Note

Ontogenetic variability in the feeding behavior of a marine amphipod in response to ocean acidification

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ABSTRACT

Global stressors like ocean acidification (OA) are expected to influence the quality or palatability of primary producers like algae. Such changes can trigger a response on algal consumers' feeding strategies, and this response may not necessarily be the same for the consumers during the ontogeny. We used a mesocosm's system to expose algae to current and projected OA conditions (390 and 1000 ppm, respectively) and then compared the feeding behavior and absorption efficiency of juvenile and adult stages of the amphipod *Orchestoidea tuberculata*. Specifically, we measured consumption rates (with and without a choice) and absorption efficiency on algae exposed and not exposed to OA. Our results show that OA affect the amphipod's consumption and feeding preferences, and that these effects were related with the analyzed ontogenetic stage (juveniles versus adults). These results support the existence of an ontogenetic change in the response of this species and others similar marine invertebrates to OA, which highlight the need to incorporate different life stages in the study of OA or others global stressors.

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1. Introduction

Near a third of all the CO_2 produced by human activities during the last two centuries has been absorbed by the ocean (Sabine et al., 2004). This has triggered a chemical process called ocean acidification (OA), a global stressor with widespread consequences for a myriad of calcifying organisms (Cooley and Doney, 2009; Nienhuis et al., 2010). Less known but equally important are the adverse effects of OA on marine non-calcifying organisms like algae (Mohite and Wakte, 2011; Jiang et al., 2010). In these organisms the OA alters their photosynthetic rates, nutritive value, and metabolic processes (Xu et al., 2010; Gutow et al., 2014; Duarte et al., 2016). These changes may affect algal quality or palatability (Poore et al., 2013; Falkenberg et al., 2013; Gutow et al., 2014) and alga-herbivore interactions (O'Connor, 2009; Duarte et al., 2016). In fact, few studies have already shown that algal changes caused by OA can alter herbivore's feeding strategies (Poore et al., 2013; Falkenberg et al., 2013; Duarte et al., 2016).

While a growing number of studies has focused on the effects of OA on different species, lesser attention has been given to evaluate the intraspecific variability (among different populations or ontogenetic stages within of a same species) in the responses to OA. This is surprising considering that wide intra-specific variation should be expected from OA, and therefore, its study is critical to understand the complex role of this stressor (Tomanek, 2010; Byrne, 2011; Kremp et al., 2012; Duarte et al., 2016). Marine invertebrate herbivores, for example, have a broad repertoire of feeding strategies to adjust for changes in the algae they consume (Cruz-Rivera and Hay, 2003; Duarte et al., 2011). These organisms optimize their diet by choosing the most nutritive alga (Pennings et al., 1993; Barile et al., 2004; Duarte et al., 2010, 2014), or by increasing the consumption rate (compensatory feeding; Cruz-Rivera and Hay, 2001) or the absorption efficiency (Simpson and Simpson, 1990) of lower quality algae. Talitrid amphipods are abundant and widespread consumers, likely ideal to study such strategies and their potential alteration by stressors like OA. For instance, juvenile and adult individuals of *Talorchestia capensis* differ in consumption rates (Muir, 1977), whereas juvenile and adult of *Talitrus saltator* exhibit distinct energetic requirements (Scapini et al., 1992). We argue that changes in food quality resulting from OA should prompt a response

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in feeding behavior that may not necessarily be the same across different ontogenetic stages.

The amphipod Orchestoidea tuberculata has an ubiquitous presence along the south-east Pacific shorelines and is an avid consumer of the brown alga Durvillaea antarctica (Duarte et al., 2010, 2011). A previous study has reported that OA reduces the quality of D. antarctica and affected the feeding behavior of juvenile individuals of O. tuberculata (Duarte et al., 2016). However, we ignore if the changes induced on the algae would prompt a similar response on adult individuals O. tuberculata. Hence, in this study we replicated a previous study (Duarte et al., 2016) using a mesocosm system for simulating OA (Torres et al., 2013; Navarro et al., 2013) to offer algae exposed to current and 2100-projected pCO_2 levels to adult individuals of O. tuberculata (Meinshausen et al., 2011). Our aim was to assess the effects of this stressor on amphipod's preference, consumption, and absorption efficiency, by conducting a direct comparison between juvenile and adult individuals of this species, a novel approximation in this kind of studies.

2. Materials and methods

Adult and juvenile individuals of O. tuberculata (hereafter amphipods) were manually collected during the summer of 2013 from Calfuco beach, South-central Chile (ca. 39°S) and held in laboratory conditions following Duarte et al. (2011). Fresh seaweed (Durvillaea antarctica) were collected from adjacent rocky shores and acclimated to laboratory conditions for 3 d in a 200 L tank containing filtered (5 μm) seawater. The tank was under 120 μmol photons $m^{-2} s^{-1}$, illuminated by white fluorescent lamps with a 12:12 h photoperiod at 15 °C (see Duarte et al., 2016 for details).

2.1. Seawater acidification system and measurements

Several disks with 20 mm diameter (~4 g) were extracted from the algal fronds and maintained in 4 L seawater plastic containers bubbled with either 390 ppm or 1000 ppm pCO_2 concentrations for 10 d (n = 8 per treatment). The pCO_2 concentrations were achieved by using a laboratory-based mesocosms designed to evaluate OA effects on marine organisms (Torres et al., 2013). The 390 ppm reflected current conditions whereas 1000 ppm reflected projected pCO_2 for the end of the century (Caldeira and Wickett, 2003; Feely et al., 2004; Meinshausen et al., 2011). To obtain 390 ppm, pure atmospheric air was bubbled into the containers. For 1000 ppm, we blended dry air with pure CO_2 to the target concentration using mass flow controllers (MFCs, www. aalborg.com) for air and CO_2 and bubbled it into the containers (Navarro et al., 2013). Dry and clean air was generated by compressing atmospheric air (117 psi) using an oil-free air compressor.

During the trials, total alkalinity (TA; Haraldsson et al., 1997) was measured every 3 d (n = 3) whereas pH, temperature and salinity were monitored daily. pH was measured in a closed 25-mL cell thermostatically controlled at 25.0 °C using a Metrohm 713 pH meter (input resistance > 1013 Ohm, 0.1 mV sensitivity, and nominal resolution 0.001 pH units) and a glass combined double junction Ag/AgCl electrode (Metrohm model 6.0219.100) calibrated with 8.089 Tris buffer (DOE, 1994) 25 °C. pH is reported on the total hydrogen ion scale (DOE, 1994). TA, pH, phosphates and dissolved silicates (Strickland and Parsons, 1968) were used to calculate the rest of the carbonate system parameters and the saturation stage of Omega, Aragonite and Calcite using CO2SYS software (Lewis and Wallace, 1998). These were set with Mehrbach solubility constants (Mehrbach et al., 1973) refitted by Dickson and Millero (1987).

2.2. Consumption with choice (food preference) and without choice, and absorption efficiency

Algal disks exposed to 390 and 1000 ppm CO_2 were offered simultaneously to groups of 4 amphipods per container (5 × 5 cm plastic bottles

with perforated lids for airflow; n = 5-10 for adults and juveniles). To prevent potential errors, identification of each algal disk in each replicate was achieved by tagging and anchoring the disks to a place previously labeled in the container using small wood matches. Trials run for 24 h at 15 °C at constant humidity and with a 12:12 photoperiod (Duarte et al., 2016). For estimation of consumption without choice, similar experiments were run by offering similar algal disks but separately (n = 5-10 for adults and juveniles). Each experimental container was matched with a reference container containing only alga that was used to estimate change in weight in the absence of amphipod consumption (Roa, 1992). At the beginning and end of the experiments, individual algal disks were weighed (± 0.0001 g) to determine mass changes. The changes in algal mass unrelated to consumption were calculated as follows (Roa, 1992; Silva et al., 2004):

$$\text{Consumption rate} = (E_{initial} - E_{final}) - (C_{initial} - C_{final})$$

where E and C denote Experimental and Control algal weights, respectively. For all the measurements conducted, rates of consumption were standardized as amount of biomass per individual amphipod (either adult or juvenile) or mg individual^{-1} d^{-1}.

Absorption efficiency was derived from the relationship between organic and inorganic matter of ingested food and fecal material (Conover, 1966):

$$AE = [(F\prime - E\prime)/(1 - E\prime) F\prime] * 100$$

where AE = Absorption efficiency (%), F′ = Proportion of organic matter in the food and E′ = Proportion of organic matter in the feces. Absorption efficiency was estimated in two parallel experiments conducted separately for juveniles and adults. To collect fecal pellets, groups of 3 amphipods (either juveniles or adults) were kept 4 d in plastic bottles with algal disks exposed to either 390 or 1000 ppm CO_2 (n = 5-10 for adults and juveniles). Feces were collected every 12 h and frozen until analysis. Simultaneously, algal disks were removed and replaced with fresh fragments. Feces were dried at 60 °C for 48 h, weighed and then burned in a muffle furnace at 450 °C for 3 h and weighed again to determine the organic and inorganic content. The same methodology was used to determine the percent organic matter in the algae exposed to the different pCO_2 levels.

2.3. Data analysis

Differences in consumption rates with choice, consumption rates without choice and absorption rates, were assessed separately using two-way ANOVAs. These analyses assessed the contribution of the ontogenetic life stage (juvenile versus adult), the pCO_2 level (390 versus 1000 ppm) and the interaction between both variables. When the interaction term was significant, additional one-way ANOVAs were conducted to compare each explanatory variable separately (Underwood, 1997). ANOVA assumptions were checked in each analysis using Shapiro-Wilk tests (normality) and Levene tests (equal variance).

3. Results

Amphipod consumption rates with and without a choice of algae (Fig. 1a,b) were significantly associated to life stage and pCO_2 level. However, their significant interaction prevented a direct assessment of each explanatory variable (two-way ANOVA p ≤ 0.011 in all comparisons; Table 1). With regards to life stage, adult amphipods consumed near ten times more algae than juveniles, both when they had a choice (Fig. 1a) or had no choice of algae (Fig. 1b). In both cases, these differences were significant (Two-way ANOVA p ≤ 0.001; Table 1). With regards to pCO_2 levels, when amphipods had the chance to choose between the two algal types, both juveniles and adults consumed as least twice as much algae maintained at current pCO_2 levels
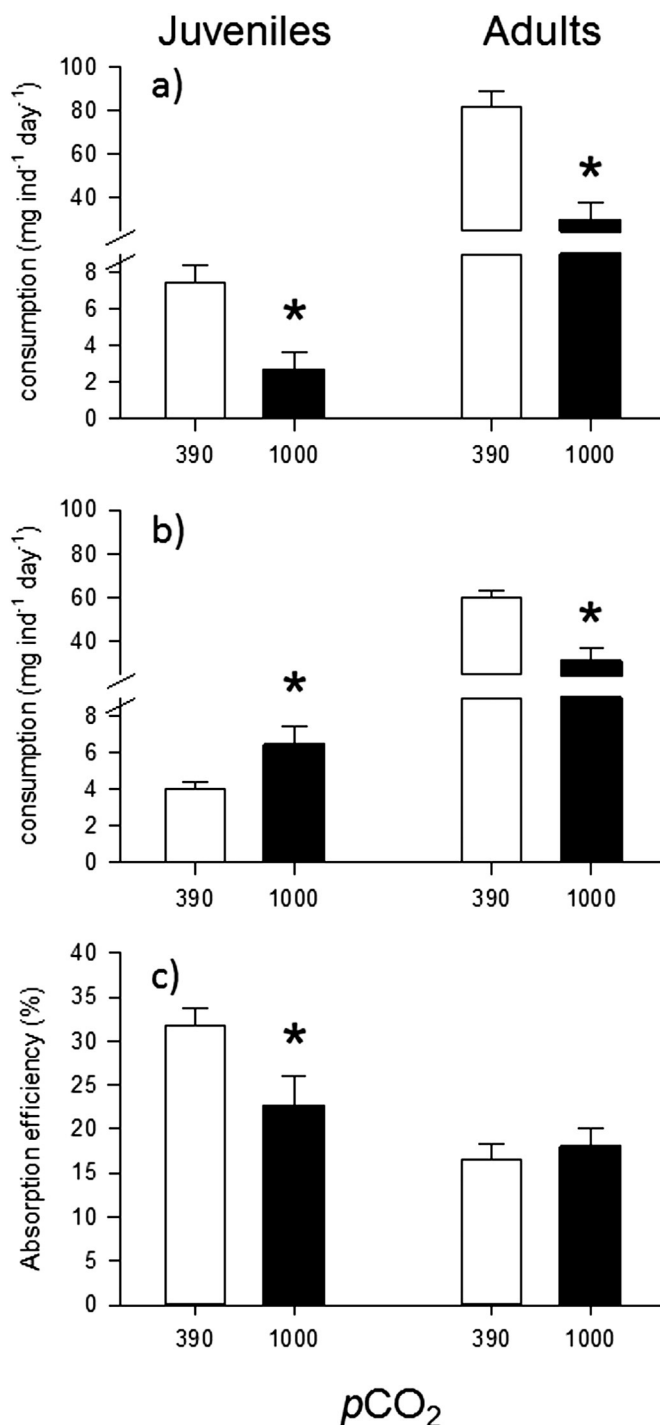
Fig. 1. Mean (± SE) rates of consumption when amphipods had a choice (a) and when they did not have a choice (b) and absorption efficiency between seaweeds maintained at 390 and 1000 ppm (open and filled bars, respectively). Asterisks identify one-way ANOVA significant differences between CO_2 treatments for juveniles and adults separately (see text).

Table 1

Summary of two-way ANOVA analyses assessing the influence of life stage (juvenile vs adult) and CO_2 levels (390 vs 1000 ppm) on the three response variables identified. With the exception of absorption, data was sqrt-transformed to meet ANOVA assumptions. Given that significant interaction terms were detected in all the analyses, separate one-way ANOVAs were run to assess the influence of stage and CO_2 on the each response variable (see Fig. 1). Significant p-values are highlighted in bold.

Response variable	Source of variation	DF	MS	p
Consumption with choice	Stage	1	181.213	<**0.001**
	CO_2 level	1	46.138	<**0.001**
	Stage × CO_2	1	8.201	**0.011**
	Error	26	1.099	
Consumption (no choice)	Stage	1	4.074	**0.003**
	CO_2 level	1	97.198	<**0.001**
	Stage × CO_2	1	9.846	<**0.001**
	Error	17	0.348	
Absorption efficiency	Stage	1	530.898	<**0.001**
	CO_2 level	1	83.468	0.135
	Stage × CO_2	1	154.053	**0.048**
	Error	18	34.077	

ANOVA p < 0.001, Table 1), but life stage interacted significantly with pCO_2 level (two-way ANOVA $p = 0.048$; Table 1) preventing a direct assessment of each variable. Separate comparisons showed that absorption efficiency of juveniles was significantly higher on algae maintained at current pCO_2 levels (390 ppm; one-way ANOVA $p = 0.041$; Fig. 1c). Meanwhile, adults exhibited no differences in absorption efficiency in relation to pCO_2 levels (one-way ANOVA p = 0.619; Fig. 1c).

4. Discussion

Our results show that OA is an important stressor for the amphipod *O. tuberculata*, and that the response of this species is more complex than anticipated. In fact, the effects of OA on the feeding behavior and absorption efficiency of the amphipod varies significantly across the ontogeny (i.e. juveniles or adults). This highlights the need for more studies focusing on intra-specific ontogenetic variation in response to this and other environmental stressors, an aspect that has not yet received enough attention.

In a previous study (Duarte et al., 2016) we conducted nutrient analyses and determined that OA (exposure to 1000 ppm) reduced significantly the nutritive quality of *D. antarctica* (i.e. amount of proteins and organic matter) in comparison to current conditions (390 ppm). Such a change likely dictated the preference of experimental amphipods for algae exposed to current pCO_2 conditions over those at 1000 ppm. Although consumption rates were 10 times higher in adults, when given a choice both life stages showed the same level of preference for the alga with the best nutritive quality. These results are in line with previous studies on herbivore preferences (e.g. Duffy and Hay, 1991; Barile et al., 2004; Lastra et al., 2008; Duarte et al., 2010), in which species like the amphipods *Gammarus mucronatus* and *Elasmopus levis*, for example, sought primarily food items with the highest nutritive value (Cruz-Rivera and Hay, 2000).

Our estimates of algal consumption without algal choice were interesting because they made evident a contrast in the response of juveniles and adults individuals of *O. tuberculata* to OA. While juvenile individuals consumed significantly more algae exposed to OA (1000 ppm), adults consumed significantly less. This type of response in juveniles has been attributed to "compensatory behavior" (Duarte et al., 2016) in which an herbivore consumes large amounts of algae of inferior nutritive value (e.g. lower proteins content, Duarte et al., 2010) to compensate for the limited amount of nutrients (e.g. Stachowicz and Hay, 1996; Cruz-Rivera and Hay, 2001). Unlike juveniles, adults remained consistent and consumed significantly more of the best quality alga (390 ppm) both when they had or not the chance to choose between algae. It is likely that adults did not use compensatory feeding simply because they did not need it in order to fulfill their nutritive

(390 ppm) than alga maintained at 1000 ppm (one-way ANOVA $p = 0.020$ in both comparisons; Fig. 1a,b). In the experiments in which amphipods did not have the possibility to choose between the two algal types, juveniles consumed significantly more algae exposed to 1000 ppm (one-way ANOVA $p = 0.020$; Fig. 1b) whereas the exact opposite was observed in adults, which significantly consumed higher amounts of algae maintained at 390 ppm (one-way ANOVA $p = 0.020$).

With regards to amphipod absorption efficiency, juveniles showed a significantly higher absorption efficiency than adults (Fig. 1c; two-way

requirements. If that hypothesis is correct, it would also explain why adults did not exhibit differences in absorption between algal types. That latter result again contrasted what was observed in juveniles, which showed significantly higher absorption efficiency on algal tissues exposed to 390 ppm, which we had previously determined to have the best nutritive quality (see Duarte et al., 2016).

Whether related to the presence of an stressor or not, consumption and absorption efficiency on a same food source can change among species and among populations of the same species (Slansky, 1993; Bowen et al., 1995; Barbehenn et al., 2004). Indeed, prior studies on adults of *O. tuberculata* have showed considerable variation in consumption and absorption rates on a same type of alga, with individuals opting or not to use compensatory feeding (Duarte et al., 2010, 2011, 2014). Although the lack of compensatory feeding in adults was not necessarily unexpected, we still lack clear explanations for the distinct feeding strategies showed by the two life stages. Some authors assume that differences between juveniles and adults are always linked to a tradeoff between energy demands associated with growth (both stages) or reproduction (adult stages) (Contreras et al., 2003; Pavia et al., 1999; Scapini et al., 1992; Jormalainen et al., 2001a, 2001b). Stressors like OA can alter tradeoffs like this, adding considerable complexity to consumer feeding strategies. Therefore, further studies on the response of different life stages to OA are a priority, particularly in Talitrid species like *O. tuberculata*. Given their dominance in terms of abundance and biomass in many coastal areas, their response to OA may have ramifications to entire coastal communities.

Acknowledgements

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