Marine Environmental Research 175 (2022) 105586



Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev





Long-term effects of contrasting pCO_2 levels on the scope for growth in the carnivorous gastropod *Concholepas concholepas*

J.M. Navarro [a,b,*], P.V. Andrade-Villagrán [c], P.H. Manríquez [d,e], C. Duarte [f], O.R. Chaparro [a]

[a] Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile
[b] Centro FONDAP de Investigación de Ecosistemas Marinos de Altas Latitudes (IDEAL), Valdivia, Chile
[c] Facultad de Ciencias, Universidad Católica de la Santísima Concepción. Concepción, Chile
[d] Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Avenida Ossandón 877, Coquimbo, Chile
[e] Laboratorio de Ecología y Conducta de la Ontogenia Temprana (LECOT), Coquimbo, Chile
[f] Departamento de Ecología y Biodiversidad, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello, Santiago, Chile

ARTICLE INFO

Keywords:
Scope for growth
Carnivorous snail
pCO_2
Exposure time

ABSTRACT

We evaluated the effect of contrasting pCO_2 levels: lower (390 μatm), moderate (700 μatm) and extreme (1000 μatm), on the scope for growth of the keystone snail *Concholepas concholepas* over an exposure period of 6 months. Juvenile snails were collected from rocky intertidal habitats and acclimated for 5 months to those pCO_2 levels. Subsequently, three groups of snails were randomly taken (n = 7 for each treatment) and reared for an additional 1 month for each of the three pCO_2 levels. Physiological traits related with energy gain and energy expenditure were quantified. The scope for growth index decreased significantly with increases in pCO_2, yielding negative values throughout the experimental period for the snails exposed to 1000 μatm pCO_2, probably due to the extra energy required to maintain their metabolic functions in balance. This suggests that future climate change scenarios with elevated pCO_2 levels could threaten the growth and other basic functions of juvenile snails of this species.

1. Introduction

The increased absorption of atmospheric CO_2 by the ocean since the industrial revolution has induced changes in the water chemistry, and a decrease in ocean pH and carbonate content (Feely et al., 2004). At present, pCO_2 concentrations in the ocean has generated a pH decrease of 0.1 units since the preindustrial age, and according to the IPCC reports (AR5), the projected decrease in open ocean surface pH until the end of the century is in the range of 0.06–0.07 for RCP2.6 and 0.3 to 0.32 for RCP8.5 (IPCC et al., 2014).

Existing evidence suggests that high environmental pCO_2 levels can produce hypercapnia, which can cause alterations in marine organisms at the physiological, morphological, and behavioral level. Hypercapnia occurs at extra or intracellular levels (Pörtner, 2004), and can impact growth, development, and survival of marine organisms (Kurihara, 2008; Widdicombe and Spicer, 2008). Bivalve species exposed to high pCO_2 levels have showed deleterious responses, such as decreases in feeding and oxygen uptake rates, that have occurred with increases of pCO_2 concentrations (Liu and He, 2012; Navarro et al., 2013; Manríquez et al., 2016; Peng et al., 2017; Meseck et al., 2020). Shirayama and Thornton (2005) found that a moderate increase in pCO_2 (200 μatm) caused a reduction in the growth rate of gastropods and echinoderm species. Navarro et al. (2013) found that high pCO_2 levels (1200 μatm) predicted for the next century have a negative impact on the physiological energetics of juvenile of the bivalve *Mytilus chilensis*, by reducing feeding, absorption, metabolic rate, and ultimately, available energy for growth. On the other hand, a decrease in carbonate content that can affect shell characteristics of different bivalves exposed to high pCO_2 levels has been described (Kurihara, 2008; Dickinson et al., 2012). In juveniles *M. chilensis*, the calcium deposition rate and total weight were negatively affected by high pCO_2 levels (Duarte et al., 2014). In addition, behavioral changes in the feeding strategies of organisms exposed to high pCO_2 levels have also been described in the larvae of the gastropod *Concholepas concholepas*, where increases in pCO_2 caused a change in food selection. Here, nanoflagellates and cyanobacteria were preferentially consumed instead of diatoms and dinoflagellates (Vargas et al., 2013). Furthermore, the exposure to high pCO_2 levels, could also affect the reproductive processes of marine organisms, such as gonadal

* Corresponding author. Instituto de Ciencias Marinas y Limnológicas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.
E-mail address: jnavarro@uach.cl (J.M. Navarro).

https://doi.org/10.1016/j.marenvres.2022.105586
Received 17 September 2021; Received in revised form 8 February 2022; Accepted 8 February 2022
Available online 10 February 2022
0141-1136/© 2022 Elsevier Ltd. All rights reserved.

development, fecundity and hatching processes (Mayor et al., 2007; Siikavuopio et al., 2007; Kurihara, 2008; Manríquez et al., 2014a).

The effects of increases in coastal pCO_2 have been described as species-specific, depending on the environmental characteristics to which the organisms are naturally exposed (Ries et al., 2009; Clements and Hunts, 2015; Vargas et al., 2017; Navarro et al., 2020). Organisms inhabiting fluctuating environments (i.e., estuaries, intertidal and upwelling zones), have been described as tolerant to ocean acidification and other environmental factors, such as temperature, when compared with those organisms inhabiting more stable environments (i.e., open ocean) (Thomsen et al., 2010; Duarte et al., 2015; Vargas et al., 2017; Navarro et al., 2020).

In scenarios with imminent changes in the chemistry of the ocean driven by the incorporation of CO_2 in the oceans, it is important to investigate how marine organisms that play key ecological and economical roles will respond. In Chile, the muricid gastropod *C. concholepas* (hereafter snail), is a keystone predator species (Castilla et al., 1979), and represents a fishery resource with a high economic importance (Leiva and Castilla, 2002). This snail is a carnivorous species that inhabits the inter and subtidal zone at Pacific South Oriental, from southern Perú to south of Chile (Stuardo, 1979). So far, existing evidence suggests that small juvenile snails display several behavioral, physiological and morphological responses when exposed to elevated temperature and pCO_2 levels. Elevated pCO_2 levels can disrupt the snails' predator perception but does not affect prey recognition (Manríquez et al., 2014a, 2014b). Additionally, regardless of whether the individuals are from northern or southern populations, elevated pCO_2 levels increase the standard metabolic rate of this snail species (Lardies et al., 2014). Based on this, physiological, morphological and behavioral responses of this snail can be modulated by changes in temperature (Manríquez et al., 2016, 2020; Domenici et al., 2017).

Since physiological, behavioural and morphological fitness traits are related to energy provisioning (Sokolova et al., 2012), the potential effects of high pCO_2 levels on the physiological energetics of *C. concholepas* might have cascading consequences on these traits. We hypothesize that energetic demands associated with keeping the homeostasis under a long-term exposure to high pCO_2 levels will threaten the physiological fitness of this gastropod. The objective of the present study was to determine the physiological energetics of *C. concholepas* to long-term (6 months) exposure to current and predicted scenarios of pCO_2 levels. For this purpose, an analysis of ingestion, absorption, oxygen uptake, and ammonia excretion were carried out in snails from southern Chile. The physiological index scope for growth was then derived to describe the integrated response of the whole organism to contrasting pCO_2 levels.

2. Materials and methods

2.1. Animal maintenance and experimental design

Juvenile snails were collected from rocky intertidal habitats of southern Chile at Calfuco (39° 44′S, 73° 38′W), and then transported to the Calfuco Coastal Aquatic Resources Laboratory of the Universidad Austral de Chile. Prior to physiological measurements, groups of twelve individuals were acclimated to three different levels of pCO_2 for five months. In three different Plexiglas aquaria (one by pCO_2 level) of 7.5 L capacity. The aquaria were filled with of filtered seawater (0.5 μm) which was supplied from the laboratory piping system (ca. 390 μatm pCO_2 during the time of this experiment) and from a mesocosm system, constantly bubbled with different concentrations of pCO_2 (700 and 1000 μatm), as described by Navarro et al. (2013) and Torres et al. (2013). During the acclimation period, the seawater was changed once a day with fresh pCO_2 treated seawater, which was supplied from two large equilibration tanks. Once a week the aquaria were cleaned, and consumed prey items removed. To maintain the pCO_2 levels in the experimental aquaria for the 390 μatm treatment (control condition) pure atmospheric air was bubbled into the aquaria. For 700 and 1000 μatm pCO_2 levels, dry air was blended with pure CO_2 to each CO_2 concentration using mass flow controllers (MFCs, www.aalborg.com), for air and CO_2. During the acclimation period, water pH and total alkalinity were monitored every 3 days in the 230 L equilibration tanks, where the corresponding treated seawater was produced. pH measurements were done in closed 25 mL cells thermostatically controlled at 25 °C using a Metrohm 713 pH meter, calibrated with 8.089 Tris-buffer at 25 °C. Temperature and salinity were measured in the equilibration tanks using a CTD (Ocean Seven 305 Plus CTD, www.Idronaut.it). Total alkalinity was measured using the method of Haraldsson et al. (1997). The resulting data along with the hydrographic data were used to calculate the rest of the carbonate system parameters and the saturation stage of Omega Aragonite and Calcite using CO_2SYS software (Lewis and Wallace, 1998), which was refitted by Dickson and Millero (1987) and set with Mehrbach solubility constants (Mehrbach et al., 1973).

Following the acclimation period (five months), seven snails between 13 and 23 mm were chosen at random from each acclimation condition (i.e., seven replicates per treatment) and transferred to plastic containers that were kept closed. Then, the snails were exposed for a period of 30 d to the corresponding pCO_2 level (390, 700 and 1000 μatm). Seawater in the experimental containers was changed every day, with pre-treated water supplied by the same mesocosm system. During the experimental period, the seven replicates (one per container) for each pCO_2 condition were immersed in a water bath set to a mean temperature (±SD) of 12 °C (±0.5). The snails were fed *ad libitum* (10 mussels per individual) using juveniles of the mussel *Perumytilus purpuratus*, with sizes ranging from 5 to 8 mm in length. The same procedure of injecting air and CO_2 in the experimental aquaria was used here to maintain the pCO_2 levels in the replicate container. Samples for seawater chemistry were collected during the experimental period from the 230 L equilibration tanks, every 3 days.

2.2. Physiological parameters

2.2.1. Organic ingestion rate (OIR)

Physiological measurements of each experimental snail were performed four times following the acclimation period, starting at the initial day and then every 10 days over the 30 days experiment.

To calculate OIR of the snails, every alternate day the empty valves of the consumed mussels from each container were collected and measured. In some cases, valves of the prey still contained tissue, which was collected and dried at 100 °C for 24 h. To calculate the food ingested, a regression curve between the shell length (L) and the soft tissue dry weight (DW) of 50 individuals (shell length: 4–19 mm) of *P. purpuratus* was used. The mussel tissue not consumed was discounted from the calculated dry weight of the flesh, in order to calculate the daily OIR value. This value was then multiplied by the organic fraction of the food (0.83), which was estimated from the combustion of the soft tissue removed from the 50 mussels, at 450 °C for 3 h.

2.2.2. Absorption Efficiency (AE) and Absorption Rate (AR)

The AE of the snails was estimated by determining the organic and inorganic matter contained in the food and feces (Conover, 1966). For each replicate snail, AE was estimated every ten days on pooled samples of feces collected every two days. Feces were filtered through pre-weighed glass fiber filters of 25 mm (Whatman® GF/C), washed with ammonium formate, dried to a constant weight at 100 °C, weighed, and then burned at 450 °C for 3 h, and weighed again to estimate the organic and inorganic fractions of the feces. The AR was calculated as the product of the organic ingestion rate and absorption efficiency.

2.2.3. Ammonia excretion (VNH_4–N)

Ammonia excretion was measured every ten days on individual snails from each replicate aquarium and incubated for 2 h in 140 mL sealed glass chambers, containing filtered (0.45 μm) seawater. Afterwards, they were immersed in a thermo regulated bath at a constant

temperature (12 °C). The volume of the experimental snails was discounted from the total volume of the chamber for the final calculation. For each treatment, one additional chamber without animals was used as the control. All chambers were filled with corresponding experimental pCO_2 seawater levels of 390, 700, and 1000 μatm. Following the incubation period, water samples (5 ml) were taken from each chamber and analyzed with the colorimetric method of Solórzano (1969).

2.2.4. Oxygen uptake (VO_2)

The oxygen uptake was determined using a Fiber Optic Oxygen Transmitter (FIBOX 3, PreSens) through oxygen sensor spots (PreSens GmbH, Regensburg, Germany) attached to the inner wall of glass chambers, hermetically sealed. Each snail from the different treatments, was placed individually in a hermetic chamber (140 mL) and the oxygen consumption was measured for 1 h. The volume of the experimental snails was discounted from the total volume of the chamber for the final calculation. For each treatment, one additional chamber without animals was used as the control. All the chambers were filled with filtered (0.45 μm) seawater previously saturated with oxygen at a salinity of 30 psμ, with the corresponding experimental pCO_2 level, and immersed in a thermoregulated bath set at 12 °C (Lauda RE112). The data obtained were recorded using the OxyView 3.51 Software program, preventing oxygen concentration from dropping below 70% saturation.

2.2.5. Scope for Growth (SFG)

This index reflects the relationship between the energy absorbed and the energy used for respiration and excretion. To calculate the SFG, the different physiological traits measured were transformed into energy equivalents, where: 1 ml $O_2 = 19.9$ J; 1 μg NH4–N = 0.0249 J, according to Elliot and Davidson (1975), and 1 mg of organic material from the diet was equivalent to 21 J (McLuski, 1981).

3. Statistical analysis

To evaluate possible differences in physiological traits between the different levels of pCO_2, through time, a 2-way ANOVA with repeated measures was used. The analyses were conducted after testing for normality (Shapiro-Wilk test), homoscedasticity (Levene test), and sphericity (Mauchly Sphericity test). When the assumptions were not met, the data were transformed. Ingestion rate was transformed to logarithm, absorption rate was transformed to square root, and scope for growth was transformed to cube root. All the analyses were performed using Statistic v. 10.0 software. A p-value ≤ 0.05 was considered significant. When necessary, multiple comparisons were included, using the post hoc Tukey test (HDS).

4. Results

4.1. Experimental seawater system

The physical and chemical characteristics of experimental seawater were previously described in a parallel study on the effects of ocean acidification on larval development and early post-hatching snails of *C. concholepas* (Manriquez et al., 2014a).

4.2. Organic ingestion Rate (OIR)

The snail OIR was affected significantly by the interaction between pCO_2 and time (Table 1, Fig. 1). A decrease in the OIR was observed from day 10 to day 30 in all treatments, although it was more evident in the snails exposed to a higher pCO_2 level, decreasing by 56%, while the lowest decrease (36%) was observed in the OIR from snails kept at 390 μatm (Fig. 1). However, there were no differences between treatments (Table 1).

4.3. Absorption Efficiency (AE) and Absorption Rate (AR)

Snail AE was affected significantly by the interaction between exposure time and pCO_2 levels (Table 1; Fig. 2). The lower AE was observed in juveniles exposed to the highest pCO_2 levels (1000 μatm) during the entire experimental time, with a mean of 33%, although with a great temporal variability. Without great variation throughout the

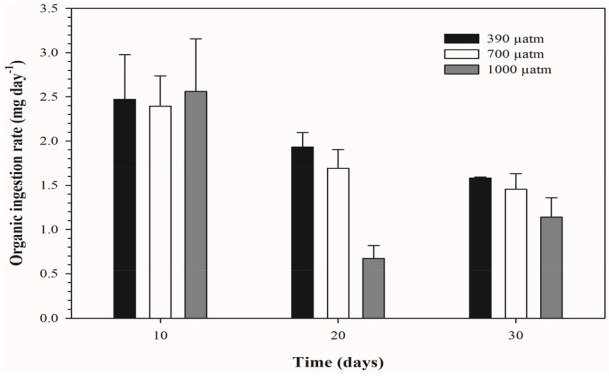


Fig. 1. A) Organic ingestion rate (mean ± se) of juvenile *Concholepas concholepas* in response to the combined effects of exposure time (0, 10, 20, and 30 days after an acclimatation period of 5 months) and pCO_2 levels (390, 700 and 1000 μatm). In each combination, 7 replicate snails were used.

Table 1
Concholepas concholepas. 2-way ANOVA with repeated measures for different physiological traits in juvenile snails exposed to different pCO_2 levels through time.

Physiological trait	Factor	SS	df	MS	F	p value
OIR	pCO_2	0.13781	2	0.0689	2.645	0.0983
	Time	0.4297	2	0.21485	19.226	**0.0000**
	pCO_2 x Time	0.12421	4	0.03105	2.7788	**0.0413**
AE	pCO_2	2.16907	2	1.08453	50.3373	**< 0.0001**
	Time	0.04185	2	0.02092	0.8754	0.4254
	pCO_2 x Time	0.48423	4	0.12106	5.0651	**0.0024**
AR	pCO_2	4.42249	2	2.21124	32.4581	**<0.0000**
	Time	0.9381	2	0.46905	7.5648	**0.0018**
	pCO_2 x Time	0.26445	4	0.06611	1.0663	0.3874
VNH_4–N	pCO_2	13662.1	2	6831	1.2133	0.3203
	Time	36356.8	3	12118.9	7.9149	**0.0002**
	pCO_2 x Time	4011.5	6	668.6	0.4367	0.8511
VO_2	pCO_2	0.18647	2	0.093234	0.076024	0.9271
	Time	0.00000	3	0.00000	0.00000	1.0000
	pCO_2 x Time	14.7188	6	2.453133	3.079247	**0.0115**
SFG	pCO_2	128.0838	2	64.04191	25.41063	**0.0000**
	Time	8.7712	2	4.38562	1.5548	0.2251
	pCO_2 x Time	16.5001	4	4.12503	1.46241	0.2340

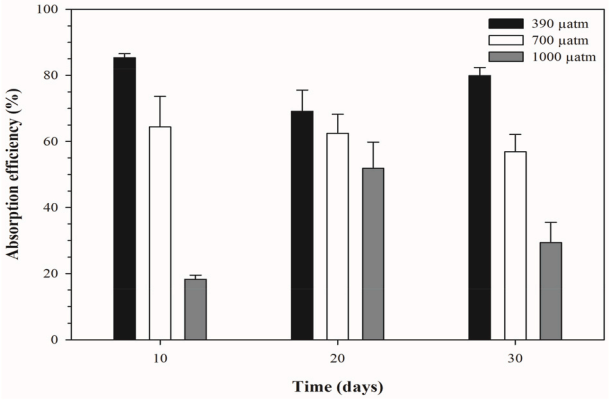


Fig. 2. A) Absorption efficiency (mean ± se) of juvenile *Concholepas concholepas* in response to the combined effects of exposure time (0, 10, 20, and 30 days after an acclimatation period of 5 months) and pCO_2 levels (390, 700 and 1000 µatm). In each combination, 7 replicate snails were used.

experiment, the highest AE occurred in snails exposed to the lowest pCO_2 level (390 µatm), with a mean of 78%.

The snail AR was also affected significantly by pCO_2 levels and exposure time, but not by the interaction of these factors (Table 1; Fig. 3). The AR of snails exposed to the control (390 µatm) was always higher (threefold) than the AR observed in snails exposed to 1000 µatm during all experimental time (Fig. 3). On the other hand, there was a decrease in the AR through the time, in all treatments (Fig. 3).

4.4. Ammonia excretion (VNH_4–N)

The VNH_4–N was affected significantly by time, but not by pCO_2 levels and the interaction of both factors (Table 1; Fig. 4). Across all treatments, a significant decrease in ammonia excretion was observed up to day 20.

4.5. Oxygen Uptake (VO_2)

The VO_2 of snails was affected significantly by the interaction between pCO_2 levels and exposure time (Table 1; Fig. 5). However, it was not affected by pCO_2 neither by time. At the beginning of the experiment (days 0 and 10 after the acclimatation period), the VO_2 of snails exposed to 1000 µatm was slightly higher than those of the control (390 µatm).

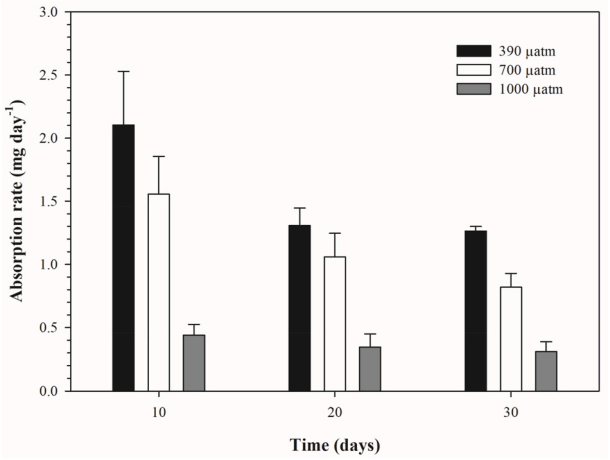


Fig. 3. A) Absorption rate (mean ± se) of juvenile *Concholepas concholepas* in response to the combined effects of exposure time (0, 10, 20, and 30 days after an acclimatation period of 5 months) and pCO_2 levels (390, 700 and 1000 µatm). In each combination, 7 replicate snails were used.

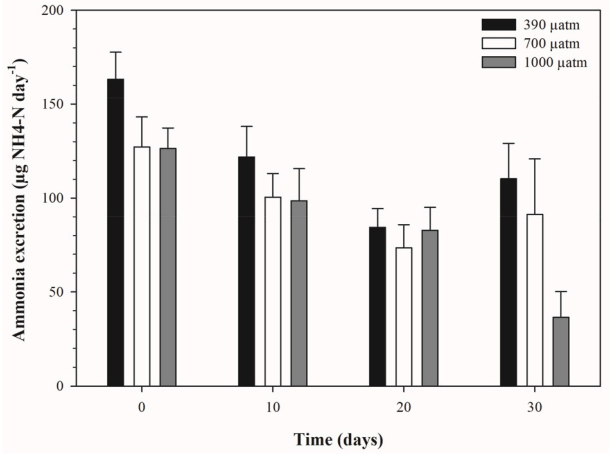


Fig. 4. A) Ammonia excretion (mean ± se) of juvenile *Concholepas concholepas* in response to the combined effects of exposure time (0, 10, 20, and 30 days after an acclimatation period of 5 months) and pCO_2 levels (390, 700 and 1000 µatm). In each combination, 7 replicate snails were used.

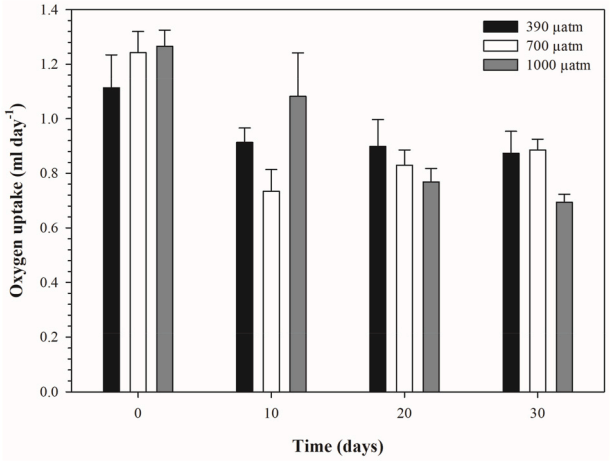


Fig. 5. A) Oxygen uptake rate (mean ± se) of juvenile *Concholepas concholepas* in response to the combined effects of exposure time (0, 10, 20, and 30 days after an acclimatation period of 5 months) and pCO_2 levels (390, 700 and 1000 µatm). In each combination, 7 replicate snails were used.

However, these snails showed a notorious decrease of VO_2 at the end of the experiment (30 days after the acclimatation period).

4.6. Scope for growth (SFG)

High pCO_2 levels reduced significantly the SFG of the juveniles of *C. concholepas*, however, no significant effects were observed for the time and the interaction between both factors (Table 1; Fig. 6). Regardless of the exposure time, the SFG measured in the snails exposed to 1000 µatm was always negative (Fig. 6). At the end of the experimental period, the SFG in the snails maintained at 1000 µatm reached mean values of -11 ± 2.5 J day^{-1}. For the snails exposed to 700 µatm, a significant decrease in the SFG was recorded. By the end of the experimental period the SFG was negative, and not significantly different from values observed in the snails exposed for the same time at 1000 µatm. On the other hand, the snails exposed to control conditions had SFG values that were always positive but decreased with increasing exposure time. The mean SFG for the control juveniles was 12 ± 4.2 J day^{-1}, which was significantly higher than the SFG observed in the snails exposed to the high pCO_2 level (Fig. 6B).

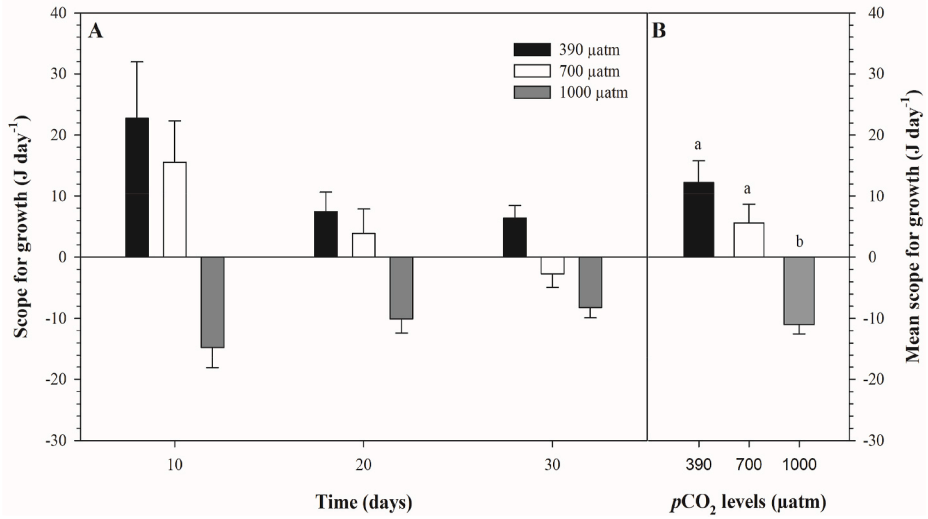


Fig. 6. The successive A) and cumulative B) combined effect of exposure time (0, 10, 20, and 30 days after an acclimation period of 5 months) and pCO_2 levels (390, 700 and 1000 µatm) on the Scope for Growth (mean ± se) of juvenile *Concholepas concholepas*. Different letters above the bars indicate significant differences.

5. Discussion

The long-term of chronic exposition (6 months) of juvenile snails *C. concholepas* to high pCO_2 levels caused alterations on their energy balance, decreasing notoriously the energy available for growth. This energetic debt is reflected in the negative values observed for the SFG of juveniles exposed 1000 µatm of pCO_2. The negative values of SFG corresponded with their low absorption rates throughout the exposition time, and higher oxygen uptake rates that were observed during the first 10 days of exposition to the highest level of pCO_2 after the acclimation period. Our results are in strong agreement with the extra energetic expenditure that calcifier organisms need to incur to maintain different processes in equilibrium i.e., the ionic balance and biomineralization, under acidification conditions (Beniash et al., 2010, Sokolova et al., 2012; Byrne and Fitzer, 2019). This could mean that an increment of total metabolic expenditure yields a redistribution of the energy that is normally allocated for other processes such as growth and reproduction (Wood et al., 2008; Beniash et al., 2010). Significant increases in the metabolism of the juvenile oyster *Crassostrea virginica* exposed to high levels of acidification (3500 µatm pCO_2, pH 7.5, for 20 weeks) has been observed (Beniash et al., 2010). This suggests that an elevated metabolism could be required for the higher energetic expenditure necessary to reach homeostasis (Beniash et al., 2010). The low amount of energy acquired by juvenile *C. concholepas* exposed to high pCO_2levels, reflected in their low OIR and AR, has also been recorded in other marine invertebrates at different development stages (Dupont and Thorndyke, 2009; Appelhans et al., 2012; Navarro et al., 2013). The juvenile clam *Ruditapes decussatus* was shown to decrease its clearance and ingestion rates, after being exposed to extreme pH (7.46) for 87 days (Fernández-Reiriz et al., 2011). Similarly, the mussel *M. chilensis* significantly reduced its clearance, ingestion, and absorption rates after being exposed during 70 days at 1200 µatm pCO_2 (7.57 pH) (Navarro et al., 2013). A different response was observed in other bivalve species, such as juveniles of *Mytilus galloprovincialis*, which presented an increase in AE and body mass, after being exposed to an acidified pH treatment (7.9–7.7) for 78 days (Fernández-Reiriz et al., 2012). It is relevant to emphasize that the exposure time to high pCO_2 in both studies was less than the chronic exposition used in our study for juveniles of *C. concholepas* (6 months). In fact, our results show that the exposure time factor has a significant effect on most of the physiological responses measured, both interacting with pCO_2 level or in a separate way.

Due to logistical issues, we have not measure physiological variables during the acclimatization period. This suggest that the measured physiological responses might represent the consequences of the experimental exposure to the pH levels in the acclimation phase (in grouped snails) along with the exposure to the same condition under individual conditions. Clements and Darrow (2018), also conclude that calcifying invertebrate feeding on elevated pCO_2 appears to be affected by exposure time, where acute acidification (<10 days) had no effect, but longer-term exposure (>10 days) did.

The species-specific response to an acidification scenario can be related with the previous exposure history to an acidified environment. Thus, different responses to high pCO_2 levels have been identified for populations inhabiting different geographic locations, suggesting high phenotypic plasticity of organisms inhabiting locations with high environmental fluctuations (Duarte et al., 2014; Vargas et al., 2017; Navarro et al., 2020).

Our study shows that ammonia excretion in juvenile *C. concholepas* was not significantly affected by an increase in pCO_2 levels. In *M. chilensis*, the exposure to high pCO_2 levels (700–1000 µatm), also did not affect their excretion rate (Navarro et al., 2013). However, other studies have shown that ammonia excretion is stimulated by increased pCO_2, because of changes in N-metabolism for acid-base regulation needs (Langenbuch and Pörtner, 2002; Thomsen and Melzner, 2010; Fernández-Reiriz et al., 2011).

The oxygen uptake in juveniles of *C. concholepas* was significantly affected by the interaction between pCO_2 level and exposure time. At the beginning of the experiment (0 days after the acclimation period), the VO_2 of snails exposed to the higher pCO_2 level was greater than at other pCO_2 levels, however at the end of experiment (30 days after the acclimation period) the individuals exposed to 1000 µatm pCO_2 decreased their oxygen uptake. Because ocean acidification has implications for acid-base regulation and metabolism (Kurihara, 2008; Widdicombe and Spicer, 2008), this reduced oxygen uptake was probably caused by the extra energy that may be required to maintain the internal acid-base balance, which can lead to reduced protein synthesis and a reduction in fitness. Manríquez et al. (2016) also identified a decrease in the oxygen consumption of juvenile *C. concholepas* after being exposed to high pCO_2 levels (1400 µatm), regardless of the rearing temperature (15 and 20 °C).

Several studies have described growth as a physiological process that might be significantly reduced under long-term exposure to elevated pCO_2 levels (Michaelidis et al., 2005; Berge et al., 2006). Shirayama and Thornton (2005) found that a moderate increase (200 µatm) from the control pCO_2 caused a reduction in the growth rate of gastropods and echinoderms. Beniash et al. (2010) found that high pCO_2 levels (pH =

7.5) inhibited both shell and soft body growth in the bivalve *Crassostrea virginica*. The present study showed that the elevated pCO_2 level (1000 μatm), that is predicted for the next century, will have a major negative impact on the SFG. This index that integrates physiological, cellular, and biochemical changes at the whole organism level represents a robust tool for measuring the effects of environmental stress on the fitness of organisms. Therefore, due to their integrative nature we suggest that SFG should be considered when ocean acidification effects on marine organisms are evaluated. Moreover, this study suggests that exposure time is an important variable to be considered in future studies aiming to evaluate the effects of pCO_2 levels on physiological traits of marine organisms. This agrees well with a recent study reporting that exposure time modulates the effects of climate change-related stressors on fertile sporophytes and early-life stage performance of a habitat-forming kelp species (González et al., 2021).

The site from which the experimental individuals were collected at southern Chile is characterized by low variability of pCO_2 (Torres et al., 2013). Furthermore, the present study reports a reduced phenotypic plasticity in terms of how the measured physiological traits (energy intake, SFG) respond to elevated pCO_2 levels. The snail *C. concholepas* is of great social, ecological and economic importance, and our results suggests that the near future climate change scenarios with elevated pCO_2 levels could threaten the growth and impact negatively snails' fitness. Considering the ecological and economic importance of this specie, the negative effects of pCO_2 will have consequences on the whole community and the national economy.

Author statement

Navarro, J.M.: Conceptualization, Writing-Original Draft, Reviewing and Editing, Supervision, Methodology, Formal Analysis, Investigation, Data curation, Funding Acquisition, Project administration. Andrade-Villagran, P.V.: Formal Analysis, Original draft preparation, Data curation, Reviewing and Editing. Manriquez, P.H.: Investigation, Methodology, Data curation, Formal Analysis, Reviewing and Editing, Supervision. Duarte, C.: Methodology, Data curation, Reviewing and Editing. Chaparro, O.R: Reviewing and Editing, Formal, Analyses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to Karin Acuña for her valuable assistance in experimental work and algal production. Maria Elisa Jara is acknowledged for running the mesocosms and rearing the snails during the acclimation phase. The study was mainly funded by the Associative Research Program, PIA ANID-CHILE (Grant Anillos ACT-132 to JMN). Additional support came from Center FONDAP IDEAL 15150003 – ANID; FONDECYT Grant 1181609 to PHM and from FONDECYT 3180316 to PA-V.

References

Appelhans, Y.S., Thomsen, J., Pansch, C., Melzner, F., Wahl, M., 2012. Sour times: seawater acidification effects on growth, feeding behaviour and acid-base status of *Asterias rubens* and. Carcinus maenas. Mar. Ecol. Prog. Ser. 459, 85–97. https://doi.org/10.3354/meps09697.

Beniash, E., Ivanina, A., Lieb, N.S., Kurochkin, I., Sokolova, I.M., 2010. Elevated level of carbon dioxide affects metabolism and shell formation in oysters. Crassostrea virginica. Mar. Ecol. Prog. Ser. 419, 95–108. https://doi.org/10.3354/meps08841.

Berge, J.A., Bjerkeng, B., Pettersen, O., Schaanning, M.T., Oxnevad, S., 2006. Effects of increased seawater concentrations of CO_2 on the growth of the bivalve *Mytilus edulis* L. Chemosphere 62, 681–687. https://doi.org/10.1016/j.chemosphere.2005.04.111.

Byrne, M., Fitzer, S., 2019. The impact of environmental acidification on the microstructure and mechanical integrity of marine invertebrate skeletons. Conserv. Physiol. 7 (1), coz062. https://doi.org/10.1093/conphys/coz062.

Castilla, J.C., Guisado, C., Cancino, J., 1979. Aspectos ecológicos y conductuales relacionados con la alimentación de *Concholepas concholepas* (Mollusca: Gastropoda: Muricidae). Biol. Pesq. 12, 99–114.

Clements, J.C., Hunts, H.L., 2015. Marine animal behaviour in a high CO_2 ocean. Mar. Ecol. Prog. Ser. 536, 259–279. https://doi.org/10.3354/meps11426.

Clements, J.C., Darrow, E.S., 2018. Eating in an acidifying ocean: a quantitative review of elevated CO_2 effects on the feeding rates of calcifying marine invertebrates. Hydrobiologia 820, 1–21. https://doi.org/10.1007/s10750-018-3665-1.

Conover, R.J., 1966. Assimilation of organic matter by zooplankton. Limnol. Oceanogr. 11, 338–345. https://doi.org/10.4319/lo.1966.11.3.0338.

Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Res. Part A 34, 1733–1743. https://doi.org/10.1016/0198-0149(87)90021-5.

Dickinson, G.H., Ivanina, A.V., Matoo, O.B., Pörtner, H.O., Lannig, G., Bock, C., et al., 2012. Interactive effects of salinity and elevated CO_2 levels on juvenile eastern oysters. Crassostrea virginica. J. Exp. Biol. 215, 29–43. https://doi.org/10.1242/jeb.061481.

Domenici, P., Torres, R., Manríquez, P.H., 2017. Effects of elevated carbon dioxide and temperature on locomotion and the repeatability of lateralization in a keystone marine mollusc. J. Exp. Biol. 220, 667–676. https://doi.org/10.1242/jeb.151779.

Duarte, C., Navarro, J.M., Acuña, K., Torres, R., Manríquez, P.H., Lardies, M.A., et al., 2014. Combined effects of temperature and ocean acidification on the juvenile individuals of the mussel *Mytilus chilensis*. J. Sea Res. 85, 308–314. https://doi.org/10.1016/j.seares.2013.06.002.

Duarte, C., Navarro, J.M., Acuña, K., et al., 2015. Intraspecific variability in the response of the edible mussel *Mytilus chilensis* (Hupe) to ocean acidification. Estuar. Coast 38, 590–598. https://doi.org/10.1007/s12237-014-9845-y.

Dupont, S., Thorndyke, M.C., 2009. Impact of CO_2-driven ocean acidification on invertebrates early life-history - what we know, what we need to know and what we can do. Biogeosci. Discuss. 6, 3109–3131. https://doi.org/10.5194/bgd-6-3109-2009.

Elliot, M., Davison, W., 1975. Energy equivalents of oxygen consumption in animal energetics. Oecologia 19, 195–2011. https://doi.org/10.1007/BF00345305.

Feely, R.A., Sabine, C.L., Lee, K., Berelson, W., Kleypas, J., Fabry, V.J., Millero, F.J., 2004. Impact of anthropogenic of CO_2 on the $CaCO_3$ system on the oceans. Science 305 (5682), 362–366. https://doi.org/10.1126/science.1097329.

Fernández-Reiriz, M.J., Range, P., Álvarez-Salgado, X.A., Labarta, U., 2011. Physiological energetics of juvenile clams *Ruditapes decussatus* in a high CO_2 coastal ocean. Mar. Ecol. Prog. Ser. 433, 97–105. https://doi.org/10.3354/meps09062.

Fernández-Reiriz, M.J., Range, P., Álvarez-Salgado, X.A., Espinosa, J., Labarta, U., 2012. Tolerance of juvenile *Mytilus galloprovincialis* to experimental seawater acidification. Mar. Ecol. Prog. Ser. 454, 65–74. https://doi.org/10.3354/meps09660.

González, C.P., Edding, M., Tala, F., Torres, R., Manríquez, P.H., 2021. Exposure time modulates the effects of climate change-related stressors on fertile sporophytes and early-life stage performance of a habitat-forming kelp species. Environ. Pol. 286, 117224. https://doi.org/10.1016/j.envpol.2021.117224.

Haraldsson, C., Anderson, L.G., Hassellöv, M., Hulth, S., Olsson, K., 1997. Rapid, high-precision potentiometric titration of alkalinity in ocean and sediment pore waters. Deep-Sea Res. Part I. 44 (12), 2031–2044. https://doi.org/10.1016/S0967-0637(97)00088-5.

IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team. In: Pachauri, R.K., Meyer, L.A. (Eds.). IPCC, Geneva, Switzerland, p. 151.

Kurihara, H., 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Mar. Ecol. Prog. Ser. 373, 275–284. https://doi.org/10.3354/meps07802.

Langenbuch, M., Pörtner, H.O., 2002. Changes in metabolic rate and N excretion in the marine invertebrate *Sipunculus nudus* under conditions of environmental hypercapnia: identifying effective acid-base variables. J. Exp. Biol. 205 (8), 1153–1160. https://doi.org/10.1242/jeb.205.8.1153.

Lardies, M.A., Arias, M.B., Poupin, M.J., Manríquez, P.H., Torres, R., Vargas, C.A., et al., 2014. Differential response to ocean acidification in physiological traits of *Concholepas concholepas* populations. J. Sea Res. 90, 127–134. https://doi.org/10.1016/j.seares.2014.03.010.

Leiva, G.E., Castilla, J.C., 2002. A review of the world marine gastropod fishery: evolution of catches, management and the Chilean experience. Rev. Fish Biol. Fish. 11, 283–300. https://doi.org/10.1023/A:1021368216294.

Lewis, E., Wallace, D.W.R., 1998. Program Developed for CO2 System Calculations. Carbon Dioxide Information Analysis Center. Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN.

Liu, W., He, M., 2012. Effects of ocean acidification on the metabolic rates of three species of bivalve from southern coast of China. Chin. J. Oceanol. Limnol. 30, 206–211. https://doi.org/10.1007/s00343-012-1067-1.

McLusky, D.S., 1981. The Estuarine Ecosystem, 2° edition. Blackie, Glasgow, pp. 37–52. Chapter 2. Primary producers.

Manríquez, P.H., Mardones, L., Jara, M.E., Navarro, J.M., Lardies, M.A., Torres, R., et al., 2014a. Effects of ocean acidification on larval development and early post-hatching traits in *Concholepas concholepas* (loco). Mar. Ecol. Prog. Ser. 514, 87–103. https://doi.org/10.3354/meps10951.

Manríquez, P.H., Jara, M.E., Mardones, M.L., Torres, R., Navarro, J.M., Lardies, M.A., et al., 2014b. Ocean acidification affects predator avoidance behaviour but not prey

detection in the early ontogeny of a keystone species. Mar. Ecol. Prog. Ser. 502, 157–167. https://doi.org/10.3354/meps10703.

Manríquez, P.H., Jara, M.E., Seguel, M.E., Torres, R., Alarcón, E., Lee, M.R., 2016. Ocean acidification and increased temperature have both positive and negative effects on early ontogenetic traits of a rocky shore keystone predator species. PLoS One 11 (3), e0151920. https://doi.org/10.1371/journal.pone.0151920.

Manríquez, P.H., Jara, M.E., González, C.P., Díaz, M.I., Brokordt, K., Lattuca, M.E., et al., 2020. Combined effect of pCO_2 and temperature levels on the thermal niche in the early benthic ontogeny of a keystone species. Sci. Total Environ. 719, 137239. https://doi.org/10.1016/j.scitotenv.2020.137239.

Mayor, D.J., Matthews, C., Cook, K., Zuur, A.F., Hay, S., 2007. CO_2-induced acidification affects hatching success in. Calanus finmarchicus. Mar. Ecol. Prog. Ser. 350, 91–97. https://doi.org/10.3354/meps07142.

Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.N., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18, 897–907. https://doi.org/10.4319/lo.1973.18.6.0897.

Meseck, S.L., Sennefelder, G., Krisak, M., Wikfors, G.H., 2020. Physiological feeding rates and cilia suppression in blue mussels (*Mytilus edulis*) with increased levels of dissolved carbon dioxide. Ecol. Indicat. 117, 106675. https://doi.org/10.1016/j.ecolind.2020.106675.

Michaelidis, B., Ouzounis, C., Paleras, A., Portner, H.O., 2005. Effects of longterm moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. Mar. Ecol. Prog. Ser. 293, 109–118. https://doi.org/10.3354/meps293109.

Navarro, J.M., Torres, R., Acuña, K., Duarte, C., Manríquez, P.H., Lardies, M., Lagos C, N. A., et al., 2013. Impact of medium-term exposure to elevated pCO_2 levels on the physiological energetics of the mussel *Mytilus chilensis*. Chemosphere 90, 1242–1248. https://doi.org/10.1016/j.chemosphere.2012.09.063.

Navarro, J.M., Villanueva, P., Rocha, N., Torres, R., Chaparro, O.R., et al., 2020. Plastic response of the oyster *Ostrea chilensis* to temperature and pCO_2 within the present natural range of variability. PLoS One 15 (6), e0234994. https://doi.org/10.1371/journal.pone.0234994.

Peng, C., Zhao, X., Liu, S., Shi, W., et al., 2017. Ocean acidification alters the burrowing behaviour, Ca^{2+}/Mg^{2+}-ATPase activity, metabolism, and gene expression of a bivalve species, *Sinonovacula constricta*. Mar. Ecol. Prog. Ser. 575, 107–117. https://doi.org/10.3354/meps12224.

Pörtner, H.O., Langenbuch, M., Reipschläger, A., 2004. Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. J. Oceanogr. 60, 705–718. https://doi.org/10.1007/s10872-004-5763-0.

Ries, J.B., Cohen, A.L., McCorkle, D.C., 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology 37 (12), 1131–1134. https://doi.org/10.1130/G30210A.1.

Shirayama, Y., Thornton, H., 2005. Effect of increased atmospheric CO_2 on shallow water marine benthos. J. Geophys. Res. 110, C09S08. https://doi.org/10.1029/2004JC002618.

Siikavuopio, S.I., Mortensen, A., Dale, T., Foss, A., 2007. Effects of carbon dioxide exposure on feed intake and gonad growth in green sea urchin. Strongylocentrotus droebachiensis. Aquac 266, 97–101. https://doi.org/10.1016/j.aquaculture.2007.02.044.

Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar. Environ. Res. 79, 1–15. https://doi.org/10.1016/j.marenvres.2012.04.003.

Solórzano, L., 1969. Determination of ammonia in natural waters by the Phenolhypoclorite methods. Limnol. Oceanogr. 14, 799–801. https://doi.org/10.4319/lo.1969.14.5.0799.

Stuardo, J., 1979. Sobre la clasificación, distribución y variación de *Concholepas concholepas* (Bruguiere 1789): un estudio de taxonomía Beta. Biol. Pesq. 12, 5–38.

Thomsen, J., Melzner, F., 2010. Moderate seawater acidification does not elicit metabolic depression in the blue mussel *Mytilus edulis*. Mar. Biol. 157, 2667–2676. https://doi.org/10.1007/s00227-010-1527-0.

Thomsen, J., Gutowska, M.A., Saphörster, J., Heinemann, A., Trübenbach, K., Fietzke, J., et al., 2010. Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. Biogeosciences 7, 3879–3891. https://doi.org/10.5194/bg-7-3879-2010.

Torres, R., Manríquez, P.H., Duarte, C., Navarro, J.M., Lagos, N.A., et al., 2013. Evaluation of a semi-automatic system for long-term seawater carbonate chemistry manipulation. Rev. Chil. Hist. Nat. 86, 443–451. https://doi.org/10.4067/S0716-078X2013000400006.

Vargas, C.A., De La Hoz, M., Aguilera, V., San Martin, V., Manríquez, P.H., Navarro, J.M., et al., 2013. CO_2-driven ocean acidification reduces larval feeding efficiency and changes food selectivity in the mollusk *Concholepas concholepas*. J. Plankton Res. 35 (5), 1059–1068. https://doi.org/10.1093/plankt/fbt045.

Vargas, C.A., Lagos, N.A., Lardies, M.A., Duarte, C., Manríquez, P.H., Aguilera, V.M., et al., 2017. Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. Nat. Ecol. Evol. 1, 0084 https://doi.org/10.1038/s41559-017-0084.

Widdicombe, S., Spicer, J.I., 2008. Predicting the impact of ocean acidification on benthic biodiversity: what can animal physiology tell us? J. Exp. Mar. Biol. Ecol. 366, 187–197. https://doi.org/10.1016/j.jembe.2008.07.024.

Wood, H.L., Spicer, J.I., Widdicombe, S., 2008. Ocean acidification may increase calcification rates, but at a cost. Proc. R. Soc. B. 275 (1644), 1767–1773. https://doi.org/10.1098/rspb.2008.0343.