomineralize using two different forms of calcium carbonate, a mixture of calcite (~17%) and aragonite (~83%) (Hubbard et al., 1981). Aragonite has a greater dissolution rate to calcite under OA conditions (Feely et al., 2004). Therefore, dissolution of the shell under lower pH may lead to mineralogical plasticity in biomineralization, as seen in this study, despite some evidence for a net decrease in calcification rate under OA (Leung et al., 2017; Li et al., 2015). There are variable effects of OAW on shell growth in the literature with the majority of the literature reporting negative impacts on growth (e.g., Fitzer et al., 2015; Lemasson and Knights, 2021). Despite this, we observed an increase in length of *M. edulis* under OA. However, the literature shows that animals calcifying under OA may prioritise investment in lower quality shell structure (i.e. greater size, weaker shell; Leung et al. 2022), which consequently may increase predation risk (Gazeau et al., 2013; Li et al., 2015; Sadler et al., 2018).

Environmental stressors can interact to influence the overall effect of a stressor on an organismal trait (Kroeker et al., 2017). The increase in growth under OA was counteracted under elevated temperature or in the presence of predator cues indicating an antagonistic relationship between these variables and biomineralization traits. In the presence of predators, mussels can induce calcification to increase shell thickness as an anti-predator response. This upregulation of calcification is a common non-consumptive effect (NCE) of predators within a prey population (Freeman, 2007). However, under environmental stress the cost of upregulating calcification increases, particularly under OA as shell dissolution increases (Nienhuis et al., 2010). Mussels may be calcifying at the same rate but reallocating the energy used to prioritise shell thickness over shell size as an anti-predator defence.
strategy. Shell thickness has been shown to decrease under OA conditions over a relatively short time scale (8 weeks) in *M. edulis* (Fitzer et al., 2015; Sadler et al., 2018). Alongside this, net calcification rate has been reported to decrease in mussels under elevated pCO₂ (Li et al., 2015). Within this study, shell thickness and net calcification rate were not recorded, however, based on the literature, we predict there was likely a trade-off between structural integrity and size of shell mussels exposed to OA (Fitzer et al., 2015; Knights et al., 2020; Sadler et al., 2018).

4.2. Condition index

CI is used to comparatively assess the reproductive condition of mussels between treatments (Knights, 2012). In this study, perhaps surprisingly, CI increased under predator presence. Given the CI calculation uses shell length and dry tissue weight, this suggests the animals are investing more in somatic tissue than length as length change did not differ between OAW treatments when cues were present. OAW had no effect on CI in mussels despite evidence suggesting otherwise in the literature. For example, temperature increase (Sunila, 1981) and enhanced food availability (Hatcher et al., 1997) both led to an increased metabolism which resulted in a greater CI of *Mytilus* sp. Low pH was also found to increase condition index in *M. californianus* (Rose et al., 2020). On the other hand, Lemasson and Knights (2021) found effects of OAW on CI to be species-specific and found no effect of OAW on CI in European flat oysters (*Ostrea edulis*). The results suggest that *M. edulis* may be prioritising reproduction and fecundity over long term survival. A similar finding was shown in *Daphnia magna*, which displayed greater investment in fecundity under size selective predation pressure (Zhang et al., 2016) and also in *M. edulis*, where gonad development was accelerated when exposed to starfish cues (Reimer, 1999).

4.3. Metabolism

Maintaining metabolic rate in response to energetic demand is essential for survival and basic functions like growth and feeding (Gazeau et al., 2013). Metabolism is closely linked to temperature, particularly in marine ectotherms (Seibel and Walsh, 2003). O₂ consumption rates (SMR), increased under OAW by 33 % for *M. edulis*, in concordance with previously reported increases in SMR in bivalves under OAW (Lemasson et al. 2018). The upregulating effect of low pH on SMR in mussels at 15 °C was, however, masked by elevated temperature. pH had no additional effect on SMR alongside elevated temperature. Similar results have been found in *M. edulis*, where temperature is the dominant factor in influencing SMR and addition of low pH stress does not affect the SMR response (Lemasson et al., 2018; Matoo et al., 2021). However, the increased SMR of *M. edulis* in response to OA at 15 °C is not well documented. OA exposure puts physiological stress on the internal homeostasis on an organism; energetic demand for acid-base regulation increases as pH of internal fluids lowers (Gazeau et al.,...
The metabolic upregulation seen in *M. edulis* was not evident in *N. lapillus*, indicating a greater resilience of *N. lapillus* to OA and supports the suggestion that some species of gastropod are more resilient to OA than bivalves (Clements and Comeau, 2019).

Change in physiology in response to an external stimulus (i.e. predator presence or OAW) can result in metabolic depression in animals over time (Gazeau et al., 2013; Seibel and Drazen, 2007). In this study, time in treatment and predator presence interacted to induce metabolic depression in *M. edulis* after just 4 weeks of exposure and may be explained by anti-predator response strategies. Animals respond in different ways to predators depending on their mobility. Mobile animals may upregulate the metabolism to escape a predator, immobile animals, such as *M. edulis*, may downregulate the metabolism to reduce predator contact through processes such as feeding (Gazeau et al., 2013; Seibel and Drazen, 2007). Alongside these findings, metabolic depression resulting from predator exposure under OA has been observed in mussels (*Brachidontes pharaonis*) exposed to crab predator cues (*Eriphia verrucosa*) (Dupont et al., 2015). The metabolic depression of *M. edulis* seen in this study may have resulted from a reduced feeding rate when predator cues were added (i.e. reduced energy acquisition), coupled with increased physiological stress of exposure conditions (i.e. offsetting shell dissolution and maintaining acid-base homeostasis) (Gazeau et al., 2013; Seibel and Drazen, 2007). *M. edulis* may have the capacity over short time scales (< 4 weeks) to maintain physiological performance under climate change stressors thereby compensating using trade-offs. However, over a longer time scale (> 4 weeks), in the presence of predators, *M. edulis* have a reduced metabolic performance which may be unsustainable and fitness-reducing as less energy is available for other physiological processes (Gazeau et al., 2013).

### 4.4. Clearance rate

Clearance rate (CR) is a semi-quantitative measurement and can be used as a measure of physiological or behavioural performance (Lemasson et al., 2018) and is closely linked to metabolic processes so that it can be used to balance energy acquisition and expenditure (Giomi et al., 2016). Increase in metabolic rate, from OAW, can be an issue if energy acquisition does not also increase. Here, a complex interaction was found between temperature, $pCO_2$, predator presence, and time in treatment. Despite increases seen in SMR as a result of OA and OW exposure, food intake (CR) did not increase under the same scenarios. Food availability or intake is a known limiting factor of animal resilience to OAW stressors (Clements and Darrow, 2018). Therefore, energetic requirements may not have been met, resulting in the decreasing trends seen over time in SMR.

From a behavioural perspective, feeding is a behaviour that increases the predation risk of an animal and can be downregulated by the animal accordingly (Křivan and Eisner, 2003). Here, cue presence
led to much greater change in CR than OAW scenario, resulting in reduced feeding of the animals when cues were present in all treatments but elevated temperature. Under elevated temperature, with predators present, feeding rate of *M. edulis* did not decrease as expected, suggesting a potential trade-off or ‘decision’ to prioritise physiological demand over predator behavioural response (Briffa et al., 2012). While this may reduce fitness in relation to predation risk, it has potential to work as a compensatory mechanism for OW as more energy is acquired to offset negative impacts of OW (Giomi et al., 2016). Animals may upregulate feeding to maintain physiological processes despite greater predation risk. This removes or alleviates food intake as a limiting factor for animal wellbeing under OAW scenarios (Clements and Darrow, 2018).

In *M. edulis*, reduced feeding rate as an anti-predator response in conjunction with the increased energy requirement observed in individuals exposed to OAW illustrates a clear juxtaposition between behavioural and physiological responses when relating to fitness maintenance. When exposed to OAW and predation, energy intake falls short of energy expenditure as seen in eventual metabolic depression. On the one hand, reduced feeding rate when exposed to cues, under OAW, indicates the mussels behavioural response (e.g., cue perception) is not impaired (Clements and Comeau, 2019). On the other, this may be detrimental to future adaption as it shows the animal is prioritising behavioural rather than physiological mechanisms of survival.

4.5. Species-specific differences and interactions

Molluscs, as a taxa, have been shown to be particularly sensitive to OAW in terms of survival, calcification, growth, and development, compared to crustaceans, fish, and algae (see review: Kroeker et al., 2013). Within the taxa, the effects of OAW exposure are often species-specific as illustrated in the growth and mortality differences found here. *N. lapillus* had no significant response to OAW scenarios either in length or wet weight whereas growth rate increased for *M. edulis*, in length and height, and decreased for wet weight. Mortality was also significantly greater in *M. edulis* under OAW conditions, whereas *N. lapillus* mortality was unaffected. The disparity between OAW responses in bivalves and gastropods has been documented in behavioural defences (see review by Clements and Comeau, 2019) with bivalves more sensitive than gastropods to OAW illustrated by predator avoidance behaviour (e.g bivalves: Clements et al., 2017; gastropods: Queirós et al., 2015).

Behavioural responses to OAW can be indicative of physiological underlying effects of OAW, such as impacts to metabolism or growth (Clements and Comeau, 2019; Gazeau et al., 2013). An increased susceptibility of bivalves to OAW over their predators may lead to incongruity in their biotic relationships. That is, if there is greater biological cost to the bivalve and no change in cost to the
gastropod predator as shown here, this may suggest potential for modification of predator-prey dynamics and wider trophic impacts.

Predation rate is a key driver in ecosystem dynamics (Holling, 1959). Change in a predator’s feeding rate has potential to destabilise lower trophic levels (Kroeker et al., 2017). In this study, elevated temperature increased *N. lapillus* feeding rates by 82%. In the literature, Quieros et al. (2015) reported that *N. lapillus* foraging distance and foraging time increased under OA suggesting an increased feeding rate. However, this may increase their own susceptibility to predation from higher trophic levels (Křivan and Eisner, 2003). Nevertheless, increased feeding of *N. lapillus* as a result of elevated temperature coupled with the negative impacts of OA on *M. edulis*, and increased SMR but reduced feeding rate, could negatively affect *M. edulis* populations. Predation risk of prey animals is reported to increase in bivalves under OA regardless of predator exposure to OAW stress (Sadler et al., 2018; Sanford et al., 2014). Increased predation leading to greater consumptive effects of *N. lapillus* on *M. edulis* may have knock-on consequences to ecosystem services and wild mussel fisheries (Lemasson and Knights, 2021; Sadler et al., 2018). However, local ecosystem effects may vary depending on functional redundancy (i.e., biodiversity) within a community and plasticity of the populations affected (Kroeker et al., 2017). Investigating OA with predation as a stressor adds ecological relevance to a study and help elucidate the interacting effects of OA in an ecologically relevant setting (Kroeker et al., 2013).

5. CONCLUSION

The relative biological cost of OA impacts individual animal fitness and will reflect into the population. The two species, *M. edulis* and *N. lapillus*, had contrasting responses in terms of growth, metabolism, and feeding to OA exposure. In addition to this, the species chosen are ecologically linked in marine ecosystems, therefore impacts to one will affect trophic relationships (Holling, 1959). Here, *M. edulis* demonstrated greater effect sizes from OA exposure than *N. lapillus*. The interaction between the two species also changed under OA exposure, exhibited through change in both NCEs (e.g., reduced CR in *M. edulis*) and CE (e.g., increased predation rate of *N. lapillus*). The differential responses of the two species and the increased feeding rate seen in *N. lapillus* indicates that under future climate change scenarios, *M. edulis* may experience greater predation risk alongside physiological implications whereas in contrast, *N. lapillus* may largely be unaffected if food is not limited. This could lead to shifts in ecosystem functioning and services depending on the functional redundancy within the ecosystem and susceptibility of different species to OA (Kroeker et al., 2017). This area of investigation would benefit from different multi-trophic interactions being explored under
OAW to further comprehend the extent of change to ecosystem services with particular emphasis on
stressor interactions. The singular and interactive effects of the stressors (pH, temperature, predator
presence, and time) on the metrics observed highlighted a mitigation of the effects of OA when
stressors interact. This supports the necessity of research prioritising interactive effects of multiple
stressors over single stressor experiments. The combination of temperature, pH and predator
presence is a far more holistic and ecologically relevant analysis of the effects of climate stressors on
organisms and clearly the interaction between stressors may mitigate the reported effects of any one
stressor on an organism. Current literature investigating singular stressors on an animal, when those
stressors are not environmentally relevant or likely (e.g., OA) has clear methodological drawbacks and
efforts should be made to rectify or enhance the current knowledge base.

ACKNOWLEDGEMENTS

Thanks to staff from the School of Biological and Marine Science, specifically Marie Palmer, Charlotte
Crowther, and Alex Fraser for technical support. Thanks to Isobel Rowe, Oscar Speed, Sarah Lane, and
Callum Scott for organism collection and husbandry support.

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Figure 1. Change in mussel (a) shell length and (b) shell height in the presence (white bars) and absence (grey bars) of whelks. Box and whiskers show: median (thick line); hinges = ±1 IQR beyond the median; whiskers = ±1SD beyond the IQR; and dots indicate outliers.
Figure 2. Localised regression (loess) of change in standard metabolic rate (SMR; mg O$_2$ L$^{-1}$ g$^{-1}$ h$^{-1}$) over time in (a) whelks, and (b) mussels with/without predators (whelks) exposed to control conditions and combinations of ocean acidification (OA), ocean warming (OW), and ocean acidification and warming (OWA). Shading indicates 95% confidence intervals around the mean.
Figure 3. Localised regression (loess) of mussel clearance rate (L h\(^{-1}\)) with (present) and without (absent) a predator under current, ocean acidification (OA), ocean warming (OW) and ocean acidification and warming (OAW) scenarios. Shading indicates 95% confidence intervals around the mean.
Figure 4. Percentage mortality of mussels after 8 days of whelk feeding. Whelks and mussels were exposed to control conditions, and combinations of ocean acidification (pCO2) and ocean warming (temperature) scenarios for 8-weeks. Box and whiskers show: median (thick line); hinges = ±1 IQR beyond the median; whiskers = ±1SD beyond the IQR; and dots indicate outliers.
Weeks
Salinity

Weeks
Total Alkalinity (mmol/L)
Appendix 1 - Carbonate chemistry parameters monitored throughout the 8-week exposure in each treatment. Treatments; 15*500 = 15°C x 500 ppm pCO$_2$, 15*1000 = 15°C x 1000 ppm pCO$_2$, 20*500 = 20°C x 500 ppm pCO$_2$, 20*1000 = 20°C x 1000 ppm pCO$_2$. (a) temperature (°C), (b) pH, (c) salinity (psu), (d) TA- total alkalinity (mmol L$^{-1}$), (e) pCO$_2$- partial pressure of carbon dioxide in seawater (μatm), (f) calcite saturation in seawater, (g) aragonite saturation in seawater. Confidence intervals (95%) around linear regression lines are shown.
Table A1- Seawater carbonate chemistry average values +/- standard deviation throughout each treatment over 8 weeks. Temperature, Salinity, pH, Total alkalinity (TA), partial pressure of CO₂ (pCO₂), calcite saturation state (Ω calcite), aragonite saturation state (Ω Aragonite).

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Temperature (°C)</th>
<th>Salinity</th>
<th>pH</th>
<th>TA (mmol L⁻¹)</th>
<th>pCO₂ (µatm)</th>
<th>Ω calcite</th>
<th>Ω aragonite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.80</td>
<td>34.69</td>
<td>8.09</td>
<td>2.25</td>
<td>297.73</td>
<td>3.51</td>
<td>2.28</td>
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<tr>
<td>± SD</td>
<td>0.51</td>
<td>1.18</td>
<td>0.06</td>
<td>0.08</td>
<td>52.35</td>
<td>0.45</td>
<td>0.30</td>
</tr>
<tr>
<td>Elevated CO₂ (OA)</td>
<td>15.14</td>
<td>34.87</td>
<td>7.83</td>
<td>2.25</td>
<td>593.47</td>
<td>2.17</td>
<td>1.41</td>
</tr>
<tr>
<td>± SD</td>
<td>0.44</td>
<td>1.33</td>
<td>0.07</td>
<td>0.07</td>
<td>107.43</td>
<td>0.34</td>
<td>0.22</td>
</tr>
<tr>
<td>Elevated Temperature (OW)</td>
<td>19.36</td>
<td>34.30</td>
<td>8.16</td>
<td>2.26</td>
<td>247.51</td>
<td>4.61</td>
<td>3.02</td>
</tr>
<tr>
<td>± SD</td>
<td>0.80</td>
<td>1.40</td>
<td>0.06</td>
<td>0.06</td>
<td>44.43</td>
<td>0.49</td>
<td>0.33</td>
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<tr>
<td>Elevated CO₂ and temperature (OAW)</td>
<td>19.47</td>
<td>34.54</td>
<td>7.87</td>
<td>2.27</td>
<td>559.15</td>
<td>2.77</td>
<td>1.82</td>
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<tr>
<td>± SD</td>
<td>0.79</td>
<td>0.92</td>
<td>0.09</td>
<td>0.05</td>
<td>145.97</td>
<td>0.50</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Table A2- Seawater carbonate chemistry average values +/- standard deviation throughout each treatment during Nucella lapillus feeding experiment (8 d). Temperature (°C), Salinity, pH, Total alkalinity (TA), partial pressure of CO₂ (pCO₂), calcite saturation state (Ω calcite), aragonite saturation state (Ω Aragonite).

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Temperature (°C)</th>
<th>Salinity</th>
<th>pH</th>
<th>TA (mmol L⁻¹)</th>
<th>pCO₂ (µatm)</th>
<th>Ω calcite</th>
<th>Ω aragonite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.55</td>
<td>35.86</td>
<td>8.13</td>
<td>2.28</td>
<td>266.84</td>
<td>3.97</td>
<td>2.58</td>
</tr>
<tr>
<td>± SD</td>
<td>0.25</td>
<td>0.64</td>
<td>0.05</td>
<td>0.04</td>
<td>36.87</td>
<td>0.26</td>
<td>0.17</td>
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<tr>
<td>Elevated CO₂ (OA)</td>
<td>15.76</td>
<td>36.00</td>
<td>7.75</td>
<td>2.31</td>
<td>738.49</td>
<td>1.97</td>
<td>1.28</td>
</tr>
<tr>
<td>± SD</td>
<td>0.30</td>
<td>0.76</td>
<td>0.05</td>
<td>0.04</td>
<td>91.53</td>
<td>0.21</td>
<td>0.14</td>
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<tr>
<td>Elevated Temperature (OW)</td>
<td>19.01</td>
<td>35.85</td>
<td>8.17</td>
<td>2.30</td>
<td>241.06</td>
<td>4.84</td>
<td>3.18</td>
</tr>
<tr>
<td>± SD</td>
<td>0.39</td>
<td>0.53</td>
<td>0.08</td>
<td>0.03</td>
<td>32.37</td>
<td>0.60</td>
<td>0.39</td>
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<tr>
<td>Elevated CO₂ and temperature (OAW)</td>
<td>18.98</td>
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<td>2.36</td>
<td>711.38</td>
<td>2.47</td>
<td>1.62</td>
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<tr>
<td>± SD</td>
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<td>0.50</td>
<td>0.11</td>
<td>0.05</td>
<td>171.52</td>
<td>0.57</td>
<td>0.37</td>
</tr>
</tbody>
</table>
Highlights

• Multiple environmental stressors act upon multiple trophic levels
• Mollusc predator and prey respond differently to future climate scenarios
• Prey are negatively impacted physiologically and behaviourally
• Predators unaffected resulting in elevated predation risk for prey
• Potential for fundamental change in trophic interactions affecting biodiversity
Author Statement

Rebecca Greatorex: Conceptualization; Investigation; Writing – Original
Antony Knights: Conceptualization; Formal analysis; Writing – Review & Editing; Supervision
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: