

Potential for acclimation of banded-dye murex, *Hexaplex trunculus* (Linnaeus, 1758) after long-term exposure to low pH

Mogućnost aklimatizacije kvrgavog volka *Hexaplex trunculus* (Linnaeus, 1758) nakon dugotrajne izloženosti niskom pH

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Abstract

Previous work on ocean acidification highlighted contrasting response between marine species and population. This so-called species-specific response was hypothesized to be partly a consequence of local adaptation to the present range of natural variability in the carbonate chemistry. Under that hypothesis, species tolerance threshold should be correlated to its environmental pH niche. This paper aims to evaluate shell growth rate of *Hexaplex trunculus*, an important predatory gastropod in benthic communities of Mali Ston Bay. A long-term experiment (310 days) was designed to test a range of pH treatments covering present and future pH levels relevant in the context of future ocean acidification (7.95-7.22 pH_r) at the sampling site. Sex had an effect on the shell growth rate irrespective of pH, and was only significant after 236 days. As growth rate in all pH treatments followed seasonal patterns correlating to changes in seawater temperature, the data were divided into 3 time periods. A positive relationship between shell growth rate (SGR, mm day⁻¹) and pH was observed for the period 1-59 days (temperature ranging between 26.5 & 18.8 °C), whereas SGR decreased significantly with pH for the following period (60-236 days, temperature ranging between 20.6 & 8.5 °C). After 236 days (temperature ranging between 27.5 & 14.1 °C), there was no significant difference in SGR among pH. Similar temperature was experienced between the first and third period and the difference in response could be explained as a consequence of an acute negative response versus a longer exposure indicating possible potential for acclimation. Our results highlight the modulating effect of temperature and the importance of long-term experiments to better assess impacts of ocean acidification on marine organisms.

Sažetak

Dosadašnja istraživanja utjecaja acidifikacije mora na morske organizme pokazala su znatno različite reakcije među srodnim vrstama te različitim populacijama iste vrste. Pretpostavlja se da je specifičan odgovor svake vrste (tzv. species-specific) djelomično rezultat adaptacije na varijaciju parametara karbonatne kemije mora u određenom staništu. U skladu s tim, granica tolerancije svake vrste trebala bi biti u koleraciji s njenom pH nišom. Cilj ovog rada je utvrditi stopu rasta kućice kvrgavog volka, *Hexaplex trunculus*, važne predatorske vrste puža u bentoskim zajednicama Malostonskog zaljeva. Dugotrajni eksperiment (310 dana) istraživanja raspona pH obuhvatio je postojeće i potencijalne vrijednosti pH u kontekstu buduće acidifikacije mora (7,22 – 7,95 pH_r) na području uzorkovanja. Značajan utjecaj spola na rast kućice zabilježen je tek 236 dana nakon početka eksperimenta, te nije ovisio o pH. Stopa rasta kućice u svim pH uvjetima varirala je ovisno o sezonskim promjenama temperature mora, pa su podaci podijeljeni u tri razdoblja. Pozitivna veza između stope rasta kućice (SGR, mm dan⁻¹) i pH opažena je između prvog i 59. dana eksperimenta pri rasponu temperature između 26,5 i 18,8 °C, dok se stopa rasta kućice značajno smanjila smanjivanjem vrijednosti pH u sljedećem periodu od 60. do 236. dana, pri čemu se vrijednost temperature kretala u rasponu između 20,6 i 8,5 °C. Nakon 236 dana, pri rasponu temperature između 27,5 i 14,1 °C nije bilo značajne razlike u stopi rasta kućice pri različitim pH vrijednostima. U prvom i posljednjem razdoblju zabilježena je slična temperatura, a razlika u odgovoru kvrgavog volka moguće je posljedica akutnog negativnog odgovora na dužu izloženost, koja ukazuje na mogućnost aklimatizacije. Rezultati rada pokazuju modulirajući učinak temperature i važnost dugotrajnih eksperimenata radi bolje procjene utjecaja acidifikacije mora na morske organizme.

KEY WORDS

ocean acidification
growth rate
shell length
acclimation
Hexaplex trunculus

KLJUČNE RIJEČI

acidifikacija mora
stopa rasta
duljina kućice
aklimatizacija
Hexaplex trunculus

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1. INTRODUCTION

The industrial revolution was a period of rapid urbanisation and mechanisation of industry. Extensive burning of fossil fuels and change of land use for intensive agriculture led to an increase of carbon dioxide levels in the atmosphere by as much as 40% as compared to pre-industrial times 250 years ago [1, 2]. The oceans absorb about 27% of the released carbon dioxide which leads to perturbation of seawater carbonate chemistry. As atmospheric CO₂ dissolves in seawater, it forms carbonic acid, and quickly dissociates into bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) ions, and release excess of hydrogen ions (H⁺), resulting in a reduction of seawater pH [1, 2, 3]. A decrease of 0.11 pH units in average surface seawater has been observed over the past 250 years, and a further decrease of up to 0.4 pH units is projected by 2100 [3]. These changes in ocean carbonate chemistry are referred to as ocean acidification.

Numerous research papers have demonstrated the effects of ocean acidification on a number of biological processes such as development, survival, reproduction, calcification, behaviour, feeding, etc. [e.g. 4, 5, 6, 7, 8]. Many marine calcifiers are known to be particularly sensitive to ocean acidification as extra energy costs associated with acid-base regulation, calcification and protection against dissolution alter the overall energy budget and fitness [7, 8]. However, a growing body of research has shown that some calcifiers are able to maintain their calcification rate or even thrive under low pH conditions [9, 10, 11]. Species sensitivity to low pH seems to be largely dependent on their ability to adapt to present local pH variability [12,13]. Under that hypothesis, physiological thresholds can be expected to be correlated to the extreme of present natural variability [14,15]. Among other modulating factors, differences in response between sexes has often been overlooked in studying the effect of ocean acidification. Studies conducted so far indicate variation in response between sex [16, 17, 18], highlighting the need to consider sex in future studies.

Mali Ston Bay (southern Adriatic Sea) is an important marine habitat with high biodiversity, protected at the national level as a special marine reserve. In addition, it is also protected

under the ecological framework of the European Union, Natura 2000 [19]. Since the Roman times this area has been known for oyster and mussel cultivation [20], with first archive records of extensive cultivation dating back in 16th century [21].

Banded-dye murex, *Hexaplex trunculus* (Linnaeus, 1758) is a common species in Mali Ston Bay. It is a gonochoristic species with internal fertilization [22]. Spawning occurs in spring when seawater temperatures rise, and females gather together to produce spawn masses [23]. The banded-dye murex undergoes intracapsular embryonic and larval development [24, 25]. As a carnivorous and opportunistic species, it preys on other invertebrates [26]. It is known for its commercial value for small scale fisheries in the Mediterranean, but can also be considered a pest in the shellfish farming areas [27]. Adult and juvenile specimens of the family Muricidae, including *H. trunculus*, can strongly influence the population dynamics of benthic communities, especially shellfish, and cause large changes in the diversity and abundance of their prey, suggesting their ecological importance [28, 29, 30].

A previous study reported a reduction in shell elasticity and dissolution of the outer shell as well as a smaller size of specimens of *H. trunculus* collected near CO₂ vents where seawater pH is naturally low [31]. The potential effect of ocean acidification on the banded-dye murex will inevitably have consequences for the structure of benthic communities in Mali Ston Bay. This paper presents a long-term experiment in which the shell growth rate of the *H. trunculus* population from Mali Ston Bay was studied under different pH conditions relevant in the context of ocean acidification and considering the natural pH variability in the Bistrina bay.

2. MATERIAL AND METHODS

2.1. Animal collection and acclimation

Mature adult gastropods were collected in August 2020 from a depth of ~ 3 m in the Bay Bistrina, part of the Mali Ston Bay (42°52'19.1 "N 17°42'02.3 "E) (Figure 1) using plastic trays filled with crushed Mediterranean mussels *Mytilus galloprovincialis* Lamarck, 1819.

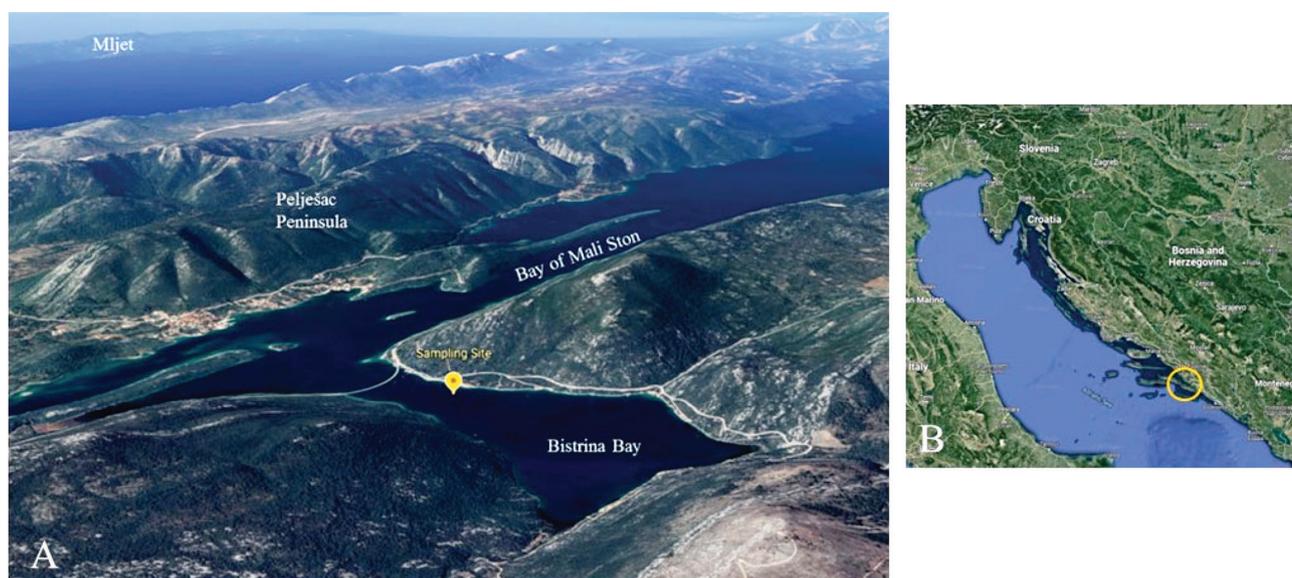


Figure 1 3D map indicating sampling site (A; yellow pin) in the study area (B; yellow circle), middle Eastern Adriatic Sea, Croatia (www.googlearth.com)

Gastropods with an undamaged shell (shell length, $SI > 45$ mm) were selected and carefully cleaned of fouling organisms with a brush. The snails were immediately transferred to the laboratory in a container with seawater. Prior to start of the experiment, they were kept in a flow-through system with filtered ambient seawater for two weeks to allow them to acclimate to laboratory conditions.

2.2. Experiment set-up

Nine pH conditions were selected for this experiment, ranging between average pH_T 7.95 and 7.22. Experimental treatments were not replicated. pH conditions cover the present (pH_T 8.07–7.74, this study) and end of the century (pH_T 7.7–7.3) range of natural variability in Bistrina Bay, and beyond. pH_T in Bistrina Bay was measured in line with the course of experiment, approximately twice a month on the seawater samples taken from the adjacent sea in front of the pump inlet (1 m depth), (Figure 2).

After an acclimation period of two weeks, 40 randomly selected individuals were placed in each of nine treatment tanks and marked with bee tags (360 snails in total). Experimental system consisted of nine separate treatment tanks (volume 130L) with a flow-through system with filtered, UV-sterilized, and aerated ambient seawater pumped from the Bistrina Bay adjacent to the laboratory facilities. The pH in each tank was manipulated separately by bubbling pure CO_2 gas from four CO_2 cylinders (one CO_2 cylinder providing gas for two tanks) and using pH controllers (Milwaukee MC122) connected to solenoid valves to maintain the desired pH. Lab grade double junction glass electrodes (Milwaukee MA911B/2) were connected to the pH controllers. Manual two-point calibration of the pH electrodes was performed twice a month with Milwaukee pH 7.01 and pH 4.01 Calibration Solution Sachets (buffer solution accuracy ± 0.01 pH). In one tank, pH was not manipulated to equilibrate with the atmospheric CO_2 concentration. Temperature (T ; $^{\circ}C$), salinity (S ; psu), dissolved oxygen concentration (DO, mgL^{-1}), and pH (on the National Bureau of Standards scale – pH_{NBS}) were measured using multiparametric probes (YSI Pro 30, Oxygen Handy Polaris, and SI Analytics Lab Meter 845, respectively). Salinity and temperature followed the ambient conditions of the

incoming seawater and values were confirmed by measuring salinity and temperature of the seawater at the pump inlet twice a month. Faeces and debris were siphoned every other day, and once a week a complete exchange of the seawater and detailed cleaning was done. The animals were fed *ad libitum* once a week with commercial mussels *M. galloprovincialis*, obtained from a local shellfish supplier. Mussels were replaced once a week. Gastropods were maintained under experimental conditions for 310 days (start: August 24, 2020; end: June 30, 2021).

2.3. Carbonate chemistry parameters

Following the guide to best practices for ocean acidification research [28] and recommended procedures [32], every 2 days pH was measured on a total scale (pH_T) by the potentiometric method after calibration with TRIS (2-amino-2-hydroxy-1,3-propanediol) buffer. Total alkalinity (TA, $\mu mol\ kg^{-1}$) was measured every two weeks using the potentiometric two-point open-cell titration method with a manual burette [33]. Other seawater carbonate chemistry parameters (pCO_2 , Ω_{Ca} , Ω_{Ar}) were calculated based on known TA and pH_{NBS} for a given salinity using CO2SYS software, with dissociation constants of Mehrbach [34] refitted by Dickson and Millero [35].

2.4. Shell growth rate

Shell length (in mm) was measured as the maximum length along the central axis, from the apex of the shell to the end of siphonal canal [36]. Measurements were performed on the same individuals nine times in total over the course of experiment. Each marked individual was measured with a digital calliper to the nearest 0.01 mm. Shell growth rate (SGR in $mm\ day^{-1}$) was calculated from changes in the shell length between observation points.

2.5. Sex effect

At the end of the experiment specimens from all treatments were frozen until further analysis. For the sex determination, shells were cracked with a bench vice to expose soft tissue. Males were determined by the presence of penis behind the right tentacle, females were determined by the vaginal opening.

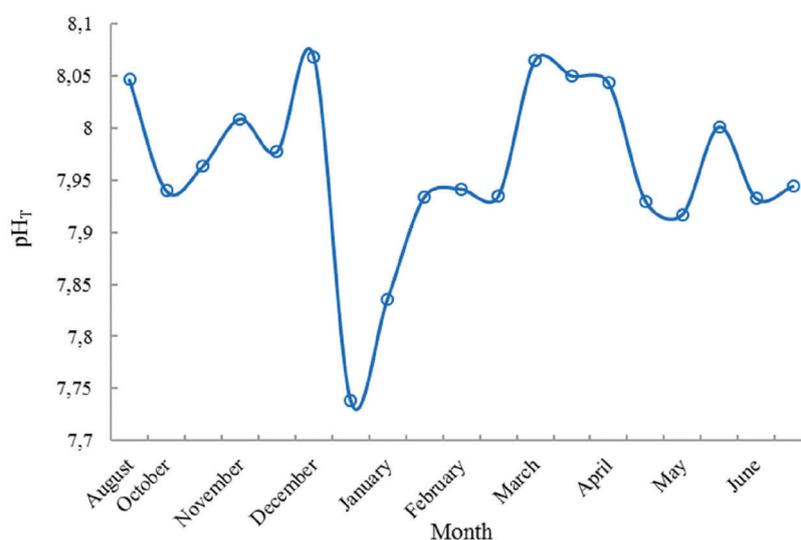


Figure 2 Measured pH_T variability in the Bistrina Bay (middle Eastern Adriatic Sea, Croatia), over the course of experiment

2.6. Statistical analysis

Statistical analyses were performed using SPSS Statistics v.26. Mean differences in temperature and salinity among pH treatments were tested with one-way ANOVA. The effect of pH on shell growth rate was tested with Generalized estimating equations procedure (GEEs) with independent working covariance matrix applied. Generalized estimation equations procedure (GEEs) models population-level effect, while accounting for repeated measures within the same individual. Estimated marginal means (EMMs \pm SE) obtained from model were used to further investigate direction of the relationship between pH and shell growth rate.

The effect of sex on shell growth rate was tested with one-way ANCOVA with sex as a fixed factor and pH as a covariate. When there was no regression between shell growth rate and pH a two-way ANOVA was applied. When significant effect ($p < .05$) was observed, a post-hoc pairwise comparison with Bonferroni correction was applied. Prior to analysis, data were tested for normality with Shapiro Wilk test and for homogeneity of variance with Levene's test.

All assumptions were met. Data are presented as mean \pm standard deviation.

3. RESULTS

3.1. Seawater parameters

Temperature and salinity varied with seasonal changes, between 8.4 and 26.6 °C for temperature and 22.6 and 35.3 psu for salinity (Figure 3). Temperature and salinity had no significant effect on the pH (one way ANOVA: $F(8, 855) = .02, p = 1.00$), $F(8, 882) = .00, p = 1.00$, respectively).

The dissolved oxygen concentration never fell below 6.28 mg L⁻¹ O₂. The pH_T of seawater in the unmanipulated pH treatment varied between 7.75 and 8.05 during the experiment, corresponding to the nearshore pH variability in Mali Ston Bay. The measured and calculated carbonate chemistry parameters are listed in Table 1.

Seawater was undersaturated in respect to calcite only at pH_T 7.22 ($\Omega_{Ca} = 0.9 \pm 0.6$), whereas undersaturation in respect to aragonite occurred at pH_T 7.42, 7.33 and 7.22 ($\Omega_{Ar} = 0.9 \pm 0.1, 0.7 \pm 0.1$ and 0.6 ± 0.1 , respectively).

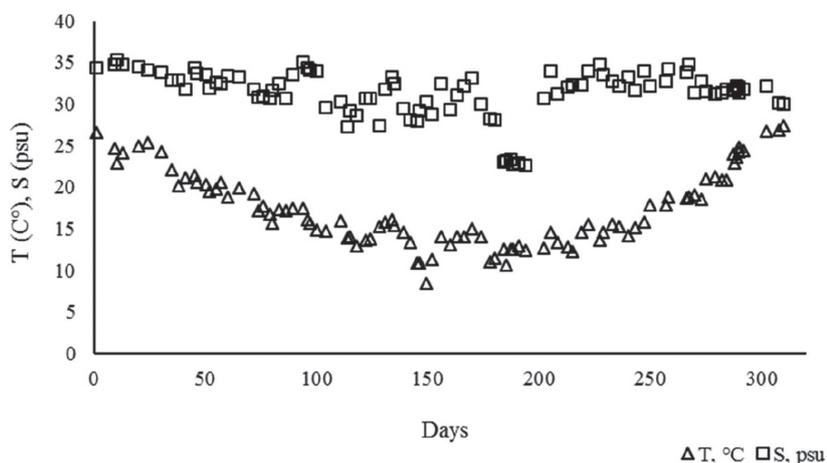


Figure 3 Temperature (T, °C) and salinity (S, psu) fluctuations showing seasonal variations

Table 1 Seawater carbonate chemistry parameters presented as mean \pm SD. Measured: Seawater pH on a NBS scale (pH_{NBS}), total scale (pH_T), salinity (S; psu), temperature (T; °C) and total alkalinity (TA; mmol kg⁻¹). Calculated: CO₂ partial pressure (pCO₂; μ atm), calcite and aragonite saturation states (Ω_{Ca} and Ω_{Ar} , respectively)

Measured					Calculated			
Target pH _{NBS}	pH _{NBS}	pH _T	S (psu)	T (°C)	TA (mmol kg ⁻¹)	pCO ₂ (μ atm)	Ω_{Ca}	Ω_{Ar}
Control	8.08 \pm 0.07	7.95 \pm 0.07	31.3 \pm 2.8	17.4 \pm 4.6	2976 \pm 216	692 \pm 18	3.9 \pm 0.8	2.5 \pm 0.6
8.1	8.07 \pm 0.07	7.95 \pm 0.08	31.3 \pm 2.8	17.4 \pm 4.6	2950 \pm 176	698 \pm 19	3.9 \pm 0.9	2.5 \pm 0.6
8.0	8.00 \pm 0.05	7.87 \pm 0.08	31.4 \pm 2.8	17.4 \pm 4.6	2940 \pm 190	809 \pm 77	3.3 \pm 0.4	2.1 \pm 0.3
7.9	7.90 \pm 0.07	7.76 \pm 0.07	31.3 \pm 2.8	17.4 \pm 4.5	2955 \pm 201	1064 \pm 99	2.7 \pm 0.4	1.7 \pm 0.3
7.8	7.81 \pm 0.05	7.64 \pm 0.07	31.3 \pm 2.9	17.5 \pm 4.6	2935 \pm 207	1335 \pm 12	2.2 \pm 0.3	1.4 \pm 0.2
7.7	7.69 \pm 0.05	7.51 \pm 0.07	31.3 \pm 2.9	17.5 \pm 4.6	2917 \pm 208	1759 \pm 17	1.7 \pm 0.2	1.1 \pm 0.2
7.6	7.60 \pm 0.05	7.42 \pm 0.07	31.3 \pm 2.8	17.4 \pm 4.5	2946 \pm 179	2187 \pm 18	1.4 \pm 0.9	0.9 \pm 0.1
7.5	7.52 \pm 0.04	7.33 \pm 0.06	31.3 \pm 2.9	17.4 \pm 4.6	2891 \pm 207	2601 \pm 24	1.2 \pm 0.2	0.7 \pm 0.1
7.4	7.43 \pm 0.05	7.22 \pm 0.07	31.4 \pm 2.8	17.4 \pm 4.6	2851 \pm 184	3221 \pm 25	0.9 \pm 0.6	0.6 \pm 0.1

3.2. Shell growth rate

Shell growth rate was calculated as the daily increase in shell length between observation points (SGR, mm day⁻¹). Growth rate in all pH treatments followed a similar trend over the course of the experiment, with periods of higher growth at the beginning and end of the experiment and periods of no growth and dissolution in between (Figure 4).

Relationship between shell growth rate and seawater temperature revealed seasonal pattern indicating that growth is highly impacted by temperature and is only positive for the temperatures above 20 °C (Figure 5).

Analysis of shell growth rate was therefore divided in three periods to account for the temperature effect: (i) first 59

days of exposure with temperature above 20°C and positive growth (SGR₁, T = 22.4 ± 2.37 °C), (ii) between 59 and 236 days with temperature below 20°C (SGR₂, T = 14.4 ± 2.29 °C), and (iii) the last 74 days with temperature above 20°C (SGR₃, T = 22.1 ± 3.9 °C).

For the first 59 days, there was a significant negative relationship between shell growth rate and pH (GEEs, W(8) = 35.882, p = .000) (Figure 6a). During the period when temperatures were below 20° C growth was negatively affected by low pH following a positive relationship (W(8) = 46.637, p = .000) (Figure 6b). During the last observation period, there was no significant effect of pH on the growth rate (W(8) = 17.245, p = .058) (Figure 6c).

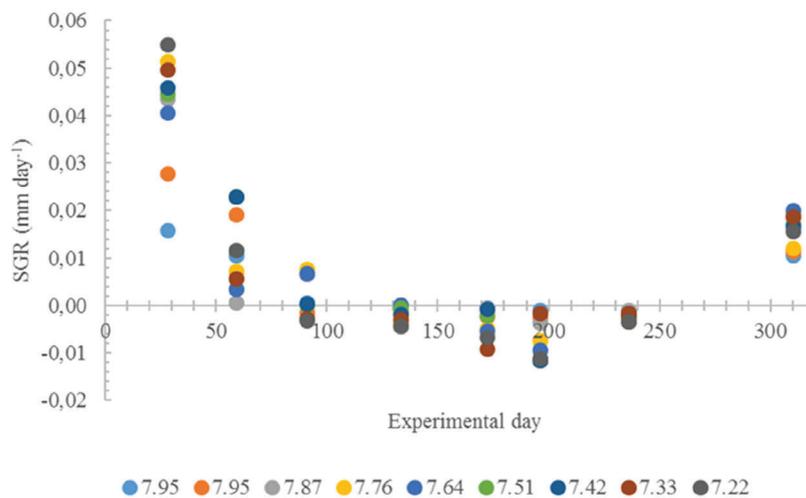


Figure 4 Shell growth rate (SGR, mm day⁻¹) of banded-dye murex, *Hexaplex trunculus*, over the course of experiment (Experimental day) showing seasonal pattern. Colored dots indicate respective pH_T

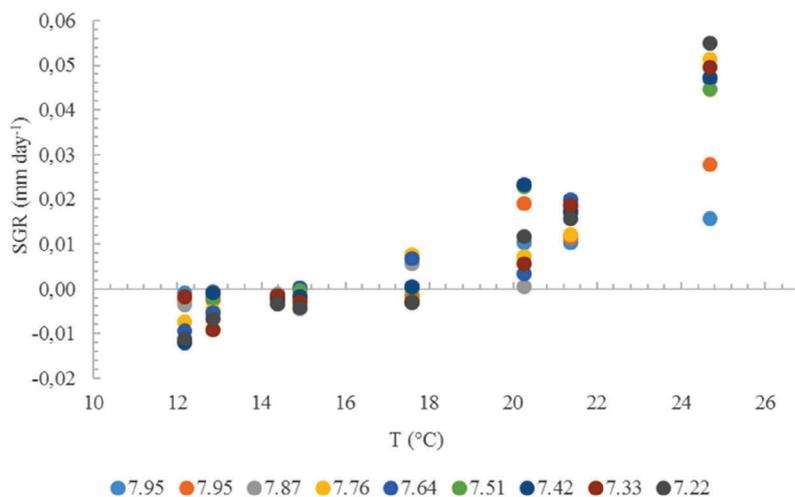


Figure 5 Relationship between shell growth rate (SGR, mm day⁻¹) of banded-dye murex, *Hexaplex trunculus* in pH treatments (colored dots) and seawater temperature (T, °C)

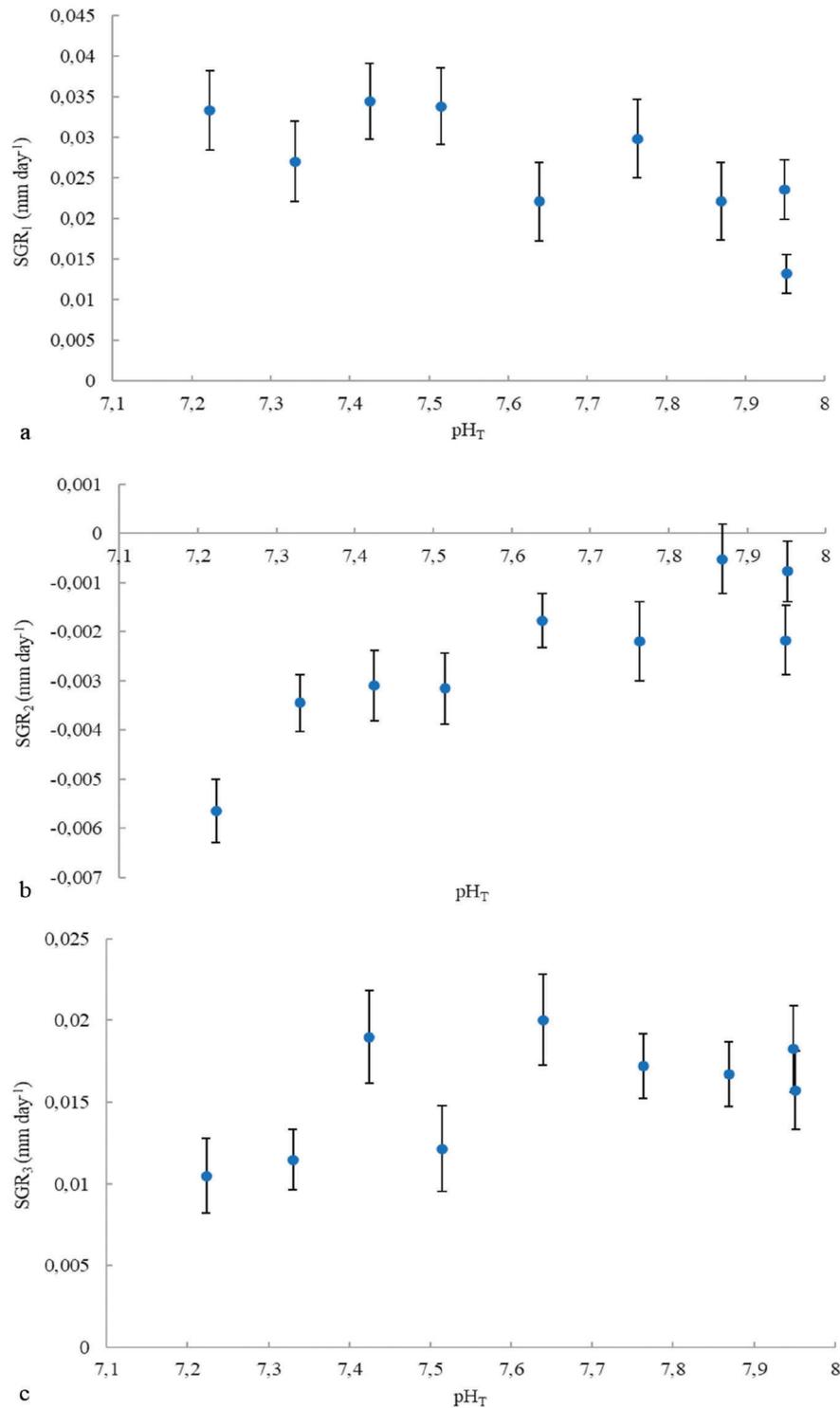


Figure 6 Relationship between estimated marginal means (EMMs ± SE) of shell growth rate (SGR, mm day⁻¹) of banded-dye murex, *Hexaplex trunculus* and pH_T for each observation period: a) SGR₁, b) SGR₂, and c) SGR₃

3.3. Sex effect

In total, 161 males and 174 females were sexed, with sex ratio of 1:1.08. Percentage of males and females in each pH is presented in Figure 7.

Sex had no significant effect on the average shell growth rate (mm day⁻¹) at the end of experiment (2-way ANOVA, ($F(1, 344) = 3.412, p = .066$)). The effect of sex on the shell growth rate in three temperature dependent (SGR₁, SGR₂, SGR₃) periods

was tested. For the SGR₁ and SGR₂ there was no effect of sex on the shell growth rate (ANCOVA, ($F(1, 344) = 1.060, p = .304$, and $F(1, 344) = .173, p = .678$, respectively)). However, in the last observation period, a significant effect of sex on the shell growth rate was observed, (2-way ANOVA ($F(1, 344) = 10.793, p = .001$)), with females having a higher shell growth rate than males ($MD = .007, SE = .002, p = .001$)

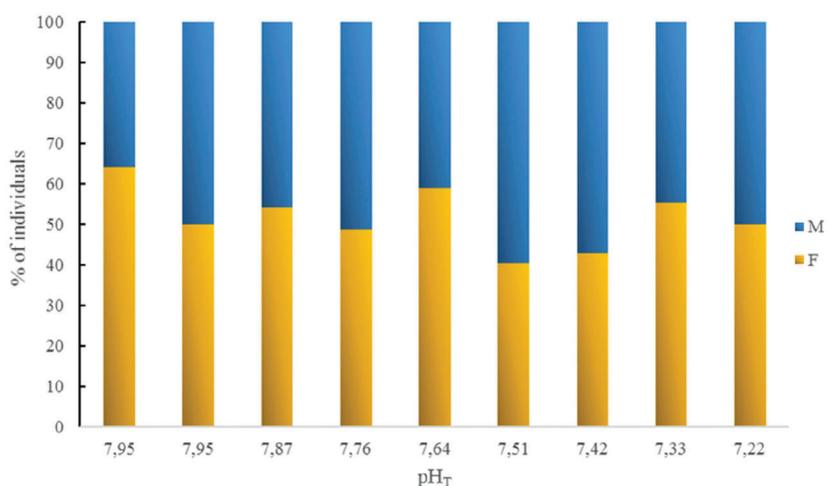


Figure 7 Percentage (%) of males (blue) and females (yellow) banded-dye murex, *Hexaplex trunculus* in pH treatments

Table 2 Summary of statistical analysis testing for the sex effect (sex) on shell growth rate (SGR) of banded-dye murex, *Hexaplex trunculus*

	Sum of Squares (Type III)	df	Mean Square	F	p
SGR _{total}					
2-way ANOVA	0.001	17	7.725E-5	1.411	0.129
sex	0.000	1	0.000	3.412	0.066
pH	0.001	8	9.558E-5	1.745	0.087
sex * pH	0.001	8	6.263E-5	1.144	0.333
Corrected total	0.019	344			
SGR ₁					
ANCOVA	0.009	2	0.004	6.105	0.002
pH	0.008	1	0.008	10.935	0.001
sex	0.001	1	0.001	1.060	0.304
Corrected total	0.258	344			
SGR ₂					
ANCOVA	0.000	2	0.000	15.010	0.000
pH	0.000	1	0.000	29.685	0.000
sex	2,686E-6	1	2,686E-6	0.173	0.678
Residuals	0.06	344			
SGR ₃					
2-way ANOVA	0.008	17	0.000	1.448	0.112
sex	0.004	1	0.004	10.793	0.001
pH	0.004	8	0.000	1.361	0.213
sex * pH	0.001	8	0.000	0.346	0.947
Corrected total	0.121	344			

4. DISCUSSION

Long-term exposure to low pH did not significantly affect the shell growth rate of banded-dye murex, despite observing an effect at the time of acute exposure and energy-limiting periods. During the first period of the experiment (temperature above 20°C), shell growth rates were positively affected by low pH, with no significant difference between males and females. Positive effect of low pH on metabolism is well-documented [e.g., 37, 14] and can translate into increasing growth rates under non-limiting energetical conditions. In our experiment, organisms were fed *ad libitum* and could then likely compensate for the extra-energetical costs associated with exposure to low pH (e.g., acid-base regulation) and increase their growth rate together with an increase in metabolism.

Over winter, when temperature decreased below 20°C, a negative growth rate was observed with a negative effect of low pH. The combination of lower metabolic rates in general during

winter, increased energetical costs for gametogenesis [23], and lower food intake [38] can explain both the negative growth rates and the inability to compensate for the extra energy costs associated with the exposure to low pH.

During the third observation period (temperature higher than 20°C), positive growth rates were observed with no effect of pH. Despite similar temperature conditions, the effect of pH on the growth rate was strikingly different between the first and last experimental periods. This can be a consequence of acclimation over longer period of time [39, 40, 41]. The time required to acclimate to a new environment can be species-specific. For example, the adult Antarctic sea urchin *Stereochinus neumayeri* took six to eight months to acclimate to the combined stressors of altered pH and temperature [42], but for the green sea urchin *Strongylocentrotus droebachiensis* 16 months passed before full acclimation was observed [43].

Acclimation and the development of compensatory mechanisms often require extended periods of time and may result in energy being expended on other physiological processes [43]. Some species seems also better equipped than others to cope with lower pH. Reductions in shell growth and calcification during the exposure to low pH have been observed in some marine gastropods [44, 45, 46]. Interestingly, gastropods from higher trophic levels can often tolerate acidification more successfully [47]. Our results support this hypothesis.

Seasonal variations in the shell growth rate are well known in molluscan species, with temperature being an important factor contributing to annual variation [48]. Within species' temperature tolerance range, metabolic and activity rates generally increase with increasing temperature [48]. In our experiment, the growth rate of banded-dye murex was highest at the beginning and end of the experiment when temperature was above 20°C and decreased significantly over winter (temperature below 20°C), suggesting a lower metabolic rate. In a previous study, a lower food consumption rate was also documented during this period [38]. For banded-dye murex winter is a period of gonadal development [23], which is known to depress or even temporarily arrest growth in marine invertebrates [48, 49]. Over period of lower seawater temperature, shell length growth was negative, indicating dissolution. The average shell growth rate of *H. trunculus* was only positive at temperatures above 20 °C, regardless of pH.

Males and females did not respond differently to pH in terms of shell growth rate. Although there is a paucity of studies in the field of ocean acidification that account for sex in the experimental design [50], there are indications that sex can modulate a species' response, especially when it comes to reproduction, gonadal tissue and gamete quality [51, 52, 53]. However, in this study, only shell growth rate was measured, and no significant effect of sex was observed, irrespective of tested pH. Further research is needed to assess whether sex modulates other physiological processes such as gametogenesis, metabolic rates, calcification, etc. Overall, while sex did not appear to have a significant effect on shell growth rate among pH treatments in this study, it remains an important factor to consider in future research on the impacts of ocean acidification.

It is important to note that during the last observation period, a significant effect of sex on the shell growth rate was observed across all pH treatments, indicating that the effect was not pH-related. Female gastropods of many species in general have a higher growth rate than males [54, 55, 56], including *H. trunculus* [57], but intra-annual variation in growth between sexes has not received as much attention. Gastropods can accumulate lipid and glycogen reserves when food is abundant, as in this study where they had access to an unlimited amount of food, and use them for energy-consuming processes or during times of food shortage [58, 59]. Although both males and females are capable of storing and using these energy reserves, differences in lipid content between the sexes throughout the year have been demonstrated. For example, males of the muricid *Nucella lapillus* (Linnaeus, 1758) had stable lipid reserves with no significant fluctuations throughout the seasons, while females had periods of extensive accumulation and depletion of lipid droplets [60]. It is possible that females only use more energy reserves to increase growth in a certain time of the year, such as periods when no energy-consuming processes (e.g., spawning,

maturation of ovaries) are occurring, as may have been the case during the last period of this study when spawning occurred.

5. CONCLUSIONS

An overwhelming body of evidence is documenting the effect of ocean acidification on marine species and ecosystems. Our ability to mitigate the impacts on marine biodiversity, including seafood, depends on our ability to understand its impact on key species, including their ability to acclimate or adapt. This study confirms that time of exposure is critical to fully understand the impact of low pH as well as the modulating role of other key environmental drivers such as temperature. While pH had an effect on banded-dye murex after an acute exposure or during energy limiting periods (e.g., winter), no effect of pH was observed over longer exposure time. Overall, banded-dye murex seems to be tolerant to pH values expected for the end of the century under ocean acidification scenarios. However, other factors not considered in this study (e.g., food levels, other stressors, ecological interactions) are likely to modulate this response. While banded-dye murex may exhibit tolerance in terms of shell growth rate over extended periods, there are other physiological processes that must be studied to fully understand their response to low pH. Therefore, further research is needed to adequately evaluate response of this species, which holds great importance in both ecological and commercial contexts.

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