Functional consequences of prey acclimation to ocean acidification for the prey and its predator

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Ocean acidification is the suite of chemical changes to the carbonate system of seawater as a consequence of anthropogenic carbon dioxide (CO2) emissions. Despite a growing body of evidences demonstrating the negative effects of ocean acidification on marine species, the consequences at the ecosystem level are still unclear. One factor limiting our ability to upscale from species to ecosystem is the poor mechanistic understanding of the functional consequences of the observed effects on organisms. This is particularly true in the context of species interactions. The aim of this work was to investigate the functional consequence of the exposure of a prey (the mussel Brachidontes pharaonis) to ocean acidification for both the prey and its predator (the crab Eriphia verrucosa). Mussels exposed to pH 7.5 for >4 weeks showed significant decreases in condition index and in mechanical properties (65% decrease in maximum breaking load) as compared with mussels acclimated to pH 8.0. This translated into negative consequences for the mussel in presence of the predator crab. The crab feeding efficiency increased through a significant 27% decrease in prey handling time when offered mussels acclimated to the lowest pH. The predator was also negatively impacted by the acclimation of the prey, probably as a consequence of a decreased food quality. When fed with prey acclimated under decreased pH for 3 months, crab assimilation efficiency significantly decreased by 30% and its growth rate was 5 times slower as compared with crab fed with mussels acclimated under high pH. Our results highlight the important to consider physiological endpoints in the context of species interactions.

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Abstract

Ocean acidification is the suite of chemical changes to the carbonate system of seawater as a consequence of anthropogenic carbon dioxide (CO_2) emissions. Despite a growing body of evidences demonstrating the negative effects of ocean acidification on marine species, the consequences at the ecosystem level are still unclear. One factor limiting our ability to upscale from species to ecosystem is the poor mechanistic understanding of the functional consequences of the observed effects on organisms. This is particularly true in the context of species interactions. The aim of this work was to investigate the functional consequence of the exposure of a prey (the mussel *Brachidontes pharaonis*) to ocean acidification for both the prey and its predator (the crab *Eriphia verrucosa*). Mussels exposed to pH 7.5 for >4 weeks showed significant decreases in condition index and in mechanical properties (65% decrease in maximum breaking load) as compared with mussels acclimated to pH 8.0. This translated into negative consequences for the mussel in presence of the predator crab. The crab feeding efficiency increased through a significant 27% decrease in prey handling time when offered mussels acclimated to the lowest pH. The predator was also negatively impacted by the acclimation of the prey, probably as a consequence of a decreased food quality. When fed with prey acclimated under decreased pH for 3 months, crab assimilation efficiency significantly decreased by 30% and its growth rate was 5 times slower as compared with crab fed with mussels acclimated under high pH. Our results highlight the important to consider physiological endpoints in the context of species interactions.

Introduction

Rising atmospheric CO_2 is altering seawater carbonate chemistry in a process termed ocean acidification. Research performed over the last decade and summarized in the recent IPCC report (IPCC, 2014) allows to conclude with high confidence that ocean acidification will affects marine organism and ecosystems for centuries to come with direct consequences for human society (Gattuso et al., 2015). The picture is rather clear at a global level but many challenges remain to allow projection of impacts at the local level (Riebesell & Gattuso 2013). These challenges include the need to consider ecology (Gaylor et al., 2015), evolution (Sunday et al., 2013) and environmental complexity (Breitburg et al., 2015; Gunderson, Armstrong & Stillman, 2016) into a field currently dominated by short term response of single-species to simplistic scenarios. This translates into practical and experimental design challenges (Cornwall & Hurd, 2015; Hurd et al., 2015) often limiting our ability to fully address these questions. An alternative approach is to dissect mechanisms behind biological response across level of organization to identify unifying principles and therefore increase our ability to project future impacts (Dupont & Pörtner, 2013).

Most published studies to date are focusing on single species responses with little consideration for interspecific interactions. For example, the direct effect of ocean acidification on mussels has been extensively studied with more than 40 studies published to date. A wide range of endpoints and techniques were used and showed effects on growth, calcification, immune response, physiology, behavior, etc. (e.g. Gazeau et al., 2013). When negative effects were documented, they were often interpreted as negative consequences for the species fitness. For example, two studies documented reduced production of byssus and mechanical performance of byssus secreted under reduced pH conditions (O Donnell, George & Carrington, 2014; Li et al., 2015). Byssus plays a key role against predation and these effects could potentially translate into increased probability of being dislodged from the substrate and consumed by the predator. However, this hypothesis was not formally tested in presence of predators. In another example, Fitzer et al. (2014) showed alteration in the ultrastructure of mussel shells from juvenile raised in decreased pH conditions. It was concluded that these changes “may prove instrumental in their ability to survive ocean acidification”. However, to fully understand the consequences of such changes on individual’s fitness, indirect effects should be considered. For example, a decrease in calcification or mechanical properties of a protective shell may not translate into negative effects in absence of predators. The mussel *Bathymodiolus brevior* is able to tolerate a wide range of natural pH environment ranging between 5.36 and 7.29 around submarine volcanoes. It was shown that shell thickness and daily growth increments decreased with decreasing pH making them potentially more sensitive to predator. However, they are able to survive in dense population at the lowest pH as their crab predators are absent from these setting (Tunnicliffe et al., 2009). Studies on these indirect impacts of ocean acidification are scarce and focus on trophic interactions (e.g. Thomsen et al., 2013) or pathogens (e.g. Asplund et al., 2014). In bivalves, the predator–prey interaction was only considered by Appelhans et al. (2012) and Keppel, Scrosati & Courtenay (2015). For example, mussels and sea star predators were cultured together and a 50% reduction in predation was observed at decreased pH as a consequence of a negative effect on sea stars (Keppel, Scrosati & Courtenay, 2015).

Energy acquisition plays a key role in a species response to ocean acidification. Some direct effects of ocean acidification on digestion physiology have already been documented (e.g. Stumpp et al., 2013). However, some indirect effects through changes in food quantity and/or quality are equally important. For example, Jin et al. (2015) showed that decreased pH can lead to accumulation of phenolic compounds in phytoplankton that can be transferred to higher trophic levels. Other studies demonstrate that exposure of phytoplankton to decreased pH decreased nutritional values with negative consequences for their grazers (Rossol et al., 2012; Schoo et al., 2013).

The aim of our study was to better understand the impact of ocean acidification on the prey-predator relationship. We focused on the relationship between the predator crab *Eriphia verrucosa* and its mussel prey *Brachidontes pharaonis*. *E. verrucosa* typically inhabits the rocky intertidal zone and spend most diurnal time within the natural cavities of the Vermetid reef that are normally exposed to the air also at high tide. During the night, crabs leave their lairs to feed and they usually rely on mussels as a first item of their diet (Elner, 1978; Elner & Hughes, 1978; Leonard, Bertness & Yund, 1999). We focused on the indirect consequences of the impact of ocean acidification on the prey (mussel) for both the prey (increased sensitivity to predator) and the predator (decreased food quality). To avoid confounding factors, the direct impact of ocean acidification on the crustacean predator was not considered. This was also supported by the fact that bivalves are expected to be more sensitive to ocean acidification than crustaceans (IPCC, 2014; Wittmann & Pörtner 2013). Two main questions were tested: (i) Does the direct impact to ocean acidification on shells' mechanical properties makes mussels more sensitive to predation? (ii) Does the direct impact of ocean acidification on mussels' condition translated into decreased performance in their predators?

Materials and Methods

Animal collection

To ensure that crabs encountered mussels before the experiment, both species were collected from the same site. Crabs (*Eriphia verrucosa*, without eggs) and mussels (*Brachidontes pharaonis,* size ranging between 18 and 29 mm) were sampled at low tide along a 3km transect on the Vermetid reefs of Capo Gallo - Isola delle Femmine Marine Protected Area (LAT: 38°11'53.28"N; LONG: 13°14'34.84"E) in June 2012 (400 mussels, experiment #1) and March-June 2013 (45 crabs and 400 mussels, experiment #2 & #3). Animals were transferred to the Laboratory of Experimental Ecology and Behaviour of the University of Palermo. Mussels were divided into six 12L tanks and crabs into 12L tanks filled with 1L of water and stones covered with white paper to avoid visual interference and filled with sea water at room temperature (18-20°C) and field salinity (38‰). Over the 10 days of laboratory acclimation, crabs were fed *ad libitum* twice a day with the mussel *B. pharaonis.* Mussels were fed *ad libitum* with *Isochrysis galbana*. Water was replaced every second days to avoid the accumulation of waste products.

Experimental design

Mussels were acclimated to two different nominal pH treatments: (i) pH 8.0, corresponding to present average pH at the sampling site; and (ii) pH 7.5, deviating from present range of natural variability and relevant for 2100 ocean acidification scenarios. pH was manually controlled through monitoring of pH (Cyberscan pH meter 510, Eutech Instruments, calibrated on the NBS scale) in 40L header tanks 8 times a day and injection of pure CO_2 directly into the aquarium when required. Water from each header tank was distributed into three 12L aquariums.

Experiment #1 (2012-2013)

The impact of pH on growth rate was tested on mussels (collected in 2012) exposed to the two nominal pHs for 50 weeks. Every week, total length (TL in mm), shell (SDW in g) and flesh dry weight (FDW in g; 80°C for 24h) were measured in 5 mussels per pH treatment. The Body Condition Index (BCI) was estimated as $\mathrm{C.I.} = \left(\frac{\mathrm{FDW}}{\mathrm{SDW}}\right) * 100$ (Davenport and Chen 1987).

Experiment #2 (2013)

The functional impact of exposure to pH was tested on mussels exposed to the two nominal pHs for >4 weeks as well as the consequences for their crab predators. Forty mussel shells from each pH treatment were evaluated for their mechanical properties at the Laboratory at the Department of Mechanical Engineering (University of Palermo). Experimental crushing tests were realized with an Instron 3367 controlled through the Bluehill 2.0 software to estimate shell's maximum breaking load (in N).

Crab feeding efficiency was then measured in a side experiment as time (s) spent in searching and manipulation behaviours. Fifteen crabs were randomly selected and starved for a period of 3 days. Each crab was filmed during six feeding trials with mussels acclimated to pH 8.0 (n=3) and pH 7.5 (n=3) for >4 weeks using a digital camera (XTC-200 Action Camera). Natural photoperiod at that time of the year was used during the trials (14h light). Feeding trials started with the addition of the mussel in front of the crab till complete prey consumption. Videos were analysed following Bateson (2003). Two behavioural endpoints were measured: (i) searching time (ST in s; Charnov, 1976; Pyke, Pulliam & Charnov, 1977), which was the time to find the prey; and (ii) handling time (HT in s; Pyke, Pulliam & Charnov, 1977; Hughes & Seed, 1981) which includes the breaking time, namely the time between picking up the prey and taking the first bite (Rheinallt & Huges, 1985), and the actual feeding action, called ingestion time. Time was measured to the nearest 1s using a digital chronometer.

Experiment #3 (2013)

To evaluate the consequences of pH exposure of the prey (mussel) on the predator growth, individual crab (5 per pH treatment) were fed for 3 months with mussels exposed to the two nominal pH for >4 weeks. Twice a week, faeces were collected, washed 3 times with 0.5 M ammonium formate (purest grade) and distilled water. They were then dried at 95°C for 24h, weighted (dry weight, DW) and incinerated in a muffle furnace at 450°C for 4 h, and weighted again (ash free dry weight, AFDW; Ezgeta-Balic et al., 2011, Sarà et al., 2013). The same procedure was performed using 10 mussels from each pH treatment. Assimilation efficiency (AE) was estimated according to Conover (1966): $AE = (F - E)/[(1 - E)F]$ where F is the ratio between ash-free dry weight (AFDW) and dry weight ratio (DW) for the food (mussel), and E is the same ratio for the faeces. Crab total wet weight (WW in gr) was also measured twice a week and growth rate (gr day^{-1}) was calculated for each individual as the coefficient of the significant relationship between WW and time.

Carbonate chemistry

The carbonate system speciation (pCO$_2$, Ωca and Ωar) was calculated from pH$_{NBS}$, temperature, salinity (38‰) and alkalinity (A_T = 2.5 mM; Rivaro *et al.*, 2010) using CO2SYS (Lewis and Wallace, 1998) with dissociation constants from the study by Mehrbach et al. (1973) refitted by Dickson & Millero (1987).

Statistical analyses

All statistical analyses were performed using the SAS/STAT software. The normality of data distributions was checked with a Shapiro-Wilk test and the homoscedasticity was tested using the Bartlett test. Linear regressions were used to test for relationships between studied parameters. Differences between pH treatments were tested using ANCOVA, 1- and 2- way ANOVAs. A 5% significance level was applied.

Results

Target pHs were reached in all experiments and were significantly different between the two treatments (Table 1; ANOVA; experiment #1: $F_{1,473}$=27.95, $p<0.0001$; experiments #2-3: $F_{1,135}$= 25.04, $p<0.0001$). This translated into significant differences for all calculated parameters of the carbonate system (Table 1; ANOVA; pCO_2: $F_{1,473}$=, $p<0.0001$; experiments #2-3: $F_{1,135}$=, $p<0.0001$; Ωca: $F_{1,473}$=, $p<0.0001$; experiments #2-3: $F_{1,135}$=, $p<0.0001$; Ωar: $F_{1,473}$=, $p<0.0001$; experiments #2-3: $F_{1,135}$=, $p<0.0001$).

Experiment #1

Mussel growth was negligible over the 50 weeks exposure period (Figure 1A; GLM: $F_{1,593}$=6.33, p=0.012) and there was no significant difference in total length between the two pH treatments (ANOVA: $F_{1,593}$=1.39, p=0.24). On the other hand, there was a significant difference between pH treatments for the condition index (Figure 1B; ANCOVA: model, $F_{1,593}$=9.56, $p<0.0001$, pH, F_1=11.52, p=0.0007). At pH 8.0, condition index was significantly increasing by 0.07 % week^{-1}

(GLM: $F_{1,290}$=57.29, p<0.0001) while it was significantly decreasing by 0.03 % week^{-1} at pH 7.5 (GLM: $F_{1,289}$=11.12, p=0.01).

Experiment #2

Maximum Breaking Load (BL) of the shell of mussel was significantly impacted by acclimation pH (Figure 2; ANOVA: $F_{1,79}$=30.51, p<0.0001). BL was decreased by 65% when mussels were acclimated at pH 7.5 as compared to pH 8.1.

Searching time (s) of the predator crab was highly variable between individuals (ANOVA 2: model, $F_{1,89}$=2.74, p=0.0005; ind(treat), F_{28}=2.76, p=0.0005) and was not significantly impacted by the acclimation pH of the mussel prey (Figure 3A; F_1=2.20, p=0.14). On the other hand, despite high and significant differences between tested individuals (ANOVA 2: model, $F_{1,89}$=2.72, p=0.0005; ind(treat), F_{28}=2.39, p=0.00024), the handling time was reduced by 27% when mussels were acclimated to pH 7.5 as compared to pH 8.0 (F_1=11.97, p=0.001).

Experiment #3

Acclimation pH of mussels had a significant impact of the crab assimilation efficiency (ANOVA: $F_{1,89}$=19.15, p<0.0001). When fed with mussels acclimated at pH 7.5, the crab assimilation efficiency decreased by 30% as compared to crab fed with mussels from the pH 8.0 treatment. Crab growth rate (gr day^{-1}; Figure 4B) was 5 times higher when fed with mussels acclimated at pH 8.0 as compared to pH 7.5 (ANOVA: $F_{1,9}$=5.82, p=0.044).

Discussion

Our experiments were designed to assess the direct impact of ocean acidification on the mussel *B. pharaonis* as well as the indirect consequences for both the mussel prey and its predator while interacting (functional consequences).

Direct impact on the prey

In the first experiment, the condition index of mussel acclimated to pH 8.0 was increasing with time. A decrease in condition index with time was observed in mussels exposed to pH 7.5. At the end of the 50 weeks exposure, the condition index of mussels kept at pH 8.0 was two times higher as compared to mussels maintained at pH 7.5. This significant difference in condition index was not associated with differences in growth.

The condition index is traditionally used by shellfishery researchers to quantify meat quality and yield in cultured bivalve mollusks (e.g. Widdows & Johnson, 1988). The condition index can be affected by multiple abiotic and biotic factors, among which reproductive status is one of the most important. As a consequence, it is used as a nonspecific bio-indicator to assess exposure to stressors, pollutants or assess spatial-temporal variability (e.g. Rebelo, Amaral & Pfeiffer, 2005). The observed decrease in condition index when exposed to decreased pH is consistent with Beesley et al. (2008) showing a reduction in health index in mussel exposed to ocean acidification. However, a 13 weeks exposure to ocean acidification did not translate into any impact on condition index in *Mytilus edulis* and *Arctica islandica* (Hiebenthal et al., 2013), highlighting the species and experimental condition specificity of response to decreased pH.

The observed decrease in condition index can be interpreted as a decrease in meat quality due to change in energy allocation. A wide range of physiological responses has been documented in mussels exposed to decreased pH; from a strong decrease suggesting metabolic depression (e.g.

Michaelidis et al., 2005) to unchanged or even increased oxygen consumption (e.g. Thomsen & Melzner, 2010). These differences can be explained by several factors including local adaptation to different pH envelopes as well as different tested scenarios. However, these physiological responses can all be attributed to compensated or uncompensated intracellular and extracellular acidosis and/or increased calcification costs. These lead to changes in energy allocation with potential consequences for mussel conditions (Melzner et al., 2009). Changes in energy allocation as well as perturbation of calcification/dissolution processes can also translate into modification of mussel shell properties. Several studies showed that exposure to decreased pH led to alteration of the shell crystalline structure and structural integrity (Asplund et al., 2014; Fitzer et al., 2014a,b). These structural changes are often hypothesized to translate into reduced protection from predators (e.g. Fizter et al., 2014a,b). Indeed, some other studies demonstrated that ocean acidification can also modify mussel shells mechanical properties (but see Hiebental et al. 2013). When exposed to decreased pH, mussel outer shell (calcite) is more brittle while the inner shell (aragonite) is softer and less stiff as compared with shells from high pH (Fitzer et al., 2015). These results are consistent with our observation that the shells from decreased pH treatment were more fragile with a 65% reduction in the maximum breaking load. A similar decrease in maximum breaking resistance of mussel shells by ~20% was observed in mussels (*Mytilus edulis*) acclimated to pH 7.4 for 10 weeks as compared to pH 8.1 (Appelhans et al., 2012).

Indirect impact on the prey

To test the potential functional consequences of these alterations in mechanical properties, we quantified the crab predator searching and handling time required to find and consume mussels exposed to different pHs for >4 weeks. As predicted, there was no significant difference in searching time but handling time was significantly decreased when mussels were acclimated to pH 7.5 as compared to pH 8.0. This somewhat contrasts with the conclusions of Appelhans et al. (2012) and Keppel, Scrosati & Courtenay (2015). Testing the impact of a small pH decrease (pH 7.9 vs 8.1) for 10 weeks on both the prey and the predator, Keppel, Scrosati & Courtenay (2015) showed a negative effect on growth of the seastar *Asterias rubens* but a positive effect in the mussel *Mytilus edulis.* This also led to a 50% decrease in the predation of sea stars on mussels, measured as per-capita consumption rate. Appelhans et al. (2012) obtained similar results while studying the impact of stronger acidification (pH 7.4 vs pH 8.1) on the relationship between two predators (the sea star *A. rubens* and the crab *Carcinus maenas*) and their mussel prey. Feeding was reduced by 56% in sea stars and by 41% in mussels. These effects were attributed mostly to the direct negative effect of pH on the predator and authors concluded that mussels may be subjected to a reduced or unaffected predation pressure under future conditions. All these results were obtained on predator-prey interactions with both partners acclimated to decreased pH and it is then difficult to distinguish between direct and indirect effects of ocean acidification on the predator-prey relationship. Our results suggests that the negative effect of decreased pH on the prey can translate into positive effect for the predator (decreased handling time due to decreased maximum breaking force required to break the shell). Using a similar design (untreated predator vs. treated prey), Appelhans et al. (2012) showed that the sea star predator ate more efficiently when preys were exposed to decreased pH. However, the functional consequences on the predator were not evaluated.

Indirect impact on the predator

Direct impact of ocean acidification on a prey can have a range of indirect consequences for the predator, an aspect that has received little attention in the ocean acidification literature. For example, ocean acidification has the potential to modulate the chemical ecology side of the predator–prey interactions. In mollusks, all but one study (Landes & Zimmer, 2012) showed some effects of decreased pH on predator–prey chemical interaction including decreased induced defense (Bibby et al., 2007), decreased avoidance or escape response (Manriquez et al., 2014; Watson et al., 2014), increased activity (Bibby et al. 2007; Manriquez et al. 2013) and modulation of byssus production rate (Li et al. 2015).

Exposure to ocean acidification can also impact prey's palatability (Dupont et al. 2014) or quality as food source with consequences for higher trophic levels. For example, exposure of phytoplankton to decreased pH can translate into decreased nutritional values with negative consequences for their grazers (Rossol et al., 2012; Schoo et al., 2013). The functional consequences for higher trophic levels were not investigated so far. Our experiment was designed to specifically test the impact of altered prey quality while exposed to decreased pH on its predator (animal-animal trophic relationship). We showed that crabs fed with mussels acclimated to ocean acidification had a 5 times slower growth rate and 30% decreased assimilation efficiency growth rate as compared with crabs fed with mussels raised at higher pH. As recently demonstrated by Sarà *et al.* (2013), a deficiency in the assimilation efficiency due to alterations in the quality of the diet is a major bottleneck in the energy balance of the organisms. They showed that only 7% of AE reduction is sufficient to significantly lower the magnitude of life history traits such as fecundity.

Conclusions

Our results highlight the importance to consider both direct and indirect effects of ocean acidification across trophic levels. We identified several key aspects of the prey-predator relationship that are sensitive to ocean acidification including prey's protection against predator and prey's nutritional quality. Acclimation of the prey to decreased pH can have indirect positive (decreased prey handling time) and negative effects (decreased growth rate) on the predator. To have a holistic understanding of the impact of ocean acidification on marine ecosystems and develop the needed predictive power for management, future experiments should be designed to focus on the different aspects of the species relationships and the underlying mechanisms.

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Figure 1. Relationship between Total Length (A, in mm) and Condition Index (B) and time in mussels exposed to two pHs (pH 8.0 and 7.5) over a 50 weeks period.

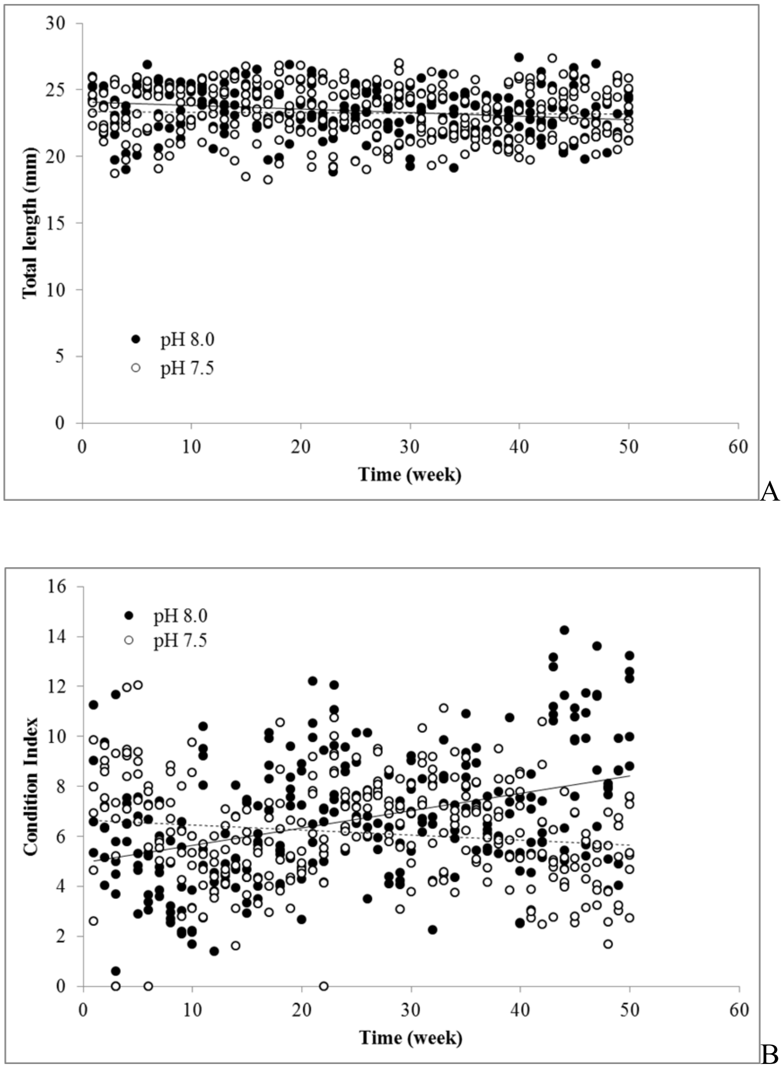

Figure 2 – Maximum breaking load (in N) of shells from mussels exposed to two pHs (pH 8.0 and 7.5) over a 50 weeks period.

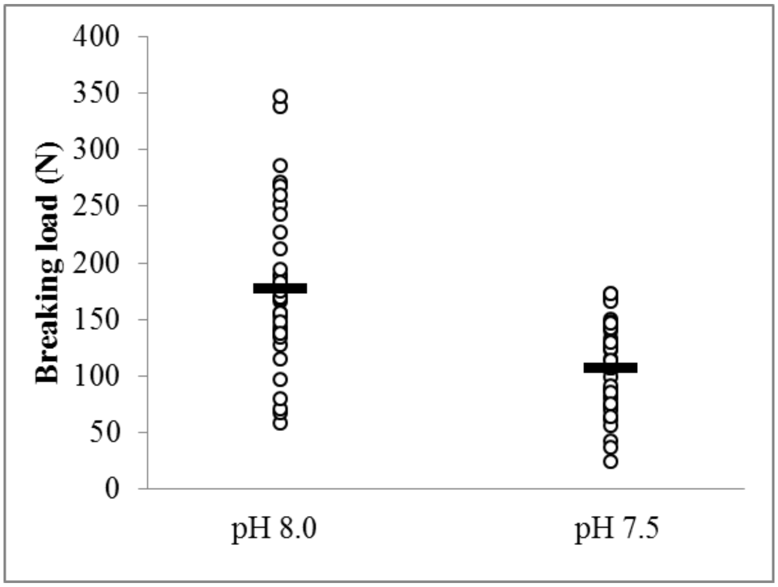

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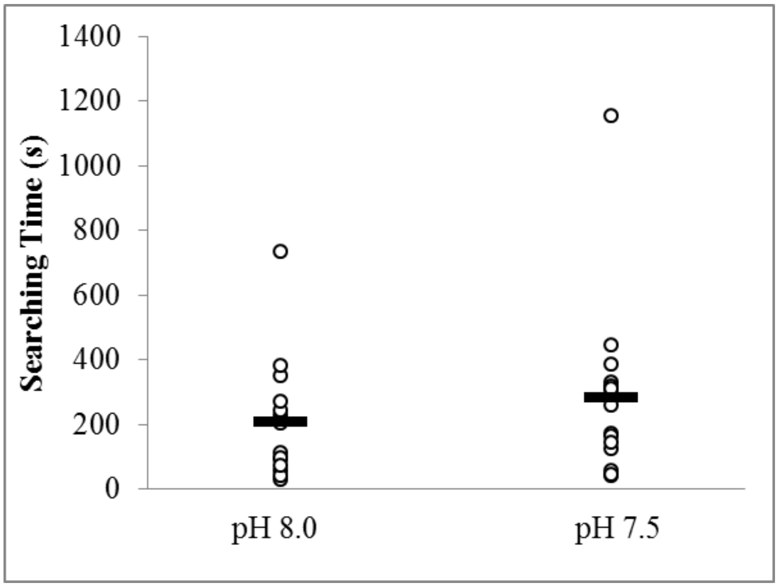
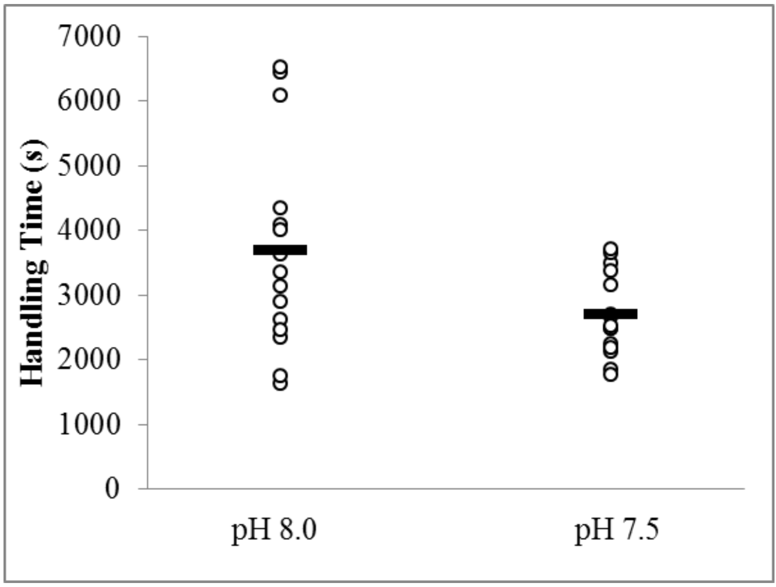
Figure 3 – Searching (A, in s) and Handling Time (B, in s) of crabs searching and eating mussels acclimated to two different pHs (pH 8.0 and 7.5) for 4 weeks.



A



B

Figure 4 – Assimilation efficiency (A) and growth rate (B, in gr day^{-1}) in crab fed with mussels acclimated at two different pHs (pH 8.0 and 7.5).

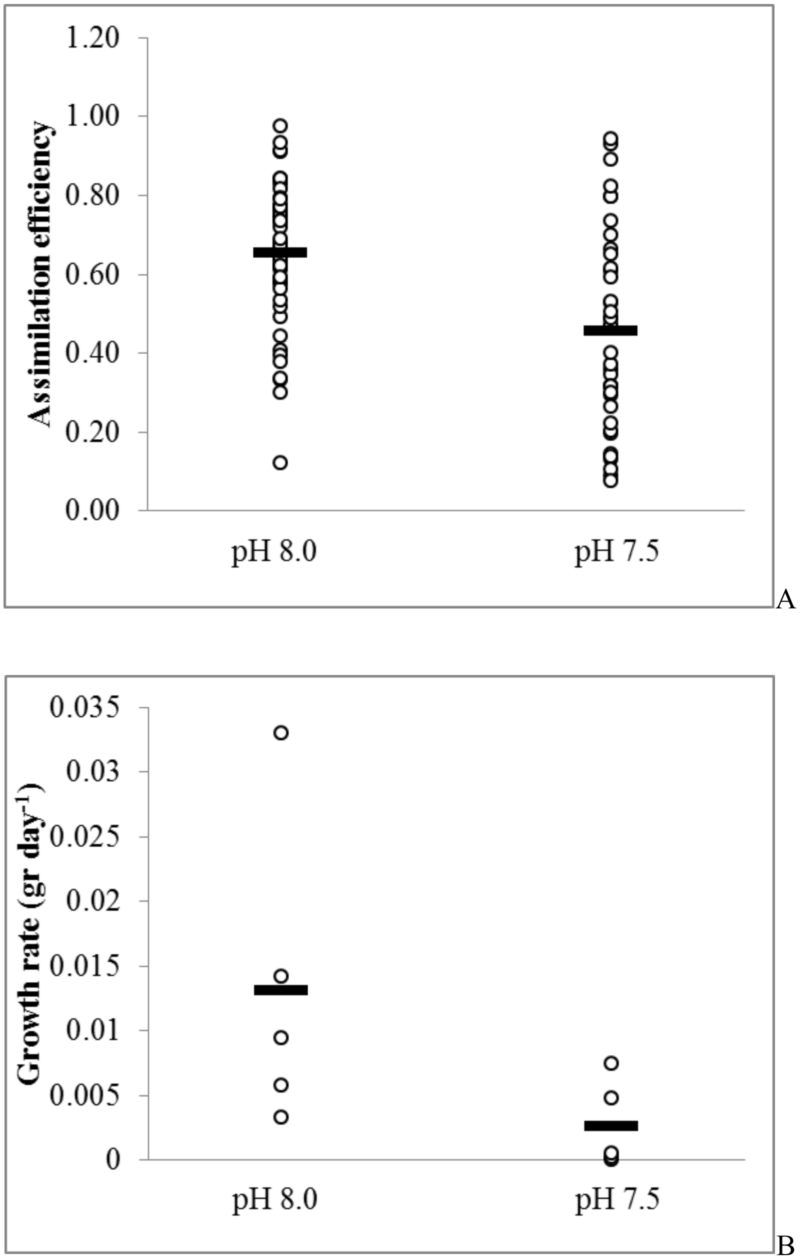


Table 1. Seawater carbonate chemistry parameters presented as mean±SEM. Seawater pH on the NBS scale (pH_{NBS}) and temperature (T; °C) were used to calculate CO_2 partial pressure (pCO_2; µatm) as well as aragonite and calcite saturation states (respectively Ωa and Ωc), for a salinity of 38 and a total alkalinity of 2500 mmol kg^{-1}.

	Measured		Calculated		
	pH_{NBS}	Temperature (ºC)	pCO_2 (µatm)	Ωca	Ωar
Experiment #1					
pH 8.0	8.01±0.01	20.48±0.26	663±9	3.80±0.05	2.48±0.03
pH 7.5	7.56±0.01	22.46±0.27	2131±26	1.60±0.02	1.05±0.02
Experiments #2-3					
pH 8.0	7.99±0.01	19.29±0.25	676±10	3.54±0.03	2.30±0.03
pH 7.5	7.46±0.01	21.05±0.24	2640±30	1.22±0.02	0.80±0.01

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