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Effect of carbonate chemistry manipulations on calcification, respiration, and excretion of a Mediterranean pteropod

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Abstract

Although shelled pteropods are expected to be particularly sensitive to ocean acidification, the few available studies have mostly focused on polar species and have not allowed determining which parameter of the carbonate system controls their cal-

- ⁵ cification. Specimens of the temperate Mediterranean species *Creseis acicula* were maintained under seven different conditions of the carbonate chemistry, obtained by manipulating pH and total alkalinity, with the goal to disentangle the effects of the pH and the saturation state with respect to aragonite (Ω_a). Our results tend to show that respiration, excretion as well as rates of net and gross calcification were not directly
- ¹⁰ affected by a decrease in pH but decreased significantly with a decrease in Ω_a . Due to the difficulties in maintaining pteropods in the laboratory and the important variability in their abundances in our study site, long-term acclimation as well as replication of the experiment was not possible. However, we strongly believe that these results represent an important step in the mechanistic understanding of the effect of ocean acidification
- ¹⁵ on pteropods physiology.

1 Introduction

The oceans play a crucial role in the global carbon cycle and store about one quarter of the anthropogenic CO₂ emissions since 1800 (Sabine et al., 2004). By limiting the accumulation of CO₂ in the atmosphere, and therefore climate change, this ocean CO₂uptake has a beneficial environmental effect. However, when CO₂ dissolves in seawater, it forms carbonic acid, and generates a decrease in pH, in the concentration of carbonate ions (CO₃²⁻) and its associated calcium carbonate saturation state (Ω). Since pre-industrial time, surface ocean pH has declined by 0.1 unit (Orr et al., 2005) and, according to model projections, a further decrease of 0.3–0.4 unit is anticipated for the end of the century (Orr, 2011). Most studies have shown a decrease in calcification rates with decreasing pH levels for organisms such as coccolithophorids



(e.g., Riebesell et al., 2000), commercial mollusks (e.g., Gazeau et al., 2007), corals and coralline algae (e.g., Langdon and Atkinson, 2005). However, some recent studies have also brought contradictory results such as no effect or even a positive effect of increasing CO₂ on calcification (e.g., Iglesias-Rodrigez et al., 2008; Ries et al., 2009). Pteropods are widely distributed holoplanktonic mollusks (Lalli and Gilmer, 1989) 5 which play a fundamental role in pelagic ecosystems, being an important food source for various predators such as zooplankton, fishes and birds (e.g., Hunt et al., 2008). The thecosome or "shelled pteropod" species produce an external fragile calcium carbonate shell made of aragonite. They contribute to the majority of the aragonite flux in the ocean that represents at least 12% of the total calcium carbonate flux (Berner 10 and Honjo, 1981). Pteropods are particularly abundant at high latitudes, where aragonite undersaturation is expected in the coming decades (Steinacher et al., 2009), but are also important components of temperate and tropical ecosystems (Bé and Gilmer, 1977). Note that their importance in the Mediterranean pelagic food web is not well doc-

¹⁵ umented. The few studies dealing with the effect of ocean acidification on pteropods have focused on high latitudes species (Orr et al., 2005; Comeau et al., 2009, 2010b; Lischka et al., 2011), and have shown sign of shell dissolution and significant reductions in calcification rates under pCO_2 levels projected for the end of the century.

Only two published studies have focused on the effect of ocean acidification on temperate and tropical pteropods; one was restricted to the larval development of a Mediterranean species (Comeau et al., 2010a), and the second one was limited to the impact of ocean acidification on respiration and excretion rates of five tropical pteropod species (Maas et al., 2012). Moreover, it is, at present, unknown whether the observed effects of ocean acidification on pteropods are due to the variations of pH, Ω

²⁵ and/or another parameter of the carbonate system. There is a strong need, for mechanistic understanding as well as modelling, to perform experiments designed to disentangle the effects of the carbonate system parameters on pteropods, as it has been recently done on corals (Jury et al., 2010). Finally, in addition to calcification, there is also a great need to evaluate the effect of these perturbations on other important



physiological processes such as respiration and excretion (e.g., Maas et al., 2012). Our study aims at providing indications on the effects of various levels of pH and Ω_a on respiration, excretion and calcification rates of the Mediterranean thecosome pteropod *Creseis acicula* and to contribute to the understanding of pteropods physiological response to carbonate chemistry changes.

2 Materials and methods

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Creseis acicula specimens were sampled, using a plankton net, in the Bay of Villefranche-sur-Mer (NW Mediterranean Sea; $43^{\circ}40'$ N, $7^{\circ}18'$ E) at the end of November 2009 and were immediately transported to the *Laboratoire d'Océanographie de Villefranche* (LOV). Organisms were on their adult life stage (length: 1.2 ± 0.2 cm). Half of the sampled organisms (n = 400) were immediately placed in 51 experimental beakers (n = 7) in which they were allowed to acclimate for few hours ($\sim 3h$) before the start of the incubations for respiration, excretion, and net calcification rates measurements. The other half was brought to the Marine Laboratories of the International Atomic Energy Agency (IAEA) for measurement of gross calcification and were also

placed into 7 experimental beakers (5 l) and acclimated for few hours (~ 3 h).

For each experiment (see Fig. 1 for the experimental set-up), four beakers were filled with seawater acidified by bubbling pure CO_2 using a continuous pH-stat system (IKS, Karlsbad), in order to obtain the following pCO_2 levels (Fig. 1, T1 to T4):

- ²⁰ 380 µatm (pH_T = 8.05), 760 µatm (pH_T = 7.80), 1200 µatm (pH_T = 7.65) and 2500 µatm (pH_T = 7.40). In the 3 remaining beakers, total alkalinity (A_T) was decreased by addition of HCI (down to about 800 µmol kg⁻¹) and pH_T was controlled and maintained at values of approximately 7.40, 7.80 and 8.05 (T5, T6 and T7, respectively). The two experiments were performed in temperature-controlled rooms (T = 19 °C). At LOV, af-
- ter the acclimation period, actively swimming pteropods (n = 20) were picked up from respective acclimation beakers, transferred to seven 250 ml BOD bottles (1 bottle per condition) filled with the experimental seawaters and incubated for 20 h. Seven extra



BOD bottles were only filled with the experimental seawaters and served as blanks. Due to the highly variable density of this species in the bay of Villefranche, it has not been possible to collect enough specimens in order to replicate this experiment.

Temperature, pH (calibrated on the total scale, thereafter referred to as pH_T) and 5 A_T were measured daily in the experimental beakers at IAEA and before and after incubation at LOV. Details on the analytical techniques and on the methods used to compute the parameters of the carbonate system are available in the supplementary material.

At LOV, respiration rates (*R*) were estimated based on oxygen uptake during the 20 h incubation in 250 ml BOD bottles. Oxygen (O₂) concentrations were semi-continuously recorded using a fiber-optic O₂ microsensors (PreSens, Planar Oxygen-Sensitive Spot, $\emptyset = 5$ mm). The microsensors were connected to an O₂ meter (OXY-4 mini, PreSens) and calibrated using a two points calibration procedure, in aerated seawater (100% air saturation) and a solution of 0.5% Na₂SO₃ (0% oxygen). O₂ consumption rates were estimated by regressing the O₂ concentration through time and corrected for O₂ consumption rates in blank incubations. O₂ saturation levels never fell below 70% saturation during the incubations.

Ammonium excretion rates (*E*) were estimated as the amount of NH_4^+ released during the incubations. Samples (20 ml) were taken in triplicates before and after incubation for each treatment (including blanks), filtered on 0.2 µm and stored at -20°C pending measurements (within 2 months). NH_4^+ concentrations were measured in triplicates using a classical colorimetric technique (Koroleff, 1983) and a JenWay 6310 (Staffordshire, UK) fluorometer. Excretion rates were corrected for changes in NH_4^+ in the "blank" incubations.

²⁵ Net calcification rates were estimated using an adaptation of the alkalinity anomaly technique (Smith and Key, 1975), taking into account the contribution of NH_4^+ excretion on changes in A_T (1 : 1 molar ratio; Wolf-Gladrow et al., 2007). Net calcification rates



 (g_{net}) were calculated as:

$$g_{\rm net} = \frac{\Delta A_{\rm T} - E}{2}$$

Where ΔA_{T} is the variation of A_{T} during the incubations corrected for changes in A_{T} in the "blank" incubations and *E* is the estimated excretion rate.

In contrast to the alkalinity anomaly technique, which provides an estimate of the balance between precipitation and dissolution of calcium carbonate, the uptake of ⁴⁵Ca provides an estimate of the amount of CaCO₃ precipitated at the edge of the shell and therefore refers to gross calcification. At IAEA, in order to measure gross calcification rates (g_{gross}), 5 L beakers, containing seawater for the different treatments, were spiked with ⁴⁵CaCl₂ (50 Bq ml⁻¹). The 7 beakers were filled with 60 pteropods each that served for both time points 0 and 48 h. Counting of radioactivity were performed on the shells of 10 pteropods sampled in triplicates per treatment and time points. An identical protocol was used under the same conditions on pteropods killed by freezing prior to incubation in order to estimate the non-biological incorporation of ⁴⁵Ca in the shell (Comeau et al., 2009).

Details on the procedure applied to normalize the rates of the different investigated processes to the weight of the incubated organisms as well as details on the statistics used to analyze the obtained dataset can be found in the supplementary material.

3 Results

- ²⁰ During the LOV experiment, pH_T at which organisms were exposed during the incubations varied between 8.02 ± 0.04 in T1 (control) and 7.39 ± 0.02 in T4 (Table 1). $A_{\rm T}$ was successfully decreased close to the targeted value of $800 \,\mu\text{mol}\,\text{kg}^{-1}$, respectively, 804.7 ± 2.0 , 792.3 ± 3.9 and $793.6 \pm 2.7 \,\mu\text{mol}\,\text{kg}^{-1}$. In T1, T2 and T3 seawater was oversaturated with respect to aragonite with mean $\Omega_{\rm a}$ of 3.1 ± 0.1 , 2.2 ± 0.1 and 1.2 ± 0.1 ,
- respectively. The seawater of the 4 other treatments was undersaturated with respect



(1)

to aragonite with mean Ω_a of 0.9 ± 0.0 , 0.3 ± 0.0 , 0.6 ± 0.0 and 0.9 ± 0.0 in T4, T5, T6 and T7, respectively. Very similar values were observed for each treatment during the IAEA experiment (Table 2). pH_T varied between 8.08 ± 0.03 in T7 and 7.42 ± 0.01 in T4. As for the LOV experiment, seawater was oversaturated with respect to aragonite in T1, T0 and T0 ($\Omega_{ab} = 0.0 \pm 0.01$, $\Omega_{ab} = 0.01$, and $\Omega_{ab} = 0.01$, and $\Omega_{ab} = 0.01$, and $\Omega_{ab} = 0.01$.

⁵ T1, T2 and T3 ($\Omega_a = 3.3 \pm 0.1, 2.0 \pm 0.0$ and 1.6 ± 0.1 , respectively) and undersaturated in the other treatments ($\Omega_a = 0.9 \pm 0.0, 0.3 \pm 0.0, 0.6 \pm 0.0$ and 0.9 ± 0.0 in T4, T5, T6 and T7, respectively).

The highest rates of respiration were measured in the control treatment (T1, $A_{\rm T} \sim$ $2563 \mu mol kg^{-1}$, pH_T ~ 8.0; $72.5 \pm 0.3 \mu mol O_2 g DW^{-1} h^{-1}$), and the lowest rates were obtained in T7 ($A_{\rm T} \sim 794 \,\mu \text{mol}\,\text{kg}^{-1}$, pH_T ~ 8.0; 37.4 ± 0.5 μ molO₂ gDW⁻¹ h⁻¹). Linear regression revealed no significant effect of pH_{T} on respiration rates (Student's t-test, p = 0.43, n = 7, Fig. 2a) while a statistically significant linear decline of respiration rates was observed as a function of decreasing Ω_a levels ($R = 33.8 + 11.5 \times \Omega_a$; $r^2 = 0.88$, Student's t-test, n = 7, p = 0.002, Fig. 2b). Ammonium excretion rates varied between 17.1 \pm 3.1 μ mol NH⁺₄ g DW⁻¹ h⁻¹ in T1 (control) and 3.5 \pm 2.2 μ mol NH⁺₄ g DW⁻¹ h⁻¹ 15 in T5 ($A_{\rm T} \sim 805 \,\mu {\rm mol \, kg^{-1}}$, pH_T ~ 7.4). As for respiration, there was no significant relationship between pH_T and excretion rates (Student's *t*-test, p = 0.056, n = 7, Fig. 2c) while a statistically significant linear decline of excretion rates was observed as a function of decreasing Ω_a levels ($E = 3 - 4.2 \times \Omega_a$; $r^2 = 0.86$, Student's *t*-test, p = 0.003, n = 7, Fig. 2d). Positive rates of net calcification (2.4 ± 2.4 and 20 $3.1 \pm 1.6 \mu$ mol CaCO₃ g DW⁻¹ h⁻¹, respectively) were measured in T1 (control) and T2 $(A_T \sim 2633 \mu mol kg^{-1}, pH_T \sim 7.8)$. In the other treatments, negative net calcification rates were measured with the lowest value of $-29.9\pm2.0\,\mu$ mol CaCO₃ g DW⁻¹ h⁻¹ found in T5. As for respiration and excretion rates, pH_T had no significant effect on net calcification rates (Student's *t*-test, p = 0.31, n = 7, Fig. 2e). Net calcification rates were 25 not linearly related to Ω_a , and a saturating hyperbolic function was used to fit the data $(g_{\text{net}} = 57 \times (1 - \exp^{(-\Omega_a/0.6)}) - 53.1; p < 0.01, n = 7, \text{Fig. 2f})$. The release of NH₄⁺ by excretion of the organisms explained between 5.6 and 78.1% of the changes in $A_{\rm T}$



during the incubations. The highest rate of gross calcification was measured in the control treatment (T1; $0.8 \pm 0.1 \,\mu$ molCaCO₃ gDW⁻¹ h⁻¹) and no active CaCO₃ precipitation occurred in T5, T6 and T7 corresponding to Ω_a levels below 1. No significant relationship was found between $g_{\rm gross}$ and pH_T (Fig. 3a). Gross calcification rates were not linearly related to Ω_a , and a logarithmic function was used to fit the data $(g_{\rm gross} = 0.39 \times \ln(\Omega_a) + 0.3; p < 0.01, n = 7, Fig. 3b).$

4 Discussion

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This is the first study on the effects of ocean acidification on physiological rates of an adult Mediterranean pteropod. The respiration of *C. acicula* was not significantly ¹⁰ impacted by pH_T but decreased as a function of decreasing Ω_a . Fabry et al. (2008) mention a 25% decrease of the respiration of the Antarctic pteropod *Limacina helicina antarctica* incubated at 789 µatm as compared to "control" conditions although information on the carbonate chemistry and on the time the organisms have been incubated were not presented. Comeau et al. (2010b) have shown that the respiration rates of *Limacina helicina* were unaffected by a decrease in pH at in situ temperature but in-

- ¹⁵ *Limacina helicina* were unaffected by a decrease in pH at in situ temperature but increased significantly with decreasing pH when placed at a higher temperature (+4°C). Maas et al. (2012) studied the respiration rate of 5 tropical pteropod species, among which four were used to migrate through an Oxygen Minimum Zone (OMZ). These migrating species were unaffected by decreasing pH levels whereas respiration rate of
- ²⁰ the non-migrating species was depressed at lower pH. In the present study, decreases of respiration rates were also found under perturbed conditions, although the observed decrease in respiration rates was better correlated with Ω_a than with seawater pH_T. The mechanisms responsible for the observed decrease in respiration rates with decreasing Ω_a remain unknown but might be linked to changes in acid-base balance, notably due to a disruption of ion transportations (e.g., Portner et al., 2005).

Our study showed that *C. acicula* excretion rates and Ω_a are significantly correlated, whereas pH does not have a significant effect. Reports on the effects of



ocean acidification on excretion rates of marine organisms are very scarce. Michaelidis et al. (2005) showed a significant increase of *Mytilus galloprovincialis* excretion rates at low pH (pH_{NBS}7.3) as compared to control conditions (pH_{NBS}8.05) both in the short (hours) and the long-term (months). Maas et al. (2012) demonstrated that the excretion rate of the non-migrating pteropod (*Diacria quadridentata*) was depressed by lower pH whereas the rates of species crossing the OMZ were unaffected. As for respiration rates, our data partly confirm the findings of Mass et al. (2012), although excretion rates were not found to be directly correlated to pH_T and declined linearly

- with decreasing Ω_a . In the present study, it has been shown that both net and gross calcification rates of *C. acicula* are mainly governed by Ω_a , whereas pH_T does not have a significant effect. Jury et al. (2010) used a similar approach, based on manipulations of the seawater carbonate chemistry, to determine which parameter controls coral calcification. They showed that the calcification rate of *Madracis auretenra* in the light was mainly
- ¹⁵ governed by the bicarbonate ion concentration $[HCO_3^-]$ and not, as expected, by Ω_a . In the present study, $[HCO_3^-]$ is not correlated with any of the physiological processes measured (data not shown). At Ω_a above 1, net calcification rates were very close to 0 and no significant differences could be highlighted with increasing Ω_a levels. The fact that the computation of net calcification rates is based on several measurements
- (see supplementary Material and Methods), the propagation of errors associated with these measurements explains the non-significance of the low net calcification signal measured at Ω_a above 1. Since gross calcification rates increased with increasing Ω_a levels above 1 (see thereafter), it appears that the alkalinity anomaly technique, as used in the present study, does not have the resolution required to estimate net calci-
- fication rates above $\Omega_a = 1$. Due to this low sensitivity above the saturation level, net calcification rates were related to Ω_a levels through a saturating hyperbolic function with no significant effect above $\Omega_a = 1$. Below saturation, net calcification rates were negative and strongly declined with decreasing Ω_a values.



The ⁴⁵Ca uptake experiment also showed that Ω_a rather than pH is a major control of gross calcification. Precipitation of CaCO₃ did not occur at low Ω_a values and gross calcification rates increased with increasing Ω_a levels for Ω_a above 1. Gross calcification rates were 30 % lower at a pCO₂ level close to the ones projected for the end of the century (880 µatm, T2) than in the control. Although this ⁴⁵Ca uptake technique ap-5 peared accurate enough to estimate gross calcification rates for this pteropod species, it must be stressed that, in contrast to the alkalinity anomaly technique, it does not allow an estimation of dissolution rates. The lack of CaCO₃ precipitation in T7 as compared to T4, corresponding to the same concentration of CO_3^{2-} but a lower concentration of HCO_3^- suggests that, even though HCO_3^- is not the driving parameter, it plays a role 10 in calcification. Indeed, $[HCO_3^-]$ was ~ 4 times higher in T4 than in T7, suggesting that HCO_3^- can be used as a secondary carbon source for calcification under low CO_3^{2-} conditions. During the IAEA experiment as well as in a previous study (Comeau et al., 2010b), the significant incorporation of ⁴⁵Ca in slightly undersaturated waters demon-

- ¹⁵ strates that pteropods are still able to precipitate calcium carbonate below the aragonite saturation level. Nevertheless, following net calcification rates estimated during the LOV experiment, the clear dissolution signal measured at $\Omega_a \sim 0.9$, demonstrates that these regulating capabilities are overtaken by dissolution and do not enable to reach positive net calcification rates.
- ²⁰ In the present study, both g_{net} and g_{gross} of *C. acicula* appeared mainly governed by Ω_a , whereas pH and [HCO₃⁻] (result not shown) did not have a clear direct effect. Similar results have been found on the Pacific oyster *Crassostreas gigas* in which larval developmental success and growth rates were not affected by the pH or [HCO₃⁻], whereas they were correlated to [CO₃²⁻] and its associated Ω_a (Gazeau et al., 2011). ²⁵ The situation is less clear in zooxanthellate scleractinian corals in which numerous
- studies have brought contradictory results (for an extensive review see Allemand et al., 2010).



Previous studies on the Arctic pteropod *L. helicina* have shown similar relationship between gross calcification and Ω_a . However, the present low Ω_a recorded in this region (e.g., Yamamoto-Kawai et al., 2009) suggests a higher exposure of high latitude pteropods to declining Ω_a (Comeau et al., 2011). Orr et al. (2005) projected that the surface water of the Southern Ocean will become undersaturated with respect to aragonite by the year 2050. The situation is even more severe in the Arctic Ocean where model projections indicate an aragonite undersaturation, on an annual average, over 10% of its area by the year 2023 (Steinacher et al., 2009).

The situation is less dramatic in the Mediterranean Sea as, for example, Ω_a values never fall below 2.9 on an annual basis in the surface waters of the bay of Villefranche (NW Mediterranean; data not shown). It results that, in contrast to high-latitude species, Mediterranean species such as *C. acicula* will experience a decrease in saturation state but will not be exposed to aragonite undersaturated conditions in the coming decades (Orr, 2011).

15 5 Conclusions

The strong relations observed between physiological processes and Ω_a , as well as the fact that organisms appear unable to produce a shell under corrosive conditions, suggest that future decrease in Ω_a will impact pteropods populations, particularly high latitudes ones, as well as the ecosystems in which they play a critical role. However,

- it must be stressed that, as *Creseis acicula*, using the current techniques, can only be maintained alive for few days in the laboratory, these experiments were conducted on animals acclimated only for few hours. Similarly Maas et al. (2012), based also their work on animals acclimated for few hours, and stressed out that the observed response of an organisms to hypercapnia in short-term might differ from a longer-term response. More efficient cultivation techniques of these notoriously fragile organisms
- ²⁵ response. More efficient cultivation techniques of these notoriously fragile organisms are necessary in order to perform long-term experiments allowing an assessment of their potential acclimation capacity to low Ω_a conditions. Nevertheless, despite these



limitations, the present study represents an important additional step in our understanding of pteropods physiology under ocean acidification scenarios.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/9/6169/2012/ bgd-9-6169-2012-supplement.pdf.

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Table 1. Carbonate chemistry of the seawater used to measure rates of net calcification, respiration and excretion (LOV experiment). Temperature, pH_T and total alkalinity (A_T , in triplicates) were measured before and after incubation, averaged values (SD) are shown. Dissolved inorganic carbon (C_T) concentration, the partial pressure of CO₂ (pCO_2) and the saturation state of seawater with respect to aragonite and calcite (Ω_a and Ω_c , respectively) were calculated using seacarb (Lavigne and Gattuso 2011) and given as mean (SD).

Treatment	Т	рН _т	A _T	CT	pCO ₂	Ω _a	Ω _c
	(°C)		$(\mu mol kg^{-1})$	$(\mu mol kg^{-1})$	(µatm)		
T1	19.3 (0.2)	8.02 (0.04)	2562.8 (1.9)	2287.1 (14.3)	475 (32)	3.1 (0.1)	4.7 (0.2)
T2	19.6 (0.7)	7.82 (0.03)	2633.4 (1.6)	2456.9 (9.7)	835 (42)	2.2 (0.1)	3.3 (0.1)
Т3	19.3 (0.2)	7.54 (0.05)	2625.9 (1.6)	2566.6 (10.3)	1671 (113)	1.2 (0.1)	1.8 (0.1)
T4	19.3 (0.2)	7.39 (0.02)	2618.0 (2.7)	2615.6 (4.0)	2427 (59)	0.9 (0.0)	1.3 (0.0)
T5	18.9 (0.1)	7.43 (0.01)	804.7 (2.0)	781.0 (1.3)	654 (5)	0.3 (0.0)	0.4 (0.0)
T6	19.3 (0.1)	7.81 (0.05)	792.3 (3.9)	700.2 (6.1)	240 (17)	0.6 (0.0)	0.9 (0.0)
T7	18.8 (0.0)	8.02 (0.05)	793.6 (2.7)	651.6 (9.0)	134 (12)	0.9 (0.0)	1.3 (0.1)



Table 2. Carbonate chemistry of the seawater used to measure rates of ⁴⁵Ca incorporation (IAEA experiment). Temperature (*T*) and pH_T were measured on 4 occasions during the 48 h incubations while total alkalinity (*A*_T) was measured in triplicates at the start of the incubations; averaged values (SD) are shown. Dissolved inorganic carbon (*C*_T) concentration, the partial pressure of CO₂ (*p*CO₂) and the saturation state of seawater with respect to aragonite and calcite (Ω_a and Ω_c , respectively) were calculated using seacarb and are given as mean (SD).

Treatment	Т	рН _т	A _T	CT	pCO ₂	Ω _a	Ω _c
	(°C)		$(\mu mol kg^{-1})$	(µmolkg ⁻¹)	(µatm)		
T1	19.2 (0.2)	8.06 (0.04)	2587.8 (0.3)	2284.5 (14.3)	426 (27)	3.3 (0.1)	5.1 (0.2)
T2	19.6 (0.3)	7.79 (0.01)	2582.2 (2.0)	2423.2 (3.1)	876 (13)	2.0 (0.0)	3.0 (0.0)
Т3	19.5 (0.1)	7.68 (0.05)	2576.8 (4.2)	2463.4 (12.2)	1164 (85)	1.6 (0.1)	2.4 (0.1)
T4	19.2 (0.2)	7.42 (0.01)	2572.3 (1.4)	2558.1 (2.3)	2202 (31)	0.9 (0.0)	1.4 (0.0)
T5	19.5 (0.1)	7.44 (0.01)	761.8 (3.1)	735.5 (2.0)	606 (9)	0.3 (0.0)	0.4 (0.0)
T6	19.5 (0.2)	7.82 (0.02)	774.6 (1.5)	681.1 (2.7)	229 (7)	0.6 (0.0)	0.9 (0.0)
T7	19.5 (0.3)	8.08 (0.03)	764.5 (0.8)	605.5 (5.2)	108 (6)	0.9 (0.0)	1.4 (0.0)





Fig. 1. Experimental set-up used ($T = 19^{\circ}$ C, S = 38). For each treatment the target A_T (total alkalinity in µmol kg⁻¹), pH_T and Ω_a (saturation state of the seawater with respect to aragonite) are indicated. pH_T was controlled by a pH-stat that bubbles pure CO₂ and by continuous bubbling of CO₂-free air in the beakers. A_T was decreased in T5, T6 and T7 by HCl addition (see text for details).





Fig. 2. Respiration **(a,b)**, excretion **(c,d)** and net calcification rates **(e,f)** of *C. acicula* as a function of pH_T and of the aragonite saturation state (Ω_a) . The dashed lines represent the 95% confidence interval of the linear and non-linear regressions, ns meaning not statistically significant (p > 0.05). The error bars are SD, except in panels **a** and **b** (respiration rates) where the vertical error bars (not visible) represent the standard error of the linear fitted model (see Materials and Methods).





Fig. 3. Rate of gross calcification based on ⁴⁵Ca uptake as a function of pH_T (a) and the aragonite saturation state (Ω_a ; (b). The dashed lines represent the 95% confident interval of the non-linear regression, ns meaning not statistically significant (p > 0.05). The error bars are SD.

