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Effects of ocean acidification on larval development and early post-hatching traits in *Concholepas concholepas* (loco)

Patricio H. Manríquez[1,*], María Elisa Jara[1], Rodrigo Torres[2], María Loreto Mardones[3], Nelson A. Lagos[4], Marco A. Lardies[5], Cristian A. Vargas[6], Cristián Duarte[7], Jorge M. Navarro[3]

[1]Laboratorio de Ecología y Conducta de la Ontogenia Temprana (LECOT), Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Avenida Ossandón 877, Coquimbo, Chile

[2]Centro de Investigación en Ecosistemas de la Patagonia (CIEP), Universidad Austral de Chile, Coyhaique, Chile

[3]Instituto de Ciencias Marinas y Limnológicas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

[4]Centro de Investigación e Innovación para el Cambio Climático (CIICC), Universidad Santo Tomás, Santiago, Chile

[5]Facultad de Artes Liberales, Universidad Adolfo Ibáñez, Santiago, Chile

[6]Laboratorio de Funcionamiento de Ecosistemas Acuáticos, Centro de Ciencias Ambientales, Universidad de Concepción, Concepción, Chile

[7]Departamento de Ecología y Biodiversidad, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello, Santiago, Chile

ABSTRACT: Larval stages represent a bottleneck influencing the persistence of marine populations with complex life cycles. *Concholepas concholepas* is a gastropod species that sustains the most important small-scale artisanal fisheries of the Chile-Peru Humboldt Coastal current system. In this study, newly-laid egg capsules of *C. concholepas* collected from 3 localities along the Chilean coast were used to evaluate the potential consequences of projected near-future ocean acidification (OA) on larval development and early post-hatching larval traits. We compared hatching time, hatching success and early survivorship of encapsulated larvae reared under contrasting average levels of pCO_2: 382 (present-day), ca. 715 and ca. 1028 µatm CO_2 (levels expected in near-future scenarios of OA). Moreover, we compared morphological larval traits such as protoconch size, thickness and statolith size at hatching. Some of the developmental traits were negatively affected by pCO_2 levels, source locality, female identity, or the interaction between those factors. Meanwhile, the effect of pCO_2 levels on morphological larval traits showed significant interactions depending on differences among egg capsules and females. Our results suggest that OA may decouple hatching time from oceanographic processes associated with larval transport and reduce larval survivorship during the dispersive phase, with a potential impact on the species' population dynamics. However, the results also show geographic variability and developmental plasticity in the investigated traits. This variation may lead to an increased acclimatization ability, facilitate the persistence of natural populations and mitigate the negative effects that OA might have on landings and revenues derived from the fishery of this species.

KEY WORDS: Hatching time · Hatching success · Early larval survival · Protoconch size · Protoconch thickness · Statolith size · Egg capsule wall thickness · Developmental plasticity

INTRODUCTION

Larval survival in marine invertebrates represents a bottleneck in the recruitment of species with complex life cycles (Rumrill 1990, López et al. 1998). Therefore, adequate larval development and survival have important implications for both the aquaculture and fisheries of species of economic impor-

*Corresponding author: patriciohmanriquez@gmail.com

tance that rely on natural restocking. In the near future, ocean acidification (OA) is expected to have negative and variable effects on marine organisms (Fabry et al. 2008, Kroeker et al. 2010, 2013). However, it has also been suggested that marine biota may be more resistant to OA than expected (Hendriks et al. 2010). It is predicted that the surface ocean pH will fall ~0.1 units by 2050 and ~0.3 units by 2100 (Brewer 1997). Responses to OA vary across taxonomic groups, life-history stages and trophic levels and may interact synergistically with warming (Harvey et al. 2013). Such a decrease in pH implies a lower availability of carbonate ions (Feely et al. 2009, Chan et al. 2012), which will affect the synthesis of calcium carbonate in marine invertebrates (Chan et al. 2012). Developing larvae of marine calcifying organisms are expected to be among the most affected by the negative consequences associated with OA (Kurihara et al. 2007, 2008a) because the calcium carbonate used in the shells and skeletons of marine organisms is first synthesised during the larval stage (Kurihara et al. 2007). Furthermore, embryonic and larval stages have a reduced capacity for compensation and short-term homeostasis in response to OA scenarios (Dupont et al. 2008, Pörtner et al. 2011, Ceballos-Osuna et al. 2013). In fact, impacts of acidified seawater on the pH regulatory system involved in calcification have been demonstrated in sea urchin larvae (Stumpp et al. 2012). Several studies have demonstrated negative effects of OA on marine invertebrate embryos and larvae, mainly associated with effects on traits such as development (Dupont et al. 2008, Ericson et al. 2010, Ross et al. 2011), growth (Dupont et al. 2008, 2010, Clark et al. 2009, O'Donnell et al. 2009, Talmage & Gobler 2009, O'Donnell et al. 2010, Sheppard Brennand et al. 2010), viability (Bay et al. 1993, Kurihara et al. 2007, Dupont et al. 2008, Kurihara 2008, Ellis et al. 2009, Talmage & Gobler 2009, Crim et al. 2011), metabolism (Beniash et al. 2010), feeding (Dupont & Thorndyke 2009, Vargas et al. 2013), shell thickness (Gazeau et al. 2010), shell formation or calcification (Kurihara et al. 2008b, Beniash et al. 2010, Noisette et al. 2014), delayed metamorphosis (Talmage & Gobler 2009) and behaviour (Doropoulos et al. 2012). Therefore, by affecting the above traits, OA has the potential to act as an additional source of larval mortality. Moreover, elevated seawater pCO_2 can significantly reduce calcification of the statolith in the cuttlefish *Sepia officinalis* (Maneja et al. 2011). However, fish maintained under different levels of pH showed larger otoliths in lowered pH because of a greater deposition of calcium carbonate in the hemolymph (Munday et al. 2011). This effect suggests that different concentrations of pCO_2 associated with OA might also have consequences for the internal calcifying structures of marine invertebrate larvae. The walls of the egg capsules protect developing embryos and larvae against lethal or sublethal effects associated with predation (Spight 1977, Rawlings 1994), desiccation (Pechenik 1978, Rawlings 1995a), osmotic stress (Pechenik 1982, 1983), UV radiation (Rawlings 1996), mechanical shock (Rapoport & Shadwick 2007, Miserez et al. 2009) or microbial growth (Rawlings 1995b, Lim et al. 2007, Kaviarasan et al. 2012). Therefore, differences in egg capsule thickness might affect the exposure of embryos or larvae to lethal and sublethal effects associated with adverse environmental conditions, such as the high CO_2/low pH conditions associated with OA.

The muricid gastropod *C. concholepas* is a rocky shore keystone predator characteristic of the southeastern Pacific coast (Castilla 1999). In this environment, this species drives the intertidal distribution and abundance of the dominant space occupier, the mussel *Perumytilus purpuratus*, and subordinate species such as barnacles, which form part of its prey assemblage (Castilla 1999). Along the Chilean coast, *C. concholepas* represents the most economically important marine resource exploited within inner inshore Management and Exploitation Areas for Benthic Resources (Chile) (Castilla 1997, Castilla et al. 1998). For *C. concholepas*, protected and inaccessible areas play an important role as seeding grounds for this species through protection of the spawning activity (Manríquez & Castilla 2001). During the reproductive season, female *C. concholepas* lay their tough egg capsules bearing developing embryos, on intertidal and subtidal rocky environments (Castilla & Cancino 1976, Manríquez & Castilla 2001) where they remain exposed to ambient seawater. As there is currently no commercial production of this species (Manríquez et al. 2008), the maintenance of the natural population of *C. concholepas* relies on high levels of hatching, dispersal and recruitment success. In *C. concholepas*, the mineralisation of the protoconch and statoliths begins within a few days after the start of development (Gallardo 1973). Therefore, the protoconch protecting the soft part of the larvae and the statoliths are 2 calcareous structures present from early development onward. Incorporation of trace elements from the surrounding seawater into the statoliths has been reported for near-hatch larvae of *C. concholepas* (Zacherl et al. 2003, Manríquez et al. 2012). This incorporation suggests that encapsulated *C. concholepas* larvae are not totally isolated from

the surrounding environment and therefore are exposed to external stressors, such as changes in seawater pH and carbonate system parameters associated with different pCO_2 levels. Therefore, we hypothesised that in future scenarios of OA, the egg capsules of *C. concholepas* exposed to elevated pCO_2 levels and reduced seawater pH might develop smaller larval shells and statoliths than those obtained from egg capsules reared at present-day pCO_2 levels. We also hypothesised that OA will have negative consequences on other larval (i.e. hatching time, hatching success and early larval survival after hatching) and egg capsule traits (i.e. wall thickness). In addition, *C. concholepas* has an extensive natural latitudinal distribution (5° to 54° S), which might have important consequences in the responses to OA. The advantage of using *C. concholepas* as a model species for investigating the consequences of OA is the presence of a discrete life stage or a pre-dispersal encapsulated larval phase that lasts ~2 mo, from developing embryos to the hatching of veliger larvae of ca. 250 µm in size (Gallardo 1973). During this phase, the embryos develop inside egg capsules attached to the rocks in intertidal and subtidal environments (Fig. 1). Large females spawn larger egg capsules, and a positive relationship between the size of the egg capsules and the number of larvae exists (Manríquez & Castilla 2001). Therefore, the average number of larvae increases linearly from 2000 to 10 000 larvae with the size of the egg capsules which can range from ca. 1 to 2.5 cm in length (Castilla & Cancino 1976, Manríquez & Castilla 2001). Another practical advantage of using *C. concholepas* is that during a reproductive event, each female cleans the surface of the rock and then lays between 100 and 800 egg capsules (Fig. 1a,b), which are cemented in clutches to the surface (Castilla & Cancino 1976, Manríquez & Castilla 2001). This allows newly laid clutches of egg capsules to be recognised in the laboratory or field (Fig. 1e,f) and for egg capsules produced by a single female to be assigned to different experimental conditions (Fig. 1c).

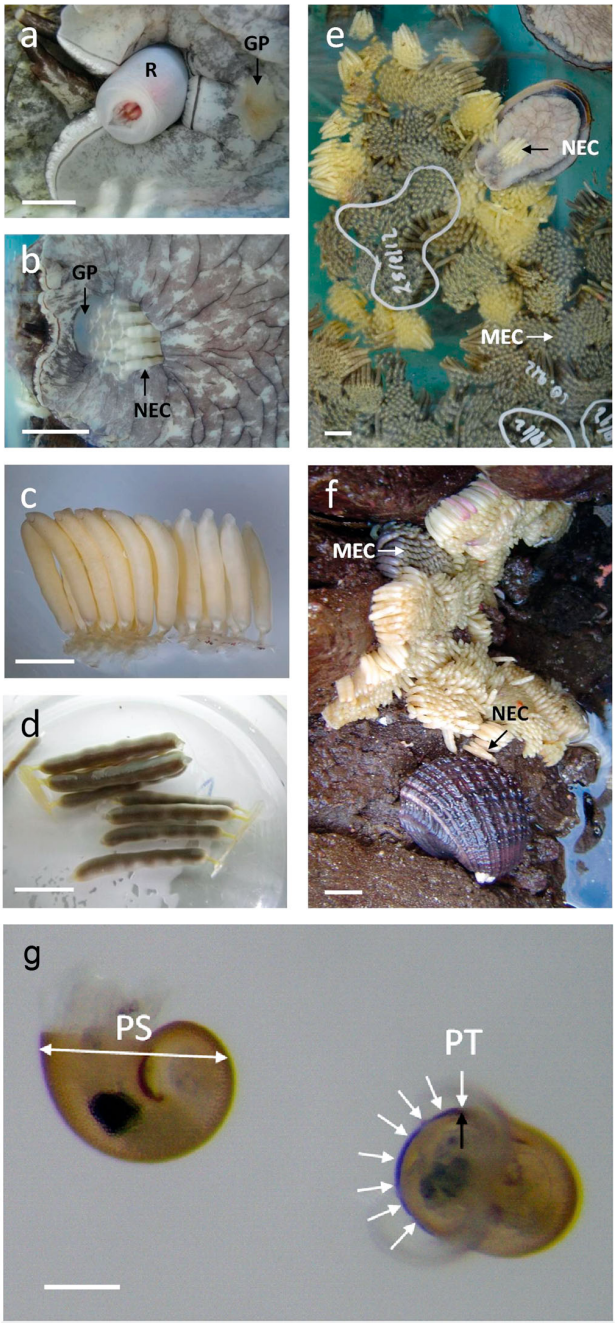

Fig. 1. *Concholepas concholepas*. (a) A female cleaning the surface of the glass aquarium with the radula prior to laying egg capsules; (b) a female laying a clutch of egg capsules on a glass aquarium surface; (c) a clutch of egg capsules newly laid by an identified female before being separated into 3 experimental groups; (d) 2 clutches of mature egg capsules at the end of the rearing period; (e) several clutches of newly laid (yellowish white colouration) and mature (brownish colouration) egg capsules on a glass aquarium surface; (f) a female laying egg capsules on a rocky intertidal surface in nature; (g) measurements made on newly hatched larvae after rearing under contrasting pCO_2 levels during the intracapsular period. R: radula; GP: gonadal pore; NEC: newly laid egg capsule; MEC: mature egg capsule. In (e), the silver line drawn on the glass surface indicates a large group of egg capsules laid by a single female. In (g), the protoconch size (lateral shell size, PS) was measured as the distance between the edge of the shell aperture and the opposite side; the protoconch thickness (the distance between the white and black arrows, PT) was measured along the outermost larval shell edge at 8 equidistant points depicted by the white arrows. Scale bars: (a–f) = 1 cm, (g) = 100 µm

MATERIALS AND METHODS

Collection of egg capsules

Newly laid egg capsules of *Concholepas concholepas* with sizes ranging from 1.5 to 2.0 cm in length and from 0.3 to 0.5 cm in diameter were either hand-collected from rocky intertidal platforms in Antofagasta (23° 38′ S, 70° 25′ W; Fig. 1f) or obtained from controlled spawning of brood individuals maintained in the laboratory at the Estación Costera de Investigaciones Marinas (ECIM) Marine Reserve at Las Cruces (hereafter Las Cruces), 34° 34′ S, 72° 02′ W, and in the Laboratorio Costero de Recursos Acuáticos at Calfuco (hereafter Calfuco), 39° 46′ S, 73° 23′ W. In the laboratory, the females were identified with plastic labels glued onto the shells with epoxy resin. In the field and in the laboratory, recently laid egg capsules of *C. concholepas* were recognised by their characteristic yellowish white colouration (Manríquez & Castilla 2001; Fig. 1b,c,e,f). Females of *C. concholepas* tend to remain close to the recently laid egg capsules (Manríquez & Castilla 2001; Fig. 1f). Thus, to ensure that the egg capsules had been laid recently, egg capsules were only collected close to females displaying egg capsule-laying activity (Fig. 1e,f). To investigate the site-specific responses and latitudinal susceptibility to OA, we used egg capsules from 3 sampling regions along the Chilean coast: Antofagasta (northern Chile) Las Cruces (central Chile) and Calfuco (southern Chile). Unfortunately, in the present study, *in situ* carbonate systems parameters during the collection of the egg capsules were not directly measured. However, a description of those parameters, measured at the same sampling sites a year before our samples were collected, is available in Ramajo et al. (2013). That study showed that the highest values of total alkalinity (A_T) (2306 µmol kg^{-1}) and pCO_2 (725 ppm) were recorded at Las Cruces. No information is available on pH levels for Antofagasta; however, the average (±1 SD) pH values in Las Cruces and Calfuco were 7.596 (±0.040) and 7.796 (±0.106) respectively (Ramajo et al. 2013). The saturation states (Ω) of calcite at the sampling sites were 4.798 (Antofagasta), 2.159 (Las Cruces) and 3.289 (Calfuco). However, the Ω aragonite values were 3.107 (Antofagasta), 1.377 (Las Cruces) and 2.095 (Calfuco) (Ramajo et al. 2013). Moreover, calcifying organisms in southern coastal regions of Chile are exposed to stress by the input of acidic freshwaters (e.g. Salisbury et al. 2008) and reduced Ω carbonate due to increased pCO_2 sequestration (Torres et al. 2011). Salinity at these 3 study sites is high and fluctuates around a mean ± SD of 33 ± 0.96 psu (Ramajo et al. 2013), and sea-surface temperature decreases from north to south over the latitudinal range of the 3 study sites, with a mean (± SD) temperature of 17.03°C (±1.23) at Antofagasta (Lagos et al. 2008); 13.2°C (±0.48) at Las Cruces (Lagos et al. 2005) and 11.20°C (±1.10) at Calfuco (P. H. Manríquez unpubl. data). This pattern highlights that females and egg capsules of *C. concholepas* are naturally exposed along the Chilean coast to different temperatures, pCO_2 levels and Ω for calcite and aragonite. Therefore, the mineralization of hard parts in *C. concholepas* from southern and central Chile should be more difficult compared to that of northern Chile populations.

Experimental procedures

Egg capsule rearing. Regardless of their origin (field or laboratory), *C. concholepas* egg capsules were reared under controlled pCO_2 laboratory conditions in Calfuco for their entire development period (hereafter acidification phase) with 3 contrasting and controlled pCO_2 concentrations (present-day: 365–398 µatm, medium: 686–745 µatm and high: 979–1077 µatm pCO_2; see Table 2). The first experimental series was conducted between May and August of 2011, and to incorporate latitudinal susceptibility, the experiments were conducted with egg capsules collected from identified females from Las Cruces and Calfuco. The second experimental series was conducted between April and July of 2012, with egg capsules collected from identified females from the same sites used in the first experimental series (i.e. Las Cruces and Calfuco) and expanded northward with the incorporation of Antofagasta as a third sampling site. Each newly laid clutch was divided evenly into 3 groups, and each group was assigned to one of the experimental pCO_2 levels (Table 1). During the acidification phase, the egg capsules were maintained in plexiglas aquariums (30 cm in length and 17 cm in width and height) filled with 7.5 l of 0.5 µm filtered seawater (FSW) conditioned at the required pCO_2 levels. Both experimental series were conducted during autumn and winter months, with seawater temperature varying between 11 and 13°C in the rearing aquaria. Conditioned seawater, from the appropriate reservoir tank (see next subsection), was used to replace the water in each of the exposure aquariums daily. Once a week, the surface of each egg capsule was cleaned with the aid of a soft artist's brush. To ensure that pCO_2 levels remained stable, each plexiglas aquaria was fitted with a plastic lid,

Table 1. *Concholepas concholepas*. Summary of females and egg capsules assigned to each average experimental pCO_2 level (Present-day: 382 µatm; medium: 715 µatm; high: 1028 µatm) and variables measured (HT: hatching time; ELS: early larval survival; PT: protoconch thickness; PS: protoconch size; SS: statolith size; HS: hatching success) at the end of the acidification phase in the newly hatched larvae. Data correspond to information generated by egg capsules collected from 2 or 3 source localities along the Chilean coast depending on the experimental series. Regarding the number of females, the egg capsules were directly observed and assigned to a single female only in Calfuco, where the brooder specimens were maintained in captivity. In Las Cruces and Antofagasta, the egg capsules were assigned indirectly to a single female

Source locality	No. of females	Sampling source per female	No. of egg capsules	No. of egg capsules assigned per pCO_2 level Present-day	Medium	High	Measured variable
First experimental series							
Las Cruces (34° S)	3	Brood-stock	46–56	14–24	13–29	14–18	HT
	2	Brood-stock	9	3	3	3	ELS
	3	Brood-stock	15	5	5	5	PT, PS & SS
Calfuco (79° S)	3	Brood-stock	58–89	22–30	19–32	17–30	HT
	2	Brood-stock	9	3	3	3	ELS
	3	Brood-stock	15	5	5	5	PT, PS & SS
Second experimental series							
Antofagasta (24° S)	3	Field	35–55	13–18	10–28	5–11	HT
	2	Field	9	3	3	3	HS
	3	Field	12	4	4	4	PT, PS & SS
Las Cruces (34° S)	4	Brood-stock	40–59	14–31	18–36	12–22	HT
	2	Brood-stock	9	3	3	3	HS & ELS
	4	Brood-stock	12	4	4	4	PT, PS & SS
Calfuco (79° S)	4	Brood-stock	54–60	13–18	11–18	21–31	HT
	2	Brood-stock	9	3	3	3	HS & ELS
	4	Brood-stock	12	4	4	4	PT, PS & SS

and a continuous stream of either air (390 µatm CO_2) or enriched CO_2 air (700 or 1000 µatm) was bubbled through the water in each aquaria via an air-stone. The methodologies used to create the required mixed gas precisely followed methods described in the literature (Navarro et al. 2013, Torres et al. 2013).

Carbonate system determination in the conditioned seawater. Conditioned FSW with different pCO_2 levels for the rearing aquariums was generated in 3 polyethylene (230 l) reservoir tanks. Each tank was filled with FSW, and a continuous stream of either air (390 µatm CO_2) or enriched CO_2 air (700 or 1000 µatm) was bubbled through the water. During the experiment, the seawater parameters — pH, temperature, salinity and A_T — were monitored in each unit 3 times a week (Table 2). The pH measurements were made in a closed 25 ml cell, thermostatically controlled at 25°C, with a Metrohm 713 pH meter. Temperature and salinity were measured using a CTD (Ocean Seven 305). The pH, A_T and hydrographic data were used to calculate the rest of the carbonate system parameters (pCO_2 and DIC) and the saturation state of Ω aragonite, using CO2SYS software (Lewis & Wallace 1998) set with Mehrbach solubility constants (Mehrbach et al. 1973) refitted by Dickson & Millero (1987).

Development traits of newly hatched larvae

Hatching time (HT). As larval development in the egg capsules of *C. concholepas* proceeds, the colour of them becomes progressively browner, indicating that the larvae are within a few days of hatching. Therefore, at daily intervals, we recorded the development of each egg capsule through changes in colour and direct observation of the larval activity inside it. For each female, the HT was measured as the number of days needed to achieve the complete hatching of all capsules from the maintained clutch. Following the visual examination, the egg capsule developmental stage was categorised as immature (IE), in development (DE), mature (ME), in hatching (IHE) or hatched (HE). IE capsules were those with the characteristic yellowish white colouration (Manríquez & Castilla 2001). DE capsules were those with a brown colouration but without evidence of larval swimming activity. ME capsules were those with brownish colouration and containing swimming larvae. IHE capsules were those with a fully open plug and larvae leaving the egg capsule. Finally, HE capsules were those that were completely empty when inspected. Egg capsules with a purple colouration were considered to be physically

Table 2. Average (± SD) conditions of the seawater used to maintain (in 2 experimental series) egg capsules of *C. concholepas* during their entire development period. Medium and High pCO_2 are based on rate of change in pH predicted by the most extreme scenario (RCP8.5 scenario) of atmospheric CO_2 for the end of the present century and the beginning of the next century, respectively. See Meinshausen et al. 2011 for further details

Level of pCO_2	pH at 25°C (pH units)	Temperature (°C)	A_T (µmol kg^{-1})	pCO_2 *in situ* (µatm)	[CO_3^{2-}] *in situ* (µmol kg^{-1} SW)	Salinity	Ω calcite	Ω aragonite
First series (May–August 2011)								
Present-day	7.83 ± 0.01	11.32 ± 0.19	2148.04 ± 1.82	398.24 ± 4.98	119.21 ± 2.22	31.96 ± 0.33	2.89 ± 0.05	1.83 ± 0.03
Medium	7.59 ± 0.01	11.32 ± 0.19	2150.35 ± 16.71	746.46 ± 16.92	72.82 ± 1.96	32.13 ± 0.31	1.76 ± 0.05	1.12 ± 0.03
High	7.46 ± 0.01	11.41 ± 0.19	2161.66 ± 15.93	1077.68 ± 19.30	57.30 ± 1.34	32.25 ± 0.28	1.30 ± 0.03	0.82 ± 0.02
Second series (April–July 2012)								
Present-day	7.89 ± 0.01	12.59 ± 0.33	2238.50 ± 11.21	365.44 ± 5.65	142.37 ± 3.95	32.32 ± 0.29	3.45 ± 0.09	2.19 ± 0.06
Medium	7.66 ± 0.02	12.60 ± 0.34	2222.99 ± 13.24	685.59 ± 21.95	87.87 ± 3.65	32.26 ± 0.31	2.13 ± 0.09	1.35 ± 0.06
High	7.52 ± 0.02	12.64 ± 0.35	2218.04 ± 10.29	979.46 ± 36.92	65.32 ± 3.16	32.33 ± 0.28	1.60 ± 0.1	1.01 ± 0.05

stressed or containing infected embryos (Spight 1977, Gallardo 1979) and were removed from the rearing containers to prevent cross-contamination. Once IHE capsules were recorded, they were removed from the experimental condition, labelled and frozen for further analysis. The HT was measured in 2 experimental series using egg capsules from Las Cruces and Calfuco (first series) and from Antofagasta, Las Cruces and Calfuco (second series). In the experiments, egg capsules produced by 3 to 4 female individuals per sample site were divided into 3 clutches and assigned to each experimental pCO_2 level (Table 1).

Hatching success (HS). HS was measured as the number of live larvae just after hatching at the end of the acidification phase. Live larvae were those larvae displaying active swimming, with the entire 2-lobed velum protruded from the larval shell and the operculum fully opened (Manríquez et al. 2013b). To obtain the *C. concholepas* newly hatched larvae (NHL), once hatching began, the egg capsules were maintained separately in individual beakers with 100 ml FSW (0.45 µm mesh size) with the same pCO_2 level used during the acidification phase. During hatching, the beakers were semi-immersed in a water bath to maintain the temperature at 13 ± 1°C. After hatching, the larvae pool was homogenised using a pipette, and 1 ml of the mixture was removed and diluted in 5 ml of 1 µm FSW. The sample was exposed to light for ca. 15 min, and the number of live larvae was then counted 3 times to calculate the percentage of live larvae in each capsule for each female. HS was only evaluated in *C. concholepas* NHL from capsules assigned to the 3 experimental pCO_2 levels and that were produced during the second experimental series by individuals from Antofagasta, Las Cruces and Calfuco (Table 1).

Early larval survival (ELS). ELS was estimated as the number of live larvae 96 h after hatching at the end of the acidification phase. We used *C. concholepas* NHL originating from 2 egg capsules laid by 2 females per locality. At the end of the acidification phase, once hatching had taken place, 6 groups of 10 NHL from each egg capsule were used to assess ELS. Each group was maintained in multi-well plates for 96 h with 10 ml of 1 µm FSW equilibrated at the same pCO_2 level used during the entire intra-capsular period. During the experiments, the plates were kept in darkness, and to maintain the temperature at 13 ± 1°C, the plates were semi-immersed in a refrigerated circulating water bath. After 96 h, the plates were removed from the water bath and exposed to light for ca. 15 min, and then, ELS was evaluated. The ELS was expressed as a percentage of the initial number of live larvae and compared between the different pCO_2 levels and source locations. The measurements of ELS were conducted with *C. concholepas* NHL from Las Cruces and Calfuco, exposed to the acidification phase in both experimental series (Table 1). After hatching, *C. concholepas* NHL are planktotrophic and rely on food particles present in the water column (DiSalvo 1988, Vargas et al. 2006). However, they can remain alive for few days relying only on internal food reserves (Manríquez et al. 2013b). Therefore, ELS in the present study was evaluated in absence of external food sources.

Morphological traits of newly hatched larvae

Statolith size (SS). SS was measured in both experimental series at the end of the acidification phase. Once hatching was detected, 4 or 5 randomly selected egg capsules were obtained from 3 or 4

females from each sampling for each pCO_2 level and were frozen for further analysis. The statolith extraction from defrosted samples followed the methodologies described by Zacherl et al. (2003) and Manríquez et al. (2012). Larvae were suspended in an equal volume mixture of 35% H_2O_2 buffered in NaOH (0.1 N) for 20 to 30 min. The released larval statoliths were collected, rinsed 3 times in distilled water and then pipetted onto a glass slide for drying at room temperature. The statoliths were then photographed using a digital camera attached to a compound microscope at a magnification of ×100. The maximum diameter (µm) of each statolith was measured using the image analysis software Image-Pro Plus.

Protoconch size (PS). The measurements of PS were made in 3 to 10 randomly selected larvae for each location and each pCO_2 level. Larvae were removed from defrosted IHE, and the same protocols for extraction of statoliths were used here to separate the flesh from the larvae shell. The shells were retained on a sieve, washed in distilled water and then were kept at room temperature in Eppendorf tubes. The shells were adhered to a microscope slide side-on to make the measurement of the protoconch (µm) easier. The shells were photographed and measured using the same method for SS described above. In the photographs, the PS was measured as the distance between the edge of the shell aperture and the opposite side of the larval shell (lateral shell length; Fig. 1g). The PS was measured in both experimental series at the end of the acidification phase using the same number of egg capsules used to measure the SS per each female and source locality (Table 1).

Protoconch thickness (PT). PT was measured in a sub-set of the same larvae used for the measurement of PS (Table 1). However, the position of the shells on the glass slide was carefully altered to place them with the shell aperture facing upward. In both experimental series, for each pCO_2 level and locality, the measurements were conducted for 2 groups of 10 randomly selected larvae, each group removed from 2 egg capsules laid by 3 different females. The PT was measured (µm) in the photographs at 8 equidistant points around the outermost larval shell edge aperture using the same method described above (Fig. 1g) and then averaged for posterior analyses.

Morphological traits of egg capsules

We measured the egg capsule wall thickness (EWT) in cross sections taken from egg capsules collected from Antofagasta, Las Cruces and Calfuco and reared over the entire developmental period in the 3 contrasting pCO_2 levels of the acidification phase. For Las Cruces and Calfuco, 2 egg capsules produced by 3 identified females were assigned to each pCO_2 level. However, for Antofagasta, only 27 egg capsules generated by identified females were available, and they were assigned in groups of 9 to each pCO_2 level. At the end of the acidification phase, when hatching started the egg capsules were frozen at −15°C, and stored until wall thickness was measured. Therefore, data reported here represent information generated from other egg capsules maintained under similar condition as in the other experiments (first experimental series). Two months later, the egg capsules were defrosted for ca. 20 min, dissected along the medial section including both sides of each capsule and washed with FSW to remove dead larvae. The cross sections were mounted on a glass slide, photographed and measured using the same method described above at 4 equidistant points. Due to practical issues, measurements of EWT were not conducted on the same egg capsules in which developmental and post hatching traits were evaluated.

Data analysis

For both experimental series, the developmental traits (HT, HS and ELS) collected from 3 different localities and reared under 3 experimental pCO_2 levels were compared using a factorial nested ANOVA model. In the model, the source of variation, in addition to the main effects (source locality and pCO_2 levels), was the egg capsules produced by a single female. Female identity was treated as a random factor, which was nested within source locality and crossed with pCO_2 levels [pCO_2 × female (source locality)]. Prior to statistical analysis, the percentage data of HT was arcsine transformed. In the case of morphological traits (SS, PT and PS), we used a similar factorial nested analysis including the factor 'capsule' produced by the same female [pCO_2 × capsule (female, source locality)] and using $\log_{10}$ transformed-data. Post hoc comparisons were not possible because the main factors (fixed) interact with the random variation introduced by the females and capsules. The effects of pCO_2 levels during the acidification phase on EWT were analysed using a 1-way ANOVA followed by a Tukey post hoc test.

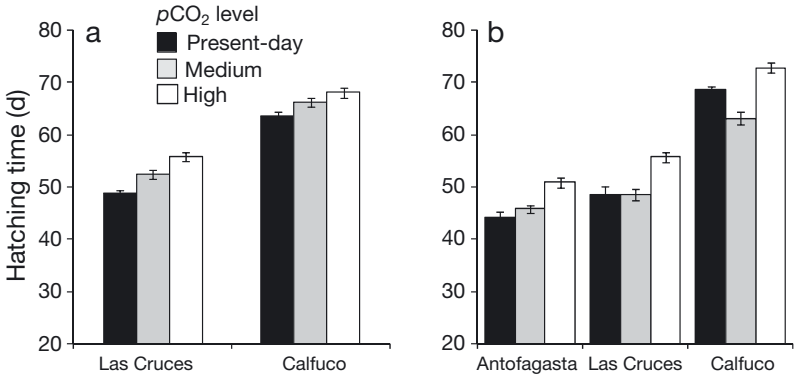

Fig. 2. *Concholepas concholepas*. Average (± SD) hatching time of larvae hatched from egg capsules reared at 3 different experimental pCO_2 levels in 2 experimental series, using egg capsules collected from (sites in north to south order) (a) Las Cruces and Calfuco in the first series and (b) Antofagasta, Las Cruces and Calfuco in the second series. See Table 2 for details of average pCO_2 levels used in each experimental series

RESULTS

Development traits of newly hatched larvae

Hatching time (HT). In both experimental series, the average HT showed a latitudinal gradient, with shorter HT in northern than southern egg capsules (Fig. 2). In the first and second experimental series and regardless of the pCO_2, the average (± SD) HT was 18.9 (± 0.07) and 4.35 (± 0.06) d longer in southern than northern egg capsules, respectively, leading to significant differences in HT among localities (Table 3). In both experimental series, longer HT were detected in egg capsules of *C. concholepas* reared at the highest pCO_2 levels (Fig. 2). In both experiments, the source locality and the interaction between pCO_2 levels and female identity had a significant effect on HT (Table 3). In the second experimental series only, the pCO_2 levels had a significant effect on HT (Table 3). In this series, the average (± SD) HT was ca. 10.03 (± 1. 11; Antofagasta), 10.02 (± 9.40; Las Cruces) and 4.9 (± 3.11, Calfuco) d longer in the acidified egg capsules reared under medium and high levels of increased pCO_2 than in those reared under present-day pCO_2 conditions (Fig. 2).

Hatching success (HS). Regardless of the egg capsule source locality and pCO_2 rearing condition, the HS was always >85%. When effects of the pCO_2 levels on HS were detected, we found a reduction of the HS ranging from 5 to 10% only in the egg capsules reared at the highest pCO_2 level (Fig. 3). The analysis indicates that the interaction of pCO_2 levels with female identity had a significant effect on HS (Table 3).

Early larval survival (ELS). When the egg capsule rearing took place at present-day and medium pCO_2, larval survival was always >95%. In both experimental series, ELS values were significantly lower at the

Table 3. *Concholepas concholepas*. Factorial ANOVAs investigating the effect of female identity (random factor), locality (fixed factor) and pCO_2 levels (fixed factor) on hatching time (HT) and hatching success (HS). Values in **bold** are significant at $p < 0.05$. Further details in 'Data analysis'. Exp.: experimental series

Response	Exp.	Source	df	MS	*F*	p
HT (d)	1	Locality	1,4	18686.2	119.03	**<0.001**
		Female (Locality)	4,8	160.4	0.68	0.623
		pCO_2	2,8	798.5	3.48	0.081
		Locality × pCO_2	2,8	100.8	0.44	0.659
		pCO_2 × Female (Locality)	8,364	236.4	4.90	**<0.001**
HT (d)	2	Locality	2,8	16635.2	6.37	**0.022**
		Female (Locality)	8,16	2869.5	9.95	**<0.001**
		pCO_2	2,16	3349	12.94	**<0.001**
		Locality × pCO_2	4,16	652.9	2.41	0.090
		pCO_2 × Female (Locality)	16,551	298.1	6.38	**<0.001**
HS (%)	1	Locality	2,8	0.16881	6.32	0.078
		Female (Locality)	3,16	0.02751	0.34	0.797
		pCO_2	2,16	0.33329	4.48	0.062
		Locality × pCO_2	4,16	0.01853	0.24	0.905
		pCO_2 × Female (Locality)	6,44	0.08079	5.99	**<0.001**

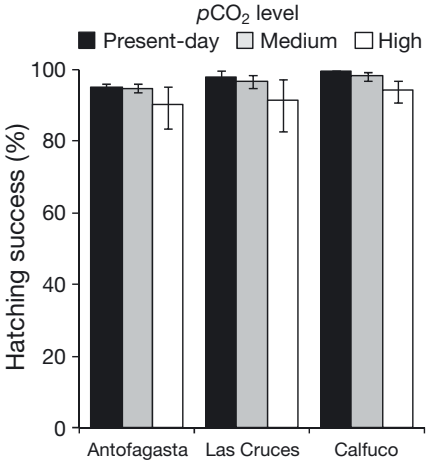

Fig. 3. *Concholepas concholepas*. Average (± SD) hatching success of larvae hatched from egg capsules collected from Antofagasta, Las Cruces and Calfuco and reared at 3 experimental pCO_2 levels. See Table 2 for details of average CO_2 levels used in each experimental series

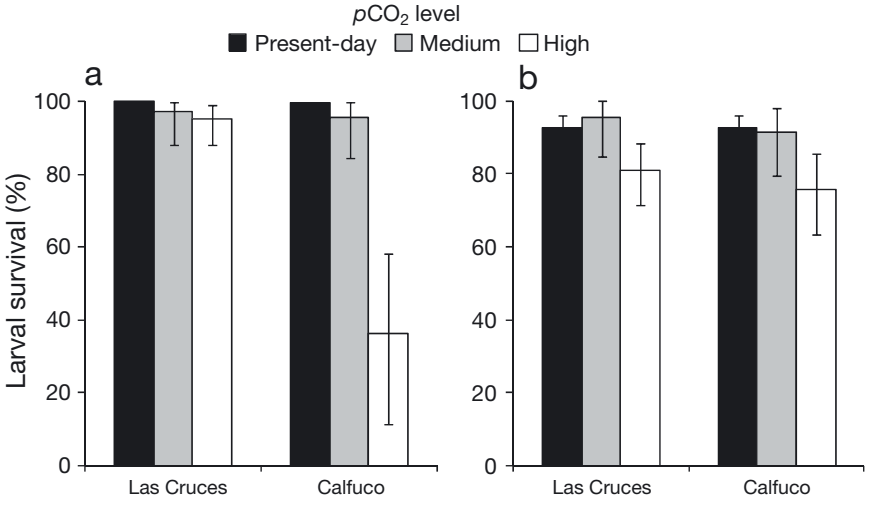

Fig. 4. *Concholepas concholepas*. Average (± SD) early larval survival of larvae hatched from egg capsules collected from Las Cruces and Calfuco, reared at 3 experimental pCO_2 levels and in 2 experimental series (a) and (b). See Table 2 for details of average CO_2 levels used in each experimental series

high pCO_2 level (Fig. 4, Table 4). In the first experimental series, locality, pCO_2 levels and the interaction between these 2 variables had a significant effect on ELS (Table 4). However, in the second experimental series, only female identity nested within source locality and pCO_2 levels had a significant effect on ELS.

Table 4. *Concholepas concholepas*. Factorial ANOVAs investigating the effect of female identity (random factor), locality (fixed factor) and pCO_2 levels (fixed factor) on early larval survival (ELS). Values in **bold** are significant at $p < 0.05$. Further details in 'Data analysis'. Exp.: experimental series

Exp.	Source	df	MS	*F*	p
1	Locality	1,2	1.305	11.49	0.077
	Female (Locality)	2,4	0.114	1.13	0.408
	pCO_2	2,4	2.368	23.6	**0.006**
	Locality × pCO_2	2,4	0.773	7.7	**0.042**
	CO_2 × Female (Locality)	4,24	0.100	1.82	0.158
2	Locality	1,2	0.071	0.22	0.688
	Female (Locality)	2,4	0.332	9.87	**0.028**
	pCO_2	2,4	0.598	17.79	**0.010**
	Locality × pCO_2	2,4	0.021	0.62	0.584
	pCO_2 × Female (Locality)	4,24	0.034	0.88	0.491

Morphological traits of newly hatched larvae

Statolith size (SS). The average SS ranged from 10 to 12 µm and from 12 to 14 µm for statoliths removed from northern (Antofagasta) and central-southern (Las Cruces and Calfuco) larvae, respectively (Fig. 5). In the first experimental series, the SS was only significantly affected by female identity nested within source locality and by the interaction between pCO_2 and capsule nested with the terms female identity and locality (Table 5). However, in the second experimental series, in which egg capsules from northern Chile (Antofagasta) were included, SS was significantly affected by pCO_2 level and the interaction of pCO_2 level with female identity and capsule (Table 5). In the second experimental series, a latitudinal gradient was detected, with statoliths from northern sites being smaller than those from southern sites (Fig. 5b).

Protoconch thickness (PT). Including all experimental factors, the average (±1 SD) PT of *C. concholepas* NHL was ~1.84 (±0.5) µm (Fig. 6). In the first experimental series, PT was significantly affected by pCO_2 and by the interaction between this variable and capsule nested with the interaction between locality and female. Moreover, in both experimental series, the variation in the mean larval PT had a significant effect of pCO_2 which was affected by the term egg capsule (Table 5).

Protoconch size (PS). The PS of *C. concholepas* NHL ranged from 230 to 260 µm, with a latitudinal gradient with larger protoconch sizes found in northern (Antofagasta) compared to central-southern Chile (Las Cruces and Calfuco) (Fig. 7). In the first

Table 5. *Concholepas concholepas*. Factorial ANOVAs investigating the effect of female identity (random factor), locality (fixed factor) and pCO_2 levels (fixed factor) on larval statolith size (SS), and larval protoconch thickness (PT) and size (PS). Values in **bold** are significant at $p < 0.05$. Further details in 'Data analysis', Exp.: experimental series

Response	Exp.	Source	df	MS	*F*	p
SS (µm)	1	Locality	1, 4	0.049	1.47	0.292
		Capsule (Female, Locality)	24, 48	0.003	0.93	0.562
		Female (Locality)	4, 8	0.035	7.05	**0.015**
		pCO_2	2, 8	0.018	3.67	0.074
		Locality × pCO_2	2, 8	0.015	3.13	0.099
		pCO_2 × Female (Locality)	8, 48	0.005	2.01	0.064
		pCO_2 × Capsule (Female, Locality)	48, 1489	0.003	8.54	**<0.001**
SS (µm)	2	Locality	2, 8	0.857	33.47	**<0.001**
		Capsule (Female, Locality)	33, 66	0.003	0.94	0.574
		Female (Locality)	8, 16	0.026	2.99	**0.034**
		pCO_2	2, 16	0.110	12.67	**<0.001**
		Locality × pCO_2	4, 16	0.036	4.11	**0.018**
		pCO_2 × Female (Locality)	16, 66	0.009	3.19	**<0.001**
		pCO_2 × Capsule (Female, Locality)	66, 1922	0.003	9.01	**<0.001**
PT (µm)	1	Locality	1, 4	1.296	1.850	0.245
		Capsule (Female, Locality)	6, 12	0.105	0.400	0.867
		Female (Locality)	4, 8	0.701	13.160	0.613
		pCO_2	2, 8	1.409	6.650	**0.020**
		Locality × pCO_2	2, 8	0.030	0.140	0.870
		pCO_2 × Female (Locality)	8, 12	0.212	0.810	0.611
		pCO_2 × Capsule (Female, Locality)	12, 354	0.263	3.680	**<0.001**
PT (µm)	2	Locality	2, 6	0.108	3.900	0.082
		Capsule (Female, Locality)	9, 18	0.004	0.810	0.613
		Female (Locality)	6, 12	0.028	1.190	0.385
		pCO_2	2, 12	0.039	1.600	0.242
		Locality × pCO_2	4, 12	0.032	1.290	0.326
		pCO_2 × Female (Locality)	12, 18	0.024	4.470	**0.002**
		pCO_2 × Capsule (Female, Locality)	18, 486	0.005	2.320	**0.002**
PS (µm)	1	Locality	1, 4	0.0001	0.03	0.876
		Capsule (Female, Locality)	24, 48	0.0009	0.64	0.877
		Female (Locality)	4, 8	0.0065	2.41	0.167
		pCO_2	2, 8	0.0011	0.42	0.669
		Locality × pCO_2	2, 8	0.0071	2.71	0.125
		pCO_2 × Female (Locality)	8, 48	0.0031	2.51	**0.023**
		pCO_2 × Capsule (Female, Locality)	48, 622	0.0013	9.88	**<0.001**
PS (µm)	2	Locality	2, 8	0.0570	4.24	0.055
		Capsule (Female, Locality)	33, 66	0.0004	1.51	0.077
		Female (Locality)	8, 16	0.0135	3.28	**0.019**
		pCO_2	2, 16	0.0071	1.77	0.201
		Locality × pCO_2	4, 16	0.0066	1.67	0.206
		pCO_2 × Female (Locality)	16, 66	0.0003	2.01	**<0.001**
		pCO_2 × Capsule (Female, Locality)	66, 809	0.0040	15.97	**<0.001**

experimental series, PS was significantly affected by female nested with locality. Moreover, in both experimental series, PS was significantly affected by the interaction between pCO_2 levels with the factors egg capsule and female (Table 5).

Morphological traits of egg capsules

The EWT at hatching ranged between 50 and 80 µm with a latitudinal gradient. The thinnest egg capsule walls were recorded in egg capsules from northern Chile (Antofagasta), and the thickest were recorded in egg capsules from southern Chile (Calfuco; Table 6). No significant differences were found in EWT in egg capsules reared in contrasting pCO_2 levels and collected from Central Chile (Las Cruces, 1-way ANOVA $F_{2,69} = 0.61$; $p > 0.05$; Table 3) and Calfuco (1-way ANOVA $F_{2,69} = 1.69$; $p > 0.05$; Table 6). However, significant differences in EWT were found in egg capsules reared in contrasting pCO_2 levels and collected from Antofagasta (1-way ANOVA $F_{2,104} = 13.35$; $p < 0.05$; Table 6). The post hoc Tukey s test indicated that egg capsules reared at current-day pCO_2 levels were significantly thinner than those reared at medium and high pCO_2 levels (Table 6).

DISCUSSION

The hatching time (HT) of *Concholepas concholepas* varied between 45 and 60 d and displayed a latitudinal gradient, with short HT recorded in northern egg capsules. When significant effects of pCO_2 levels were recorded, the longest HT were measured in egg capsules reared at the high levels. The reproductive, dispersive larval and recruitment phases in *C. concholepas* are concentrated in clear and consecutive temporal windows of a few months (Manríquez & Castilla

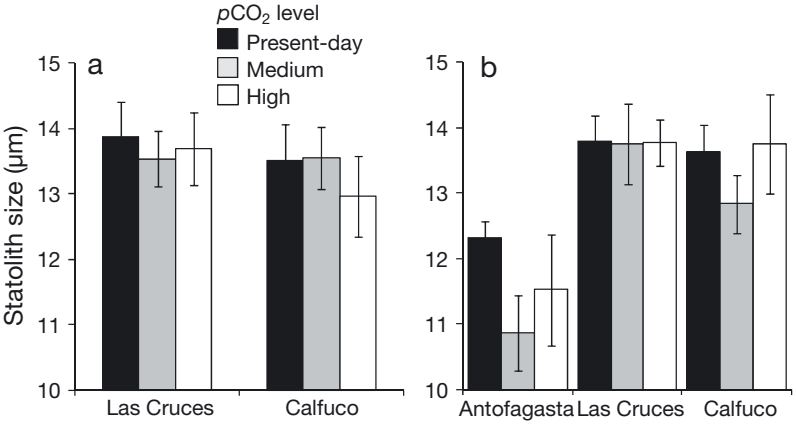

Fig. 5. *Concholepas concholepas*. Average (± SD) statolith size of larvae hatched from egg capsules reared at 3 experimental pCO_2 levels in (a,b) 2 experimental series, using egg capsules collected from (sites in north to south order) (a) Las Cruces and Calfuco and (b) Antofagasta, Las Cruces and Calfuco. See Table 2 for details of average pCO_2 levels used in each experimental series

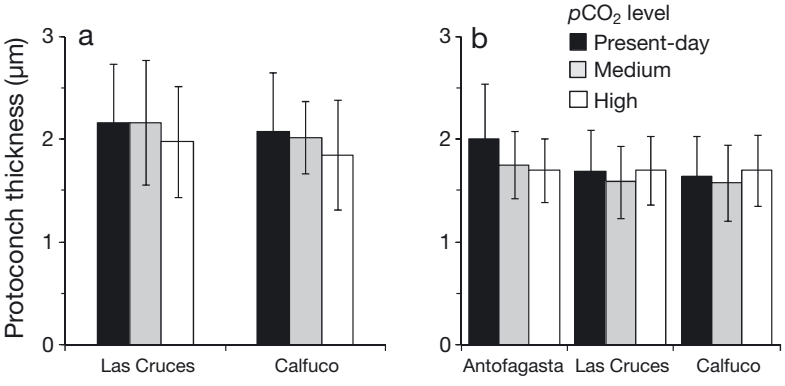

Fig. 6. *Concholepas concholepas*. Average (± SD) protoconch thickness of larvae hatched from egg capsules reared at 3 experimental pCO_2 levels in (a,b) 2 experimental series using egg capsules collected from (sites in north to south order) (a) Las Cruces and Calfuco and (b) Antofagasta, Las Cruces and Calfuco. See Table 2 for details of average pCO_2 levels used in each experimental series

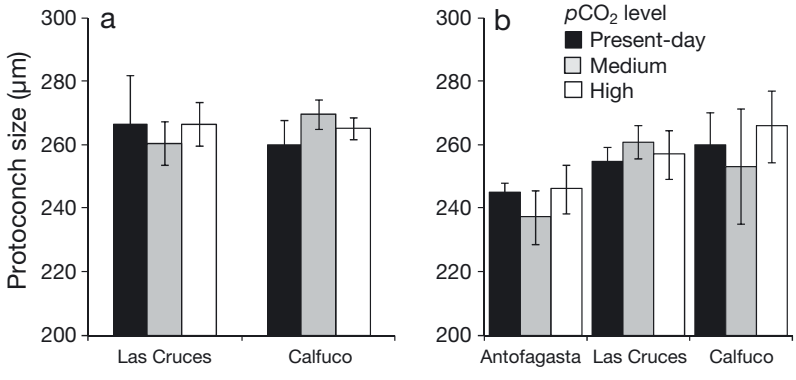

Fig. 7. *Concholepas concholepas*. Average (± SD) protoconch size (µm ± SD) larvae hatched from egg capsules reared at 3 experimental levels of CO_2 in (a,b) 2 experimental series using egg capsules collected from (sites in north to south order) (a) Las Cruces and Calfuco and (b) Antofagasta, Las Cruces and Calfuco. See Table 2 for details of average pCO_2 levels used in each experimental series

Table 6. *Concholepas concholepas*. Average (± SD) length (cm) and wall thickness (µm) of egg capsules maintained during the entire development period at different levels of CO_2. The egg capsules are from 3 source localities along the Chilean coast. For egg capsules collected from Las Cruces and Calfuco, wall thickness was measured in slices taken from 6 egg capsules produced by 3 different females (2 egg capsules per female). For Antofagasta, 9 egg capsules produced by 9 identified females were measured. A 1-way ANOVA followed by a Tukey post hoc test determined whether egg capsule thickness varied among the 3 pCO_2 levels per source locality. Results followed by the same superscript letter are not significantly different from one another ($p > 0.05$)

Source locality	Egg capsule length (±1 SD)	Egg capsule wall thickness in each pCO_2 level		
		Present-day	Medium	High
Antofagasta (23° S)	0.80 (0.27)	60.94 (9.56)[a]	56.00 (7.33)[b]	65.57 (6.36)[c]
Las Cruces (34° S)	1.57 (0.39)	64.96 (10.17)[a]	66.51 (9.75)[a]	67.92 (7.71)[a]
Calfuco (79° S)	1.56 (0.31)	76.77 (13.09)[a]	76.97 (14.01)[a]	71.41 (7.55)[a]

2001, 2011, Martínez & Navarrete 2002). This suggests that the sublethal extension in HT as recorded in the present study (i.e. Antofagasta: 9 to 11 d; Las Cruces: 7 to 9 d; Calfuco: 4 to 5 d) might cause a decoupling between the time when larvae hatch and the presence of optimal feeding and dispersive conditions in the adjacent coastal seawater. However, contrasting results between both experimental series with egg capsules from Las Cruces suggest the existence of intra-population variability in HT in response to pCO_2 levels.

Hatching success (HS) was always higher than 85%, suggesting that experimental pCO_2 levels did not have any drastic consequences for intra-capsular development. However, significant effects of the interaction between pCO_2 levels and females nested within source locality suggest that OA might have negative effects later during the larval phase. In fact, early larval survival (ELS) showed more severe consequences at high pCO_2 levels, with mortality reaching up to 30%. Therefore, the effects of elevated pCO_2 levels on ELS suggest that OA might have more drastic consequences in *C. concholepas*, taking into account their long larval dispersal phase of 3 mo (DiSalvo 1988).

The statolith size (SS) of *C. concholepas* shows a less clear pattern of variation with respect to pCO_2 levels. However, significantly smaller statoliths were recorded at medium and high pCO_2 levels (Fig. 5). The average SS measured in egg capsules collected from central (Las Cruces) and southern Chile (Calfuco) were consistent between both experimental series, in close agreement with the smallest protoconch size (PS) at hatching recorded in northern Chile (Antofagasta). The above pattern suggests that mineralisation of larval shell and statoliths in *C. concholepas* is plastic, probably with a latitudinal component of variation. At the beginning of embryonic development in gastropods, bio-mineralization begins by the accumulation of calcium present inside the egg capsules, but then requires an uptake of calcium from the environment (Marxen et al. 2003). In the present study, the egg capsules were exposed for most of the developmental time to relatively stable seawater conditions in the laboratory (ca. 2 mo). Consequently, shell mineralisation in egg capsules of *C. concholepas* should reflect conditions present during the acidification phase and not site-specific conditions present when the egg capsules were laid. However, those egg capsules were laid by females morphologically and physiologically adapted to local conditions. Thus, latitudinal differences in larval traits found in the present study could reflect local adaptation of females to the different climatic factors, such as temperature regime, present along the Chilean coast. In fact, geographical adaptations in thermal tolerance of crustacean larvae have been reported in 2 different populations inhabiting central and southern waters along the Chilean coast (Storch et al. 2009). The above trends serve to emphasize that the study of larval traits in response to OA should consider local adaptation and the existence of latitudinal gradients in relevant variables such as temperature, pH and carbonate systems parameters. The only published study describing the latitudinal variation of pH and the carbonate system parameters in Chile shows a clinal variation of the surface sea temperature with the highest temperatures in northern Chile (Ramajo et al. 2013). Moreover, that study shows a lack of clinal latitudinal variation in total alkalinity and pCO_2 along the Chilean coast, with minimum values of Ω for calcite and aragonite in central Chile and the highest values in northern Chile (Ramajo et al. 2013). Therefore, larvae hatched from egg capsules collected in central Chile were produced by females theoretically more adapted to more corrosive seawater. The existence of female local adaptations to acidified seawater will theoretically

improve the larval performance in comparison with larvae hatched from females adapted to different conditions. However, the present study provides no evidence in support of local adaptation by the spawning stock that may have positive consequences on larval traits at hatching. When negative effects of OA on the investigated larval traits were found, they were mainly concentrated in the highest experimental pCO_2 levels and did not match any latitudinal cline. Protoconch thickness (PT) of *C. concholepas* measured at the outermost larval shell edge was mainly affected by increased pCO_2 levels and their interaction with egg capsule and female identity. This suggests that despite the variability among larvae and females, OA might increase the vulnerability of *C. concholepas* newly hatched larvae (NHL) to physical damage to the shell and predation after hatching. The Ω for calcite and aragonite in the second experimental series was CO_2 supersaturated. Therefore, differences in seawater temperature and the Ω of the 2 main forms of $CaCO_3$ cannot be used to explain differences in PT between both experimental series. However, for Ω > 1, several field studies have recorded reduced calcification rates at elevated pCO_2 (Kleypas et al. 2006 and references therein).

Our results regarding the effects of pCO_2 levels on egg capsule wall thickness (EWT) were not conclusive enough to explain sublethal and lethal responses found in *C. concholepas* NHL. EWT at hatching was not significantly different between pCO_2 levels when capsules were collected from Las Cruces and Calfuco. However, significantly thicker walls were found in egg capsules collected from Antofagasta and exposed to medium and high pCO_2 levels. To our knowledge, studies regarding consequences of OA on EWT are not available. However, intra-specific variations in the thickness of the egg capsule wall of marine gastropods in response to UV radiation have been reported (Rawlings 1996). However, the role of egg capsule wall thickening in *C. concholepas* as a mechanism for protecting developing larvae from the consequences of OA remains speculative and deserves to be investigated further. Since larval encapsulation seems to be a good mechanism for protecting the intra-capsular development, the properties of the egg capsule wall should play an important role in avoiding the potentially negative effects of near-future elevated pCO_2. The egg capsule wall of *C. concholepas* limits oxygen diffusion (Cancino et al. 2000, Brante 2006), and thus, larval development is negatively affected if the extra-capsular oxygen concentration is reduced (Cancino et al. 2003). In our experiment, the egg capsules were maintained in motionless seawater with continuous supply of a mix of air and CO_2 to achieve the required seawater acidification levels; therefore, oxygen supply was not limited. Moreover, the egg capsules were frequently cleaned to remove from their surface mobile and sessile invertebrates that reduce the intracapsular oxygen tension and the rate of calcification, as has been described in other gastropods (Cancino et al. 2000). Oxygen diffusion limitation from the surrounding seawater into the perivitelline fluids though the egg envelope seems to be a critical factor in late embryonic development of cuttlefish *Sepia officinalis* (Gutowska & Melzner 2009). However, unlike most species studied that display a decreased calcification in acidified scenarios (see Fabry et al. 2008), *S. officinalis* embryos are able to form the internal shell under conditions that impair calcification. Gutowska & Melzner (2009) propose the existence in *S. officinalis* of a mechanism linked to retention of excretory CO_2 by embryos already adapted to cope with relatively high pCO_2/low pH values. However, the existence of this type of adaptation that enables the encapsulated embryos/larvae to cope with acidified scenarios is unknown for *C. concholepas*.

In the present study, corrosive seawater, characterised by a Ω < 1 for aragonite, was obtained only in results for the highest pCO_2 levels in the first experimental series (Table 2). Therefore, in this series, net shell corrosion and significant shell dissolution was favoured (Fabry et al. 2008), with expected negative consequences on shell size and thickness and other potential sublethal and lethal effects. This result could in part explain the significantly lower larval survival and smaller statoliths recorded in the first experimental series at high pCO_2 levels. However, negative effects on other larval traits were also recorded in the second experimental series when the Ω calcite and Ω aragonite were not corrosive (Ω > 1). This pattern suggests that the effects of OA on *C. concholepas* NHL traits might occur regardless of whether Ω are corrosive or not.

In the present study, the consequences of OA on larval traits were measured for no longer than 96 h post hatching. Post-hatching mortality is an important issue in *C. concholepas* because this species has a long larval dispersal period of ~3 mo (DiSalvo 1988). Therefore, we cannot exclude the possibility that other lethal or sublethal effects of OA on *C. concholepas* might emerge as carry over effects later during late larval and post-metamorphic stages. However, logistical problems associated with long-term larval rearing in *C. concholepas* and the total

lack of progress in cultivating this species prevent these types of experiments from being conducted. Finally, in coastal rocky intertidal habitats, *C. concholepas* egg capsules may face low pH associated with freshwater discharges (Salisbury et al. 2008), which are common along the central-southern Chilean coast (Dávila et al. 2002). Moreover, natural CO_2 supersaturated surface waters are commonly present along the northern and central Chilean coast (21° to 37° S, Torres et al. 2011). Therefore, the extremely variable conditions present in intertidal habitats could explain the absence of more drastic effects of elevated CO_2 levels on developing and morphological larval traits evaluated in the present study. In nature, if some encapsulated larvae are tolerant or adapted to those high CO_2/low pH conditions, they may act as a source of pre-adapted genotypes facilitating adaptation to future OA scenarios. The present study agrees well with previous studies that have shown the vulnerability of early developmental stages to pCO_2 levels (Przeslawski et al. 2005, Byrne 2011, Bartolini et al. 2013, Byrne & Przeslawski 2013). Our results show the existence in *C. concholepas* NHL of a developmental plasticity in terms of responses to different pCO_2 levels. This plasticity is an important mechanism by which a single genotype can alter its developmental processes and phenotypic outcomes as a permanent response to different environmental conditions during the early ontogeny (Müller & Wagner 1991). The developmental acclimatization has the potential to eventually produce some benefits to the individual involved in terms of energetic costs (Hoffmann 1995) and is crucial in the formation of evolutionary novelties (West-Eberhard 2003). However, the energetic cost of developmental plasticity of larval traits in *C. concholepas* in the face of changes in pCO_2 levels remains unanswered and should be a subject of future studies. A recent study has reported that encapsulation in *Crepidula fornicata* did not protect embryos and larvae from deleterious effects of OA (Noisette et al. 2014). In that study, intracapsular acidosis as a consequence of elevated pCO_2 in the extra-capsular seawater had significant and negative effects on the occurrence of morphological larval abnormalities and on the intensity of birefringence, used as a proxy for the shell mineralisation. In our study, larval abnormalities were rarely detected in the 3 experimental pCO_2 levels and will be reported in a future paper. This suggests that larval encapsulation in *C. concholepas* is a good mechanism for protecting the intra-capsular larval development in this species against the negative effects of near-future elevated pCO_2. The buffer capacity of intracapsular fluids (Ellis et al. 2009) appears to be the most likely mechanism involved in reducing the effects of OA on encapsulated larvae. Even so, the lethal effects found in the present study in terms of reduced HS and ELS in response to OA might have important consequences on the dispersive larval phase and on the annual production of *C. concholepas*. Between 1997 and 2008, the annual landings of *C. concholepas* (metric tonnes × 10^3) and price per tonne exported (US$ × 10^3) were about 2.5 and 17 respectively (Gelcich et al. 2010). Therefore, a reduction of ELS of up to 30%, as reported in the present study under predicted near future OA scenarios, might result in annual losses of ~0.8 × 10^3 t of landings or 5.1 × 10^3 US$ per exported tonne of *C. concholepas*. Our results provide insight into the potential consequences of OA on the early dispersive phase of *C. concholepas*, which may affect the economic revenues provided by this species. However, the results also show geographic variability and developmental plasticity in the investigated traits, which may lead to an increased acclimatization ability, facilitate the persistence of natural populations and mitigate the negative effects that OA might have in other marine invertebrate species with similar life histories. This variability suggests that encapsulated larvae are pre-adapted for life in this particular habitat and therefore less susceptible to changes in pCO_2/low pH conditions as expected in near future OA scenarios.

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