

Copepod performance under CO₂-induced acidification: the case of *Acartia grani* and *Oithona davisae*

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Abstract

This experimental study aimed to evaluate the direct impact of two distinct pH levels, one “Control” (pH_{NBS}: 8.17) and one “Low pH” (pH_{NBS}: 7.75) on the feeding, respiration and reproductive output of two marine copepods: the calanoid *Acartia grani* and the cyclopoid *Oithona davisae*. Adult copepods collected from laboratory cultures were preconditioned for 4 consecutive days at a dinoflagellate suspension (*Akashiwo sanguinea*) prepared with filtered sea water pre-adjusted at the targeted pH values via CO₂ bubbling. Water acidification had no direct effect on the examined vital rates for any of the two copepod species. Our results lend support to the generally observed lack of direct influence of the seawater pH decrease projected at the end of the century on the group of copepods.

Keywords: marine copepods, ocean acidification, reproduction, feeding, respiration

1. Introduction

Climate-driven changes in the marine environment have been largely associated with the anthropogenic rising of the atmospheric carbon dioxide partial pressure (pCO₂), projected to increase from the present levels of ~400 ppm to values of 700-1000 ppm depending on the considered emission scenario (IPCC, 2007). Elevated pCO₂ in the atmosphere have been driving, in addition to the global warming, a concurrent change in the seawater carbonate chemistry and an associated pH drop (of 0.3-0.4 units by the year 2100, IPCC, 2007) widely known as ocean acidification (OA).

Copepods, a major group of secondary producers in the planktonic food web, have been expected to be relatively tolerant in the direct CO₂ effect mainly due to the lack of calcium carbonate formations in their exoskeleton. This view has been largely supported by a series of manipulative laboratory experiments testing copepod response even under severe acidification regimes, related with deep ocean CO₂ sequestration strategies (e.g. Kurihara et al., 2004; Mayor et al., 2007; Zhang et al., 2011). The high resilience of the group has been also verified when aspects of copepod performance were tested at CO₂ levels relevant to forecasts for the end of the century (e.g. Zhang et al. 2011; McConville et al., 2013). In contrast to this overall robustness, distinct physiological responses have been reported for certain copepod species at near future pCO₂ concentrations (e.g. Fitzer et al., 2012; Vehmaa et al., 2012; Engström-Öst et al., 2014; Cripps et al., 2014). Remarkably, some late works also reported reduced survival on the early stage progeny of a couple of species (Lewis et al., 2013; Cripps et al., 2014) or even their adults (Lewis et al., 2013). These findings seem to challenge the general copepod resilience under the direct near future OA impact, but whether or not such response variation lies on a species-dependent sensitivity or others factor are also involved is not yet clear. Here, we studied the direct impact of OA on marine copepods through a metabolic approach. We assessed the impact of pCO₂/pH levels on several vital rates (feeding, respiration, egg production and hatching success) of two representative marine copepod species, the calanoid *Acartia grani* and the cyclopoid *Oithona davisae*, under a climate relevant scenario (end of century).

2. Materials and methods

Recently matured individuals of the species *A. grani* and *O. davisae* were collected from laboratory cultures. Feeding, respiration and reproduction (egg production and hatching success) of

females of both species were assessed under two pH levels (Control: 8.17, Low pH: 7.75 at NBS scale) after a 4-day preconditioning at the selected conditions. During the acclimation period and the main experiments, copepods were offered a food suspension (50 cells mL^{-1} of the dinoflagellate *Akashiwo sanguinea*) prepared by diluting an exponentially grown algal culture (grown at $18 \pm 1^\circ\text{C}$, 18:6h L:D cycle) with $0.1 \mu\text{m}$ filtered sea water (FSW) adjusted at the targeted pH values. pH adjustment of the FSW was performed via pure CO_2 injections through a solenoid valve controlled automatically by Aqua-medic™ pH computers and probes. For the acclimation, groups of 40 females and 10 males of *A. grani* (to ensure fertilization) were pipetted into 2300 mL Pyrex screw-cap and groups of 300 females and 100 males of *O. davisae* in 625 mL Pyrex screw-cap bottles (2 bottles per pH treatment). The copepods were daily sieved out and placed in fresh food suspension (discarding dead animals if present).

For the assessment of feeding and egg production rates, four replicated groups of 10 *A. grani* and 40 *O. davisae* females were incubated in 625 mL Pyrex screw-cap bottles and 75 mL plastic cell-culture flasks, respectively (the number of incubated animals assured no stress conditions). In both cases other five replicates with food suspension served as initial (1) and controls (4) for each species. Initial samples were immediately fixed in acetic Lugol' s solution (2 %), and the rest were incubated for 24 h at 18°C under light cycle identical to the dinoflagellate growth. At the end of incubation, control and copepod bottles were either carefully poured through a $200 \mu\text{m}$ mesh to collect females (for *A. grani*) or entirely fixed in Lugol (for *O. davisae*). In the case of *Acartia*, 30 mL of the bottle contents were sampled first for the microscopic estimation of algal concentration, while the rest of suspension was subsequently sieved through a $40 \mu\text{m}$ mesh to collect the spawned eggs. A subsample (20%) of eggs was incubated for 72 hours in Petri dishes with FSW of the same pH as maternal incubation, and the hatching rate was calculated. The rest of the eggs for each bottle were preserved and counted under stereoscope to estimate the egg production rate (EPR, eggs female⁻¹ day⁻¹). The percentage of ovigerous *Oithona* females (i.e., carrying egg sacs) (OF) corresponding to the feeding experiments was determined and the clutch size (number of eggs per female) was estimated in 10 arbitrarily selected OF from each bottle. The average population EPR computed for the ensemble of ovigerous and non-ovigerous females was calculated using the egg-ratio method according to Uye & Sano (1995). Hatching success was estimated by incubating individually ovigerous females in 75 mL culture flasks (22 replicates for each pH treatment) and monitoring them for 83 h in distinct time intervals (from 7 to 16 h). Clearance rates, ingestion rates and the average food concentrations in the feeding experiments were computed according to Frost (1972), after microscopic determination of the concentration of food suspensions.

To test the effect of pCO_2/pH on the respiration activity of copepods, ca. 16 females of *A. grani* and ca. 73 females of *O. davisae* were incubated in 18 mL plastic vessels (potentially induced stress cannot be excluded but would be equal between treatments), filled with pCO_2 equilibrated FSW ("Control" and "Low pH") and acclimatized to the experimental temperature (18.1°C). For each experimental condition we used two vessels containing copepods and two additional vessels containing equilibrated FSW without animals that served as controls. All vessels were closed by a silicone cork without trapping air bubbles and placed for ca. 18 h in the darkness in a water bath at the experimental temperature. During the incubation, oxygen concentration measurements of each bottle were taken every minute by optode (4- or 10-channel fiber optic oxygen transmitter OXY-4 Pre-Sens®) probes fitted in the cork. At the end of the incubation, the number and condition of copepods was checked and counted (mortality was negligible). Respiration rates ($\text{nmol O}_2 \text{ cop}^{-1} \text{ day}^{-1}$) were calculated from the difference in oxygen concentration change rate, estimated as the slope of the temporal variation, between the control and experimental bottles, multiplied by the water volume in the experimental bottle and divided by the number of live females. The first two hours of the incubation log were discarded to disregard any effects of handling stress and allow for stabilization of the readings.

3. Results

Average clearance (Control: 34 mL cop⁻¹ day⁻¹, Low pH: 39 mL cop⁻¹ day⁻¹) and ingestion rates (Control: 1213 cells cop⁻¹ day⁻¹, Low pH: 1393 cells cop⁻¹ day⁻¹) of *A. grani* were not statistically different (t-tests, p>0.05) between the two conditions. Difference was neither found in the EPR (Control: 56 eggs cop⁻¹ day⁻¹, Low pH: 58 eggs cop⁻¹ day⁻¹) and hatching success (Control: 95 %, Low: 94 %) of this species between the two treatments (t-test, p>0.05). Similarly to *Acartia*, our results revealed no significant differences (t-tests, p>0.05) in the clearance (Control: 0.32 mL cop⁻¹ day⁻¹, Low pH: 0.29 mL cop⁻¹ day⁻¹) and ingestion rates (Control: 14 cells cop⁻¹ day⁻¹, Low pH: 13 cells cop⁻¹ day⁻¹) of *O. davisae* on a pH basis. Clutch size (Control: 7 eggs cop⁻¹, Low pH: 8 eggs cop⁻¹), EPR (Control: 1.81 eggs cop⁻¹ day⁻¹, Low pH: 1.87 eggs cop⁻¹ day⁻¹) and egg hatching success (Control: 90 %, Low pH: 97 %) of the cyclopoid copepod were also comparable (t-tests, p>0.05) between treatments. Although the average respiration rate of *A. grani* in the “Low pH” treatment seemed higher (65 nmols O₂ cop⁻¹ d⁻¹) compared to the “Control” (35 nmols O₂ cop⁻¹ d⁻¹), this difference was not of statistical significance (t-test, p>0.05). Respiration rates of *O. davisae* were similar (ca. 5 nmols O₂ cop⁻¹ d⁻¹) between the two pH treatments (t-test, p>0.05).

4. Conclusions/Discussion

The impact of increased pCO₂ concentrations on the reproduction of marine copepods has been studied by several experimental works indicating a particular copepod resistance at the acidification conditions projected by the end of the century (e.g. Zhang et al., 2011; McConville et al., 2013) and a negative effect only at extreme pCO₂/pH levels associated to carbon capture and storage leakage processes (>2000 ppm e.g. Zhang et al., 2011; Kurihara et al., 2004; Mayor et al., 2007). However, recent studies have reported a variety of copepod reproductive response under near future acidification scenarios, thus questioning the so far documented resilience of the group. For instance, Cripps et al. (2014) observed that during short-time incubations at 1000 ppm CO₂ the egg production of *Acartia tonsa* was significantly decreased and egg hatching success may be reduced by 10% after parental pre-exposures involving both female and male specimens. Contrarily, a stimulating acidification effect has been reported on the reproductive output of *Acartia bifilosa* (Engström-Öst et al., 2014) but also of *Acartia* sp. (Vehmaa et al., 2012) and the harpacticoid *Tisbe battagliai* (Fitzer et al., 2012). In the present study we found no influence of the tested pH levels in the egg production and hatching success of the calanoid *Acartia grani* and the cyclopoid *Oithona davisae*. Our results confirm the resistance of copepods under near future pCO₂ levels as reported in other cases (e.g. Zhang et al., 2011; McConville et al., 2013) and underline an existing high variability in species sensitivity within the copepod group, even between congeners (Kurihara et al., 2004; Zhang et al., 2011).

Copepod feeding and respiration activity have been barely examined under ocean acidification scenarios (Li & Gao, 2012; Hildebrandt et al., 2014). In our experiments, both *A. grani* and *O. davisae* presented similar feeding and respiration rates at the two experimental treatments. Similarly, Hildebrandt et al. (2014) reported no effect on the respiration of the arctic species *Calanus glacialis* and *Calanus hyperboreus* when exposed at 3000 ppmv of CO₂ for several months. On the other side, *Centropages tenuiremis* has been reported to cope with increased external acidity related with 1000 ppm of CO₂ by increasing its respiration and food acquisition, in order to compensate for the extra energy demand required for the acid-base regulation (Li & Gao, 2012). The ability for such acid-base regulation and the associated energy demand appears to be species-specific. Conclusively, it seems quite likely that our target species may have compensated from a physiological and biochemical point view the imposed OA stress, without facing sufficient energy-budget constraints to affect their vital rates.

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6. References

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