Aquatic Toxicology 212 (2019) 37–46

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Trophic transfer of paralytic shellfish toxin (PST): Physiological and reproductive effects in the carnivorous gastropod *Acanthina monodon* (Pallas, 1774)

Paola V. Andrade-Villagrán[a,b], Jorge M. Navarro[a,c,*], Samyra Aliste[a], Oscar R. Chaparro[a], Alejandro Ortíz[a,c]

[a] Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile
[b] Centro de Investigación en Biodiversidad y Ambientes sustentables (CIBAS), Facultad de Ciencias, Universidad Católica de la Santísima Concepción. Concepción, Chile
[c] Centro Fondap de Investigación Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Universidad Austral de Chile. Valdivia, Chile

ARTICLE INFO

Keywords:
Trophic transfer
Paralytic shellfish toxin
*Acanthina monodon*physiology
Reproduction

ABSTRACT

Harmful algal blooms can adversely affect different levels of the trophic chain, from primary consumers, such as bivalve molluscs, to higher links such as large fish, birds and mammals, including humans. Among secondary consumers, it has been described that carnivorous gastropods can accumulate these toxins when they prey on bivalves that have been exposed to toxic microalgae; these could also harm human health. In Chile, frequent events of harmful algal blooms caused by the dinoflagellate *Alexandrium catenella* have been described. This organism produces paralytic shellfish toxin (PST) which has been identified in some carnivorous gastropods. The objective of this research was to identify the physiological and reproductive response of the carnivorous gastropod *Acanthina monodon* fed on the Mytilid *Perumytilus purpuratus*, which had previously been maintained on a diet containing PST. Specimens of *A. monodon* showed a decrease in ingestion and absorption rate when they consumed PST indirectly through their diet. The oxygen consumption rate was also affected by the diet-time interaction. The variations of these parameters were reflected in the scope for growth, since the available energy was lower in gastropods exposed to toxic diet. Consumption of PST had a negative effect on the reproduction of *A. monodon*, since intoxicated adults presented lower egg-masses and delayed start of oviposition. We observed a delay in the development of the embryos inside the capsules, and a lower number of hatched juveniles, although these few juveniles from intoxicated parents accomplished higher growth rates during the next 6 months. We may therefore suggest that toxin transfer, from harmful microalgae through the trophic chain, can generate deleterious effects on the physiological energetics of the organisms that consume them, affecting their reproductive capacity and early ontogenetic development.

1. Introduction

The effects of harmful algae on marine organisms, especially those consumed by humans, have been widely studied (Bardouil et al., 1993; Basti et al., 2015; Bricelj et al., 2010; Le Goïc et al., 2013; Navarro et al., 2014). Bivalve molluscs have been the principal subject group of these studies, since they are among the primary consumers of toxic microalgae. Their filter-feeding system means that they can accumulate high toxin doses, mainly in the digestive tissue (Bricelj et al., 1990; Marsden and Shumway, 1992; Setälä et al., 2014). The intensity of the effect of toxins on bivalve molluscs appears to be species-specific, but also depends on the development level of individuals (Wang et al., 2006; Bricelj et al., 1996, 1998, 2010). Thus, the clearance rate of adult *Ostrea chilensis* exposed to a toxic diet containing PST is 25% lower than that of individuals fed with a non-toxic diet (Garrido, 2016); however, PST causes greater alterations in the feeding activity of juvenile *O. chilensis*, with a dramatic decrease in their clearance rate (ca. 95%) (Navarro et al., 2016). Despite the damage caused by these toxins to some bivalves, there are species, such as mytilidae, that acquire resistance to toxins, which makes them very effective bioaccumulators (Fernández-Reiriz et al., 2008; Navarro et al., 2011). In the bivalve *Mytilus chilensis* exposed to a diet consisting of 50% *Alexandrium catenella*, a rapid intoxication process was observed. A saxitoxin concentration of 116 µg SXT eq./ 100 g tissue was identified on the second day of exposure to the toxic diet, exceeding the safety levels for human health (80 µg SXT eq./ 100 g). By day 9 of toxic diet exposure, a concentration of 1601 µg

* Corresponding author.
E-mail address: jnavarro@uach.cl (J.M. Navarro).

https://doi.org/10.1016/j.aquatox.2019.04.017
Received 19 March 2019; Received in revised form 25 April 2019; Accepted 28 April 2019
Available online 30 April 2019
0166-445X/

SXT eq./ 100 g tissue was recorded (Navarro et al., 2011). On the other hand, in species that are more sensitive to the presence of toxic microalgae, alterations have been observed in the feeding and respiration rates (*Crassostrea gigas*: Bardouil et al., 1993; *Ostrea edulis*: Lesser, 1993; *Ruditapes philippinarus*: Li et al., 2001; *Pinctada fucata martensii*: Basti et al., 2015; *Ostrea chilensis*: Navarro et al., 2016). The scope for growth (SFG) is another physiological parameter that often present decreasing in individuals exposed to harmful algae (Li et al., 2002; Navarro et al., 2016). On other hand, deleterious effects caused by the direct contact of gametes from marine invertebrates, with harmful algae has been observed, identifying a decrease in sperm motility and cell damage in the spermatozoa of some bivalves (Haberkorn et al., 2010; Le Goïc et al., 2013; Rolton et al., 2015, 2016).

However, despite the large volume of research about the effects of toxic microalgae on the physiology and reproduction of bivalves, there is little information about the effects of toxins acquired indirectly through the consumption of intoxicated prey, on the physiological processes of higher trophic level organisms, considering that bivalves act as toxin bioaccumulators, making them primary links in the food chain. The presence and accumulation of toxins from harmful algae have been identified in different taxonomic groups, such as gastropods, crustaceans, cephalopods, fish, birds and marine mammals (Sierra-Beltran et al., 1997; Scholin et al., 2000; Costa et al., 2005; Sephton et al., 2007; Deeds et al., 2008; Lopes et al., 2013). This accumulation may have its origin in the direct consumption of harmful algae, or in the ingestion of toxic prey. In the gastropod *Xantochorus cassidiformis*, trophic transference of PST through the ingestion of intoxicated bivalves has been identified (Maturana, 2005).

The coast of Chile is frequently exposed to harmful algae blooms (HABs), mainly due to the presence of the dinoflagellate *Alexandrium catenella*, which produces paralytic shellfish toxin (PST). Compagnon et al. (1998) recorded high concentrations of saxitoxin in the carnivorous gastropods *Concholepas concholepas* (9164 µg STX eq / 100 g tissue) and *Argobuccinum ranelliformis* (14,057 µg STX eq /100 g tissue), during a bloom of *A. catenella* on the southern coast of Chile (Darwin channel, 1996). On the other hand, among the few studies of physiological responses, Maturana (2005) does not record effects on feeding and growth for individuals of the gastropod *Xanthochorus cassidiformis* exposed to a diet (the clam *Mulinia edulis*) intoxicated with *A. catenella*; nevertheless, the same study does highlight a possible effect on reproductive success, identifying a lower percentage of capsules laid per female exposed to the toxic diet.

The frequency of harmful algal blooms has been increasing in the world's oceans (Anderson et al., 2012; Anderson, 2014). In the case of Chile, the first record of the dinoflagellate *Alexandrium catenella* occurred in 1972, in the Magallanes Region. Its distribution subsequently extended northwards, reaching the Aysén Region in 1994 and Chiloé Island in 2002, where the recorded concentration reached values of 779,000 cells L^{-1}, with toxicity over 20,000 µg of STX eq./ 100 g tissue (Clement et al., 2002). During 2016, the distribution range continued to extend northwards, encompassing areas with no previous records of PST events and reaching the Los Ríos Region (39° 45′S) (Buschmann et al., 2016).

Considering the increase in the frequency, intensity and geographical distribution of *Alexandrium catenella* blooms in Chile, there is concern to identify how organisms of higher trophic levels respond physiologically and reproductively to the indirect intake of the toxins (PST) through the ingestion of toxic prey (bivalves). The carnivorous gastropod *Acanthina monodon*, which inhabits a large part of the Chilean coast, was used as the model species for this investigation.

The muricid gastropod *Acanthina monodon* is described as an active predator on mytilidae and cirripedia of the rocky intertidal and shallow subtidal zone (Osorio, 1979, Poblete et al., 1987). In Chile this gastropod is distributed between Coquimbo (29° 54′ S; 71° 15′ W) and Cabo de Hornos (55° 58′ S; 67° 16′ W). It is a dioecious species with internal fertilization and direct development. The embryos develop inside capsules that are deposited by the female on rocky substrates (Gallardo, 1979). Together with the embryos, the female lays nurse eggs inside the capsule; these serve as a food source for the development and growth of the viable embryos, which hatch after approximately 55 days (Gallardo, 1979).

The objective of this research was to evaluate the physiological energetics and reproductive responses of the gastropod *Acanthina monodon* under exposure to a toxic diet composed of the bivalve *Perumytilus purpuratus,* previously intoxicated with paralytic shellfish toxins by feeding with *A. catenella*.

2. Materials and methods

2.1. Obtaining samples and acclimation

Acanthina monodon snail specimens were collected from the rocky intertidal of Calfuco beach (39° 46′ S; 73° 23′ W), located on the coast of Valdivia in southern Chile. At the date of collection (June 2014), this site did not present records of harmful algal blooms (HAB), therefore the absence of exposure to Paralytic Shellfish Toxins (PST) is assumed. Adult gastropods (n = 150) with sizes between 35 and 40 mm shell length were collected at random. In parallel and in the same place, specimens of the bivalve *Perumytilus purpuratus* were collected and used as prey, with a size range between 15 and 30 mm in length. Individuals of *Acanthina monodon* were exposed to an acclimation period of 10 days, kept in filtered sea water with permanent aeration and constant salinity and temperature (30 psu and 14 °C). The water was changed every 4 days and they were fed *ad libitum* with a diet composed of the bivalve *P. purpuratus*.

2.2. Cultivation of microalgae and diet preparation

The non-toxic microalga *Isochrysis galbana*, used in the preparation of both mussel diets (toxic and non-toxic preys, was cultivated using enriched, filtered (0.45 µm) and sterilized seawater (f/2 medium; Guillard, 1975) at 25 °C and a salinity of 30 under a photoperiod of 14/10 h (L/D). The mean luminous intensity for the culture was 61.34 ± 4.19 µmol m^{-2} s^{-1} as measured using a LI−COR L1-250 A Light Meter. The dinoflagellate *Alexandrium catenella* was isolated in 2011 from Coldita Island, south of Chiloé, Los Lagos Region, by the Fisheries Development Institute (Instituto de Fomento Pesquero, IFOP). These dinoflagellates were cultivated in filtered seawater (0.45 µm) enriched with L1 culture medium (Guillard, 1995) at a temperatura of 14 °C and a salinity of 30, under a photoperiod of 14/10 (L/D), with a mean luminous intensity of 59.53 ± 0.25 µmol m^{-2} s^{-1} as measured using a LI−COR L1-250 A Light Meter. As *A. catenella* cells were non homogeneously distributed within the culture, bottles were gently homogenised before samples were taken for diet preparation, (see Navarro et al., 2006b for more information about culture conditions of *A. catenella*). Both cultures were used in their exponential growth phase.

The non-toxic diet corresponded to expose for 4 days the mussels *P. purpuratus* to 100% the non-toxic microalga *Isochrysis galbana*. By other hand, the toxic diet consisted in exposing for 4 days the mussel preys to a mixture of 30% (by weight) of *Isochrisys galbana* and 70% (by weight) of the toxic dinoflagellate *Alexandrium catenella* for 4 days.

The diets for the mussel preys, were supplied continuously by a peristaltic pump (Masterflex) for a period of 4 days before the bivalves were delivered as food prey to the *Acanthina monodon* specimens. New individuals of *P. purpuratus* (toxic and non-toxic) were offered to *A. monodon* every 4 days, and these conditions were maintained for 60 days. Several physiological parameters (organic ingestion rate, absorption efficiency, oxygen consumption, excretion rate and scope for growth) were measured every 10 days. Analyses carried out during the experiments indicated a mean toxicity of 1198.03 ± 166.72 µg of STX equiv/100 g tissue in *P. purpuratus* fed with the toxic diet. The

concentration of PST present in the soft tissues of the mussels used as prey was determined by High Performance Liquid Chromatography (HPLC) with post-column derivatization and fluorescence detection, following the method described by Franco and Fernandez-Vila, 1993. The analyzes were carried out by the Marine Toxins Laboratory of the Instituto de Fomento Pesquero of Punta Arenas (IFOP), Chile.

2.3. Experimental design

We used 20 specimens of *A. monodon* (shell length: 35–40 mm), which were taken at random and kept individually in aquaria with a volume of 4 L. All the experimental aquaria were maintained with filtered seawater, with permanent aeration, constant salinity (30) and submerged in a temperature-controlled bath (14 °C); the water was replaced every 4 days. Ten specimens of *A. monodon*, kept individually one in each aquarium, were fed with the non-toxic diet composed of 3 specimens of the bivalve *Perumytilus purpuratus* per each snail (ad libitum). The other ten aquaria were designated as the toxic group, where the specimens of *A. monodon* were also fed with 3 *P. purpuratus* each, which in turn had been fed with the toxic diet.

To identify the effects of the toxic diet on fecundity and embryonic development of *A. monodon*, 8 aquaria of 50 L each were used with 15 specimens of *A. monodon* per aquarium for 120 days (including the gametogenic period). The aquaria were maintained with a recirculation system of filtered seawater with constant aeration, at a temperature of 14 °C and salinity of 30 psu. The gastropods corresponding to 4 aquaria were fed *ad libitum* a non-toxic diet composed of *P. purpuratus* previously fed with a pure culture of the microalga *I. galbana*; while the gastropods kept in the remaining 4 aquaria were fed with the toxic diet, composed of specimens of the bivalve *P. purpuratus*, previously intoxicated (70% *A. catenella* + 30% *I. galbana*). The diets offered to both groups of snails corresponded to 3 *P. purpuratus* for each individual of *A. monodon*, replaced with new mussels every 4 days.

Daily observations were made to detect the presence of egg-masses of the gastropod *A. monodon*. Once present, the eggs were removed from the aquaria and placed in a 2 L glass aquarium to complete their incubation. These aquaria were submerged in a thermoregulated bath at 14 °C, 30 salinity, with constant aeration and seawater exchange every two days.

2.4. Physiological measurements

2.4.1. Organic ingestion rate (mg/day/ind)

The organic ingestion rate (OIR) was quantified individually for each experimental specimen of *A. monodon*, fed with a toxic or non-toxic diet. Empty valves of the prey *P. purpuratus* were collected each day; in some cases, valves still contained prey tissue. The shells were measured and the remaining tissue was dried at 100 °C for 24 h. To calculate the food ingested by *A. monodon*, a regression curve of shell length (L) versus dry weight (DW) of the soft tissue of *P. purpuratus* was used ($DW = 11.64\,L^{2.63}$; $R^2 = 0.97$). The tissue not consumed by *A. monodon* was discounted from the dry weight of the meat calculated to obtain the corrected value of the daily ingestion rate; this was later multiplied by the organic fraction of the food (0.87), which was estimated by combusting the soft tissue of 10 individuals of *P. purpuratus* at 450 °C for 3 h.

2.4.2. Absorption efficiency (%) and absorption rate (mg/day/ind)

The absorption efficiency (AE) was determined by the gravimetric method of Conover (1966), which assumes that an animal can digest and absorb the organic component of the food but not the inorganic fraction. For this analysis, the faeces of *A. monodon* from both experimental groups were collected and filtered in 25 mm diameter Whatman GF/C glass fibre filters, previously washed, dried and burned. Each filter with faeces was washed with an isotonic solution of ammonium formate (NH4COOH) to remove the salts present in the faeces. Subsequently, the dry weight (100 °C for 24 h) and organic and inorganic matter (450 °C for 3 h) of the faeces were obtained. The faeces produced by each gastropod were collected throughout the experiment and pooled every 10 days for analysis of the AE. The organic fraction of the prey was determined in the same way as the faeces, by drying and subsequently burning the meat of *Perumytilus purpuratus*. The absorption rate was calculated as the product of the absorption efficiency and the organic food intake, according to Bayne et al. (1985).

2.4.3. Oxygen uptake (ml/day/ind)

Oxygen uptake (VO_2) was determined using a PreSens fibre optic equipment, FIBOX 3, through oxygen sensors attached to the inner wall of hermetically sealed chambers. The oxygen consumption of each individual of *A. monodon*, from the toxic and non-toxic treatments, was measured: the individual was incubated for 1 h in filtered seawater (1.0 µm), previously saturated with oxygen, inside the respiration chambers (140 ml). Respiration chambers without animals were used as a control for each treatment and for each day of measurement. All the chambers were kept submerged in a temperature-controlled water bath at 14 °C and measurements of the oxygen dissolved in the seawater were recorded; the oxygen concentration was not allowed to fall below 70% saturation. The data obtained were recorded using the OxyView 3.51 Software program.

2.4.4. Excretion rate (µg NH4-N /day/ind)

The excretion rate (ER) was determined by the colorimetric method of Solórzano (1969). After 1 h of incubation, a water sample of 5 ml was taken in synchronisation from each of the breathing chambers described above, both the experimental chamber and the control chamber without animals. A calibration curve was made using a set of solutions with known ammonium concentrations. The samples were kept in the dark for 2 h, and their absorbance was subsequently read at a wavelength of 640 nm using an Optizen POP uv/vis model spectrophotometer.

2.4.5. Scope for growth (J/day/ind)

Scope for growth (SFG) is a physiological index that indicates the energy available for growth of the organism, for the formation of both somatic and reproductive tissues. This index reflects the relationship between the energy absorbed and the energy used for respiration and excretion, expressed in Joules (Widdows, 1985). To calculate the SFG, the different physiological parameters 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 = 19.5 J, using the value obtained by Navarro et al. (2006a) for *Semimytilus algosus*, another sympatric species of mytilid bivalve.

2.5. Measurement of reproductive parameters

2.5.1. Fecundity, intracapsular embryo development and hatching

The egg-masses extracted from toxic and non-toxic aquaria were counted and monitored through embryo development. The total number of capsules per egg-mass was counted and the day of oviposition was recorded, to later identify the hatching time. Three capsules were taken at random from each egg-mass, on days 7 and 55 of embryonic development. A stereoscopic microscope (Zeiss model Stemi 2000-C) was used to photograph and then determine the number and size of the embryos, the number of nurse eggs and the level of embryo development in each capsule using the software Image-Pro Plus. At the beginning of embryo development (day 7) it was impossible to differentiate the nurse eggs from the embryonic eggs, so both were considered in analyses. The embryo size recorded on 7 day corresponds to egg diameter; the offspring size recorded on 55 day corresponds to shell length.

2.5.2. Growth of juvenile hatched

To identify a possible carry-over effect of the parents' diet (toxic or non-toxic) on the growth of the offspring, a monthly record of the juvenile shell length was kept over a period of 6 months. Earliest capsules laid, both by toxic (n = 6 capsules) and non-toxic parents (n = 9 capsules), were extracted from the aquaria and kept, separately, in glass chambers (volume = 2000 ml). Each chamber was provided with seawater previously filtered (1.0 µm) and sterilized by UV light. The capsules were maintained with constant aeration and under controlled conditions of temperature (14 °C) and salinity (30). The water was changed every two days. Once the hatching process was complete, the number of hatched juveniles per capsule was counted and the shell length of each was recorded, using a Nikon D90 camera attached to a stereoscopic microscope (Zeiss model Stemi 2000-C). Each image obtained was later analysed using the image analysis program Image proplus. Once the number and size of the hatched juveniles were obtained, they were kept in small chambers with seawater under the conditions described above. The juveniles were fed *ad libitum* with the non-toxic bivalve *Semimytilus algosus* collected from the same environment inhabited by *A. monodon*.

2.6. Statistical analysis

Analysis of variance with repeated measures was performed to evaluate the effect of time, diet and their interaction on the different physiological parameters. The corresponding tests of normality, homoscedasticity and sphericity were carried out to verify the assumptions of the statistical analysis. In order to identify the effect of the diet consumed by adults on reproductive aspects, a one-way analysis of variance was performed, also verifying that the assumptions of normality and homoscedasticity were met. When one of the assumptions was not met, the data were transformed to their square root (physiological measurements AR, VO2, SFG). When data were not normal or homoscedastic, a nonparametric analysis was performed using the Mann-Whitney *U* test For the all statistical analyses, a significance of 0.05 was used. All analyses were performed using the STATISTICA 7.0 software.

3. Results

Paralytic shellfish toxin was observed in all *A.monodon* samples analyzed (n = 8) from toxic group, identifying a mean concentration of 225.29 ± 33.56 µg SXT eq/ 100 g tissue. Conversely, PST presence was not detected in control individuals (non-toxic diet).

3.1. Physiological measurements

3.1.1. Organic ingestion rate (OIR)

Both the exposure time and the diet had a significant effect, separately, on the OIR of *A. monodon* (two-way Anova repeated-measures: Time: $F_{5, 90} = 3.46$, $p = 0.006$; Diet: $F_{1, 90} = 4.63$, $p = 0.045$, Fig. 1A). A lower mean OIR was observed in the individuals exposed to the toxic diet (3.74 ± 0.55 mg/day, mean ± SE) than in those fed with non-toxic prey (6.08 ± 0.78 mg/day, mean ± SE), during all the days of measurement (Fig. 1B). On the other hand, there was an increase in the OIR during the course of the experiment, specifically until day 50, for both treatments (Fig. 1). The increase in the OIR was more marked in the specimens of *A. monodon* fed with toxic prey, with quadrupling of values between day 10 (1.79 ± 1.20 mg/day) and day 50 (7.65 ± 1.63 mg/day). In both treatments there was a decrease at day 60.

3.1.2. Absorption efficiency (AE) and absorption rate (AR)

There was an effect of the interaction between diet and exposure time on AE (two-way Anova repeated-measures: Diet * time: $F_{5, 90} = 9.71$, $p < 0.001$, Fig. 2A). The mean AE during the 60 days of treatment was similar for the specimens fed with each diet (toxic: 54 ± 2.2%, non-toxic: 56 ± 2.8% mean ± SE, two-way Anova repeated-measures: Diet: $F_{1, 90} = 0.67$, $p = 0.79$, Fig. 2B). The AE was variable over time for both toxic and non-toxic gastropods, however the time effect, separately, was not significant (two-way Anova repeated-measures: Time: $F_{5, 90} = 0.00$, $p = 1.00$).

On the other hand, AR was affected, separately, by diet (two-way Anova repeated-measures: diet: $F_{1, 90} = 4.84$, $p = 0.04$, Fig. 2C) and time (two-way Anova repeated-measures: time: $F_{5, 90} = 3.653$, $p = 0.004$, Fig. 2C). The mean AR was higher in *A. monodon* specimens fed a non-toxic diet (3.6 ± 1.4 mg/day, Fig. 2D) than in the intoxicated group (2.1 ± 0.3 mg/day, Fig. 2D). We also observed that the AR of toxic gastropods increased over time approximately until day 50, diminishing on day 60. The AR of non-toxic gastropods was more variable over time, reaching its highest values in the final days of the experiment (50–60).

3.1.3. Oxygen uptake (VO_2)

The oxygen uptake of *A. monodon* was significantly affected by the interaction of the diet consumed and the exposure time (two-way Anova repeated-measures: Diet * time: $F_{6, 108} = 2.64$, $p = 0.019$, Fig. 3A). The results indicate that there was a decrease in VO_2 from the beginning (day 0) to the end of the experiment (day 60), both in gastropods fed a toxic diet (day 0: 4.14 ± 1.09 ml/day, day 60: 1.61 ± 0.40 ml/day, mean ± SE) and in those with a non-toxic diet (day 0: 4.21 ± 0.97 ml/day, day 60: 1.91 ± 0.27 ml/day, mean ± SE). However, a large variation in oxygen uptake was observed over the period of exposure to the diets, decreasing towards day 20 and then increasing markedly to day 30, when a greater VO_2 was detected in the specimens fed with a toxic diet (Fig. 3A). Nevertheless, the diet, separately, did not have a significant effect on oxygen uptake (two-way Anova repeated-measures: diet: $F_{1, 108} = 0.58$, $p = 0.45$, Fig. 3B).

3.1.4. Excretion rate (ER)

The diet*time interaction did not affect significantly the ER of *A. monodon* (two-way Anova repeated-measures: diet*time: $F_{6, 108} = 0.63$, $p = 0.70$, Fig. 4A). However, a significant increase in the ER was observed over the experimental period, specifically from day 10 to day 50, in gastropods fed with either diet (two-way Anova repeated-measures: Time: $F_{6, 108} = 5.10$, $p < 0.001$, Fig. 4A). In the group of gastropods exposed to a toxic diet, the average ER during the entire experimental period was 624.7 ± 49 µg NH_4-N/day per individual. The mean ER identified in the group fed a non-toxic diet was 608.2 ± 54 µg NH4-N/day per individual (two-way Anova repeated-measures: diet: $F_{1, 108} = 0.14$, $p = 0.70$, Fig. 4B).

3.1.5. Scope for growth (SFG)

The SFG was affected significantly by the diet and the time separately (two-way Anova repeated-measures: diet: $F_{1, 90} = 12.88$, $p = 0.002$; time: $F_{5, 90} = 4.12$, $p < 0.001$, Fig. 5A). This index showed a significant increase over time in both experimental groups. However, the SFG of *A. monodon* was not affected significantly by the diet*time interaction (two-way Anova repeated-measures: diet*time: $F_{5, 90} = 1.97$, $p = 0.09$, Fig. 5A). The gastropods fed with diet containing the toxic dinoflagellate *A. catenella* had a negative SFG for most of the time, presenting, on average, a SFG of -22 ± 9 J/day (Fig. 5B). Similarly, individuals fed a non-toxic diet had negative values at the beginning of the experiment, however the values increased markedly during the later days of the experiment, to reach a mean SFG of 20 ± 13 J/day (Fig. 5B).

3.2. Reproductive parameters

The specimens of *A. monodon* exposed to a non-toxic diet produced a greater number of ovipositions (5) than the specimens fed a toxic diet (2) during the entire experimental period, which lasted 120 days. We

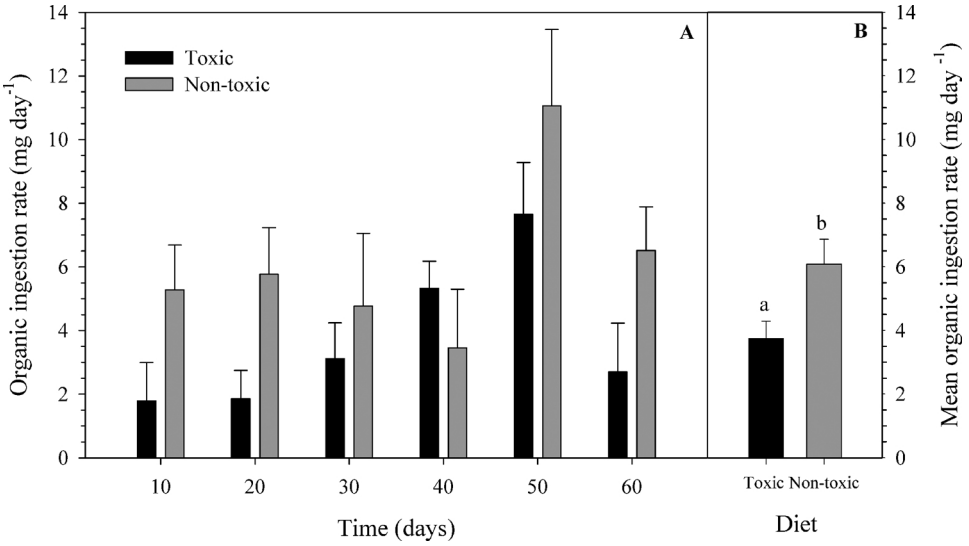

Fig. 1. *Acanthina monodon*. A) Organic ingestion rate (OIR) in individuals fed a toxic (*P. purpuratus* fed with *A. catenella*) and non-toxic diet (*P. purpuratus* fed with *I. galbana*) through the experimental period. Values correspond to mean (n = 10) ± standard error. B) Mean OIR during the whole experimental period, for toxic and non-toxic individuals. Values correspond to mean (n = 60) ± standard error. Different letters indicate significant differences.

also observed a delay in the start of oviposition in individuals fed a toxic diet (oviposition started: day 35) compared to those fed a non-toxic diet (oviposition started: day 8). No significant differences were observed in the number of capsules per egg-mass between gastropods fed a toxic (102 ± 3.5 capsules / oviposition) and non-toxic diet (95 ± 37 capsules / oviposition) (one-way Anova: $F_{1,\ 5} = 0.013$, $p = 0.91$).

3.2.1. Intracapsular embryo development

The time of intracapsular development, from oviposition to

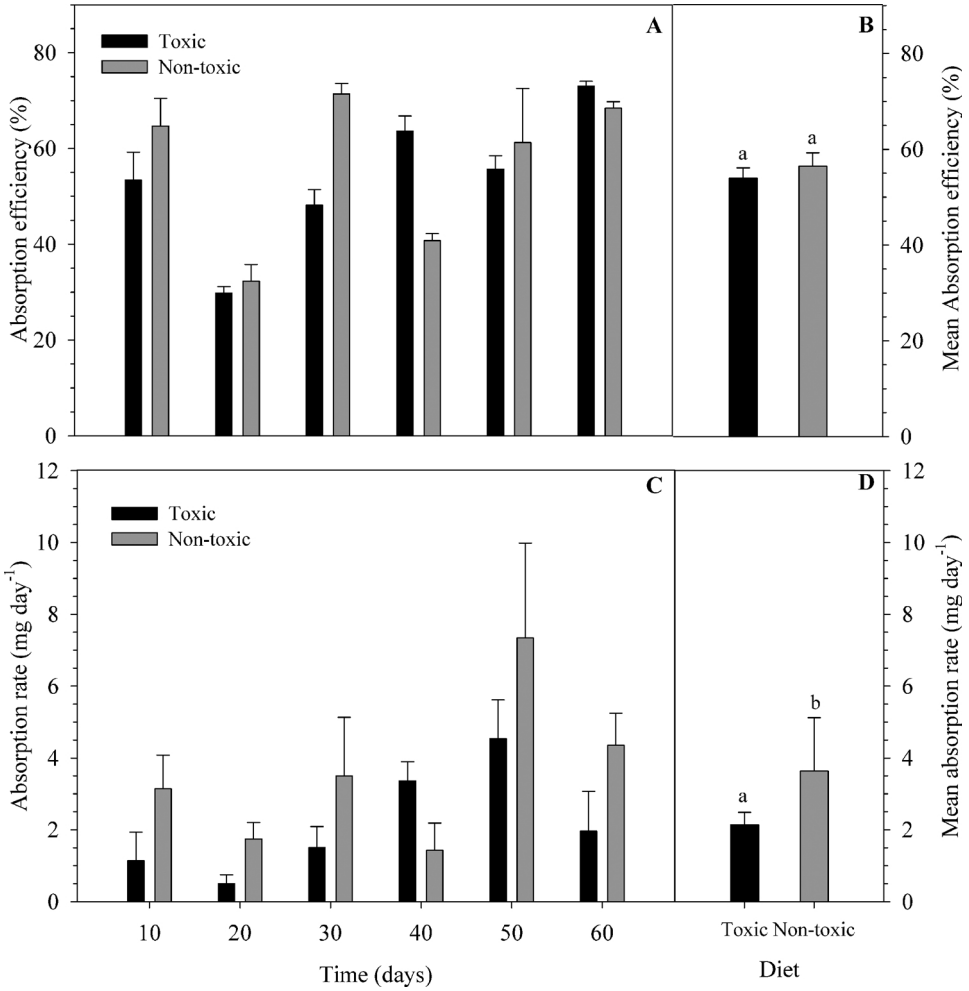

Fig. 2. *Acanthina monodon.* A) Absorption efficiency and C) absorption rate on each measurement day for each diet (toxic and non-toxic).Values correspond to mean (n = 10) ± standard error. B) Mean absorption efficiency and D) mean absorption rate, for toxic and non-toxic diet during the whole experimental period. Values correspond to mean (n = 60) ± standard error. Different letters indicate significant differences.

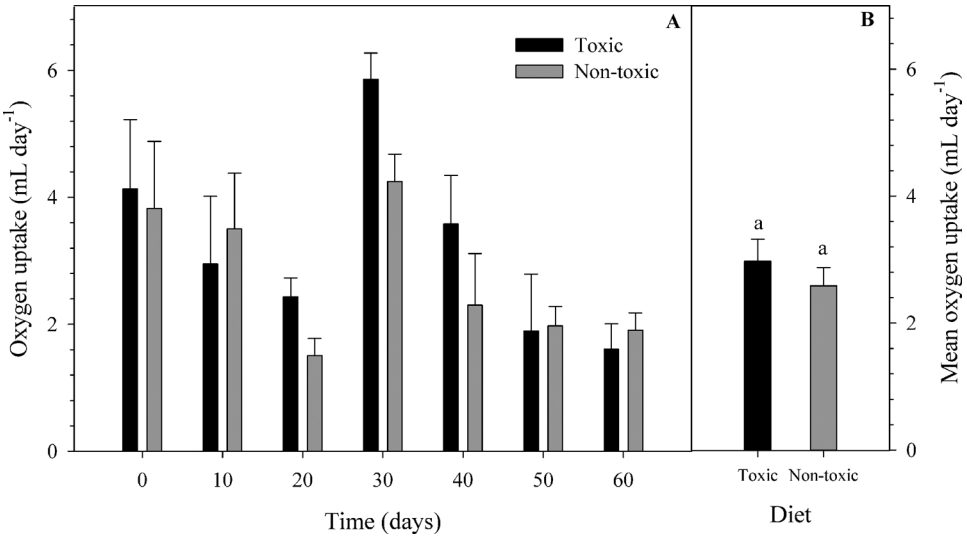

Fig. 3. *Acanthina monodon*. A) Oxygen uptake in specimens exposed to a toxic (*P. purpuratus* fed with *A. catenella*) and non-toxic diet (*P. purpuratus* fed with *I. galbana*), through the experimental period. Values correspond to mean (n = 10) ± standard error. B) Mean oxygen uptake for the whole experimental period, for each diet. Values correspond to mean (n = 60) ± standard error. Different letters indicate significant differences.

hatching, was not significantly affected by the diets supplied to the parents (one-way Anova: $F_{1, 5} = 3.33$, $p = 0.12$). Capsules from parents fed with a toxic diet took an average of 66 ± 0.5 days to hatch, while capsules produced by non-intoxicated parents took 70 ± 1.2 days until hatching. The consumption of a toxic diet by *A. monodon* does not cause significant effects on the size of the embryos produced on day 7 (Mann-Whitney U-test: U = 27, p = 0.16, Table 1), or on day 55 of embryo development (one-way Anova: $F_{1, 18} = 0.026$, $p = 0.87$, Table 1). On day 7 of intracapsular development, the number of eggs (embryos + nurse egg) per capsule did not present differences between capsules from toxic and non-toxic parents (one-way Anova: $F_{1, 19} = 1.30$, $p = 0.26$, Table 1). Similarly, the number of total offspring (veligers + pre-hatch juveniles) per capsule until day 55 did not vary between treatments (Mann-Whitney U-test: U = 45, p = 1.0, Fig. 6A). However, on day 55 of intracapsular development differences were observed in the embryo development level, specifically in the percentage of pre-hatch juveniles per capsule, between capsules from parents fed toxic and non-toxic diets (one-way Anova: $F_{1, 19} = 138.9$, $p < 0.0001$, Fig. 6B). The mean percentages of pre-hatch juveniles per capsule were 0% and 91% for egg masses laid by intoxicated and non-intoxicated gastropods respectively. Differences were also identified in the percentage of veliger larvae per capsule at the end of development (day 55), which was significantly higher in capsules from toxic (100%) than non-toxic parents (9%) (one-way Anova: $F_{1, 19} = 33.65$, $p < 0.0001$, Fig. 6B). One of three capsules taken randomly from 1 egg-mass from toxic parents did not present embryo development. On the other hand, the number of nurse eggs per capsule at day 55 was significantly higher in capsules from toxic (192 ± 90 nurse eggs) than non-toxic parents (15 ± 7 nurse eggs) (one-way Anova: $F_{1, 19} = 6.20$, $p = 0.022$). Despite the differences in embryo development level, the percentage of total offspring developed, in relation to the initial number of eggs, does not change between capsules from intoxicated (7.6% ± 3.2%) and non-intoxicated parents (7.9% ± 0.7%) (one-way Anova: $F_{1, 19} = 2.63$, $p = 0.12$), at least until day 55 of intracapsular development.

3.2.2. Hatching and juvenile growth

The hatching process was observed in a total of 13 capsules from non-toxic parents and 9 capsules from toxic parents, identifying a significant higher number of live juveniles hatched from the capsules from non-toxic parents (one-way Anova: $F_{1, 20} = 17.41$, $p = 0.0004$). The mean number of juveniles hatched per capsule from toxic parents (21.1 ± 3.7 juvenile/capsule, mean ± standard error) corresponds to 44% of the juveniles hatched from capsules from non-toxic parents (48.8 ± 4.8 juvenile/capsule). No differences were observed in the hatching size between juveniles from toxic (1.16 ± 0.010 mm, n = 211 juvenile) and non-toxic parents (1.17 ± 0.018 mm, n = 204 juvenile) (one-way Anova: $F_{1, 413} = 0.21$, $p = 0.64$, Fig.7). However,

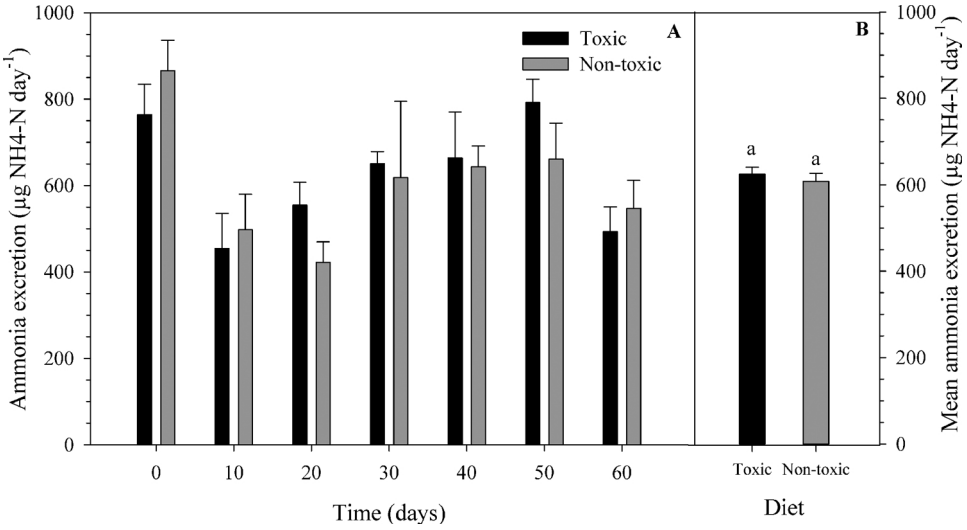

Fig. 4. *Acanthina monodon*. A) Ammonium excretion rate in specimens exposed to a toxic (*P. purpuratus* fed with *A. catenella*) and non-toxic diet (*P. purpuratus* fed with *I. galbana*), through the experimental period. Values correspond to mean (n = 10) ± standard error. B) Mean ammonia excretion for the whole experimental period of each diet. Values correspond to mean (n = 60) ± standard error. Different letters indicate significant differences.

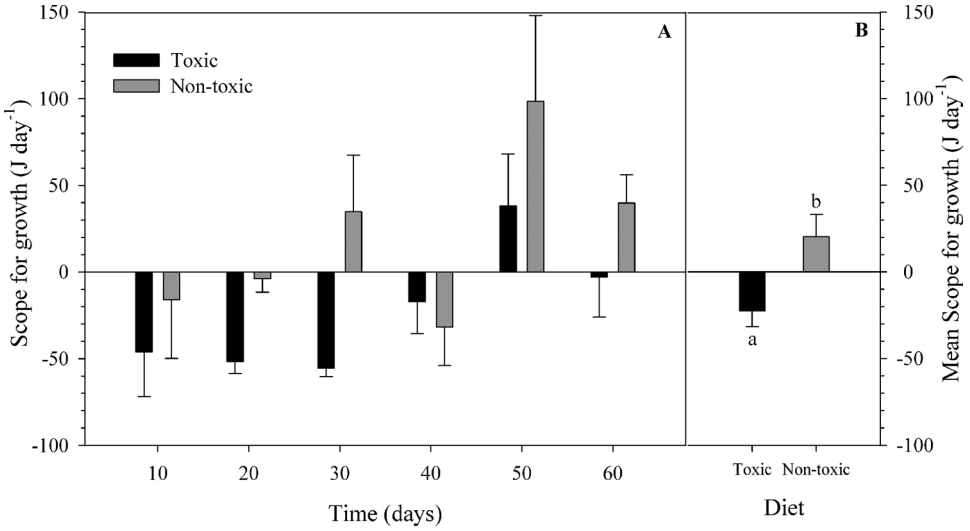

Fig. 5. *Acanthina monodon*. A) Scope for growth in adult specimens exposed to toxic (PST) and non-toxic diet, through the experimental period. Values correspond to mean (n = 10) ± standard error. B) Average of scope for growth for each diet considering the whole experimental period. Values correspond to mean (n = 60) ± standard error. Different letters indicate significant differences.

Table 1
Acanthina monodon. Mean amount and size of embryos per capsule, at the beginning (day 7) and end (day 55) of intracapsular development, for capsules from females fed a toxic (*P. purpuratus* fed with *A. catenella*) and a non-toxic diet (*P. purpuratus* fed with *I. galbana*). The number and size of embryos for day 7 includes embryos and nurse eggs, because they do not present morphological differences at the beginning of development. Different letters indicate significant differences for each day, between capsules from intoxicated and non-intoxicated females.

	Day	Toxic	Non-toxic
N° of embryos	7	461 ± 14 a	392 ± 37 a
	55	34 ± 14 a	28 ± 1.7 a
Embryo size (μm)	7	224 ± 1.8 a	238 ± 6.6 a
	55	953 ± 46 a	942 ± 32 a

from month 1 a significant increase was observed in the size of juveniles from toxic parents (one-way Anova: $F_{1,\ 97} = 6.68$, $p = 0.011$, Fig.7), and this trend was maintained until month 6 (one-way Anova: $F_{1,\ 33} = 5.00$, $p = 0.03$, Fig.7).

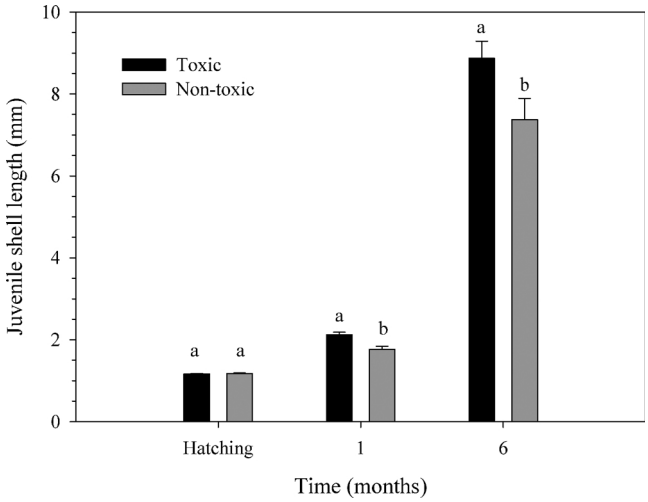

Fig. 7. Juvenile growth from toxic and non-toxic parents. Juvenile shell length at hatching (Toxic: n = 211 juvenile; Non-toxic: n = 204 juvenile), month 1 (Toxic: n = 41 juvenile; Non-toxic: n = 57 juvenile) and month 6 (Toxic: n = 17 juvenile; Non-toxic: n = 18 juvenile). Different letters indicate significant differences for each time. Values indicate mean ± standard error.

4. Discussion

Trophic transfer, through diet, of toxins produced by harmful algae has been described in different marine invertebrates; in the specific case of carnivorous gastropods, an accumulation of toxins has been identified in different tissues, but principally in the digestive glandule (Ito

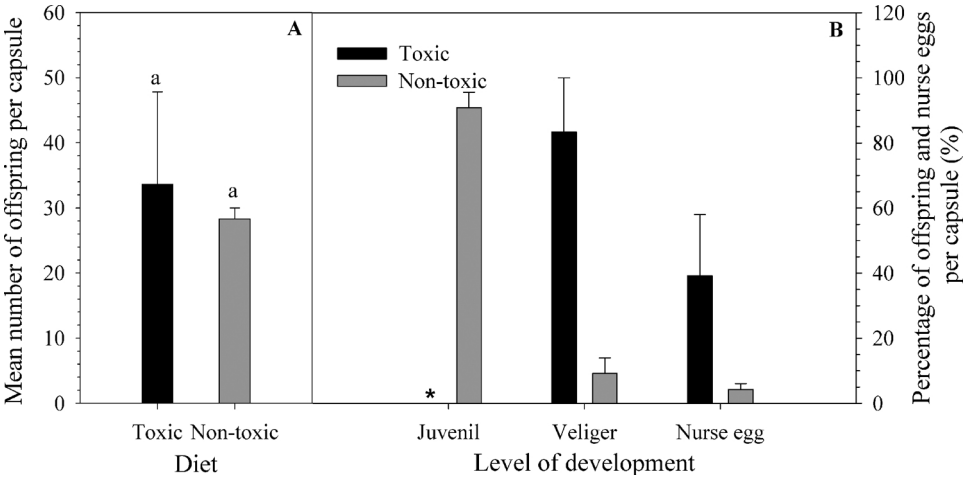

Fig. 6. *Acanthina monodon*. A) Mean number of total offspring (veliger + juveniles) per capsule at day 55, in capsules from females fed a toxic (n = 6 capsules) and a non-toxic diet (n = 15 capsules). Different letters indicate significant differences. B) Variation of the offspring percentage, in different development stages (juveniles, veliger larvae) and nurse eggs per capsule, at day 55 of intracapsular development, from females fed a toxic (n = 6 capsules) and non-toxic diet (n = 15 capsules). * indicate zero. Values indicate the mean ± standard error.

et al., 2004; Maturana, 2005; Choi et al., 2006; Deeds et al., 2008; Harding et al., 2009). The snail *Acanthina monodon* also presented transfer and accumulation of paralytic shellfish toxins after exposure to a toxic diet. After 60 days of exposure to prey intoxicated with *A. catenella*, the gastropods accumulated 225.29 ± 33.56 µg SXT eq./100 g, exceeding the safety limit for human health (80 µg SXT eq./ 100 g). Higher toxin concentrations have been observed in other carnivorous gastropods from Chile (*Concholepas concholepas*: 9164 µg STX eq / 100 g tissue; *Argobuccinum ranelliformis*: 14,057 µg STX eq / 100 g tissue, Compagnon et al., 1998), although there is no information about the effect of PST accumulation on the physiology and reproduction of these organisms.

The effects caused by harmful algae on marine invertebrates depend on several factors, such as exposure time and the concentration of the ingested toxin. (Wang et al., 2006; Bricelj et al., 2010; Basti et al., 2015). In *Acanthina monodon*, our results indicate that in most of the physiological parameters quantified, there is an exposure time effect which works separately from or in interaction with the diet. This result differs from that described by Maturana (2005), who indicates that consumption of the clam *Mulinia edulis* containing PST does not affect the physiological energetic of the gastropod *Xanthochorus cassidiformis*. According to our results in individuals of *A. monodon* fed with PST-intoxicated prey, is possible to observe changes in the organic ingestion rate (OIR) which decreases significantly in specimens fed a toxic diet. However, an increase may be observed in this parameter over time in animals exposed to the toxic diet, suggesting that they become acclimated to the diet, although, the OIR in non toxic individuals, always was high. Lance et al. (2007) described similar results for juveniles of the freshwater gastropod *Lymnaea stagnalis* exposed to the toxic cyanobacterium *Planktothrix agardhii,* where the feeding parameters increased through the time. A similar pattern was observed for the absorption rate (AR) in *A. monodon*. The gastropods fed with a non-toxic diet presented a higher AR than those fed a toxic diet during most of the experimental period, although no differences were observed in the absorption efficiency between gastropods feeding with different diet (toxic and non-toxic).

Despite presenting great variation in oxygen uptake over time, *Acanthina monodon* showed an evident decrease in oxygen consumption between the beginning and end of measurements, which is highlighted by the statistical significance of the interaction between diet and time. This response was observed in both groups of snails (toxic and non-toxic), suggesting possible acclimation to laboratory conditions, rather than to the effect of PST. However, the oxygen uptake, from day 20 onwards, is higher in intoxicated animals than in the control group (non-toxic), suggesting a higher metabolic expenditure, probably due to detoxification processes and toxin biotransformation (Bricelj et al., 1990; Choi et al., 2006). However, the oxygen uptake seems to be species-specific; in juveniles of *Ostrea chilensis* exposed to a toxic diet with *A. catenella*, a decrease in oxygen consumption was observed as compared to the control group (Navarro et al., 2016); while Marsden and Shumway (1993) concluded that oxygen uptake was unaffected in five species of filter feeding bivalves after 1 h of exposure to *Alexandrium tamarense*.

A significant effect of PST on the excretion rate of the mussel *Mytilus chilensis* has been described by Navarro and Contreras (2010). This response was associated with the capacity of the mussels to degrade the PST toxin, which is a rich source of nitrogen (Pérez, 1998). The present study shows that excretion rate was not affected by the paralytic shellfish toxin, suggesting that this is species specific and depends on multiple factors, such as the concentration and toxicity of the algae, the history of exposure to toxic blooms (Bricelj et al., 2005) and differences in digestive function (Leverone et al., 2007).

For *A. monodon*, the high values of oxygen uptake in conjunction with low ingestion and absorption rate observed in intoxicates gastropods, were reflected clearly in the scope for growth (SFG), parameter that remains negative most of the experimental time. In gastropods fed with non-toxic diets, however, the SFG was also negative at the beginning of the experiment, but positive values for the end of the period were recorded. Accord to results, the experimental time also had an effect on SFG, in separately way. The negative SFG during the first days of the experiment, in both groups (toxic and non-toxic), could be due to a process of acclimation to laboratory conditions. Maturana (2005) observed that negative SFG values in *Xanthochorus cassidiformis* were related to the lower energy absorbed, as occurred in *A. monodon.* Similar results have been described for different filter-feeder bivalve species: Navarro and Contreras (2010) reported lower SFG values in *Mytilus chilensis* at the beginning of the experiment as a result of the decrease in clearance rate and absorption efficiency when exposed to a diet containing *Alexandrium catenella*. Similar results were reported by Li et al. (2002) for the clam *Ruditapes philippinarum* and the mytilid *Perna viridis*. A decrease in absorption efficiency has also been described in the razor clam *Tagelus dombeii* after 12 days of exposure to diets containing PST (Fernández-Reiriz et al., 2013). These different findings show the relevant role that play the presence of *A. catenella* on the fitness of marine invertebrates.

Feeding on a toxic diet can also generate alterations in processes that depend directly on the energy availability (Haberkorn et al., 2010). Effects on the reproductive process have frequently been observed in bivalve molluscs that consume toxic microalgae (Haberkorn et al., 2010; Basti et al., 2015; Le Goïc et al., 2013; Rolton et al., 2018). Le Goïc et al., 2013 showed alterations in the spermatozoa quality of adult oysters exposed to the dinoflagellate *Alexandrium catenella*, suggesting a potential negative impact on sperm motility and future oocyte fecundation. On the other hand, reabsorption process of gametes present in the follicle gonadal tissue was reported in the bivalve *Ostrea chilensis* intoxicated with PSP (Oyarzun, 2015). According to our results, indirect consumption of PST seems to affect oviposition in the gastropod *A. monodon*, delaying the start of the process and decreasing the production of egg-masses. A similar situation was detected in the muricid gastropod *Xanthochorus cassidiformis*, where the production of egg-masses in toxic snails decreased a 55% regarding the toxic gastropods (Maturana, 2005). These values are comparable to those obtained in the present study, where *A. monodon* exhibited a higher percentage of postures in the control group (71%) than in the toxic group (29%). A more extreme effect was identified in the freshwater gastropod *Lymnea stagnalis*, after being exposed to toxic cyanobacteria, where a complete suspension of reproductive activity was observed (Lance et al., 2007).

The toxic diet supplied to adult *A. monodon* did not affect the total number of embryos produced per capsule, either at the beginning or at the end (day 55) of intracapsular development, nor the number of capsules per egg-mass. According to Lardies and Fernandez (2002), the number of eggs per capsule in *A. monodon* at the beginning of intracapsular development varies between 390 and 1175 in the natural environment, a much wider variation than the values obtained in this study, where mean values of 461 and 392 eggs per capsule were recorded in the toxic and control group respectively. According to our results, the percentage of developed embryos (juveniles + veligers) per capsule at day 55 is not affected when parents consume a toxic diet. However, despite not finding differences in the total number of embryos at the end of the intracapsular period (day 55), significant differences were observed in the level of embryo development between capsules from toxic and non-toxic parents, suggesting that the consumption of a diet contaminated with PST by adults of *A. monodon* delays embryo development inside the capsule. This situation could be related to a lower amount of energy available, due to the decrease in the absorption rate presented by parents who consumed a toxic diet, probably affecting the quality of the nurse eggs. It should be noted that there were egg-masses, from toxic parents, where no capsule presented embryo development throughout the whole monitoring period the egg stage. However, considering only those capsules where embryo development occurred, there was no difference between the two experimental groups in the size of embryos in an advanced development stage (d 55),

indicating that capsules from intoxicated adults have mainly large veliger larvae, similar to the pre-hatch juveniles observed in capsules from non-intoxicated parents.

On the other hand, it appears that the number of viable embryos in capsules from toxic parents decreases from day 55 of intracapsular development until hatching, although the hatching time did not vary significantly between capsules from non-toxic (70 days) and toxic parents (66 days). Competition for food (nurse eggs) is likely to occur in capsules from toxic parents during this last period of intracapsular development, allowing the few hatched juveniles to acquire a higher potential for growth by consuming more food; this is reflected in their greater shell length from the first month of extracapsular development onwards. However, the presence of nurse eggs in capsules at the advanced development stage is infrequent, since the nurse eggs are consumed completely in the first (trocophora) stage of embryo development (Büchner-Miranda et al., 2018). Therefore, the presence of nurse eggs on day 55 of embryo development could be attributed to an effect of the toxic diet. The effects on the offspring, of the bivalve *Mercenaria mercenaria,* produced by adults exposed to harmful algae were described by Rolton et al. (2018). After adult individuals were exposed to high concentrations of *Karenia brevis* (which produces neurotoxic shellfish toxin), there was a decrease in fertilization success (*in vitro*) and an increase in the percentages of abnormalities and mortalities among the larvae from intoxicated parents. These results suggest that consumption of a toxic diet (PST) by adult broodstock could have latent effects on the offspring, causing a decrease in the number of potential new recruits to the population; however those with larger size could have better chances of survival.

In conclusion, the indirect consumption of PST through diet in *A. monodon* causes changes in its physiological energetics response, modifying the energy available for growth and reproduction which vary over the exposure time. This exposure of adult individuals to PST can generate deleterious effects on the reproductive capacity of A. *monodon*, decreasing fecundity (number of egg-masses), delaying embryo development inside the capsules and decreasing the number of juveniles hatched.

Acknowledgments

The authors are grateful to Elizabeth Encalada for their valuable assistance during the experiments. This study was funded by the Comisión Nacional de Investigación Científica y Tecnológica de Chile (CONICYT − CHILE) with research grants to JMN (FONDECYT 1161420 and 1120470). Additional support came from FONDAP IDEAL 15150003 CONICYT.

References

Anderson, D., 2014. Harmful Algae 2012, Proceedings of the 15th International Conference on Harmful Algae. International Society for the Study of Harmful Algae 2014HABs in a Changing World: a Perspective on Harmful Algal Blooms, Their Impacts, and Research and Management in a Dynamic Era of Climatic and Environmental Change2014. HABs in a Changing World: a Perspective on Harmful Algal Blooms, Their Impacts, and Research and Management in a Dynamic Era of Climatic and Environmental Change ISBN 978-87-990827-4-2.

Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, M., 2012. The globally distributed genus Alexandrium: Multifaceted roles in marine ecosystems and impacts on human health. Harmful algae 14, 10–35.

Bardouil, M., Bohec, M., Cormerais, M., Bougrier, S., Lassus, P., 1993. Experimental study of the effect of a toxic microalgae diet on feeding of the oyster *Crassostrea gigas T* thunberg. J. Shellfish Res. 12, 417–422.

Basti, L., Nagai, S., Go, J., Okano, S., Nagai, K., Watanabe, R., Suzuki, T., Tanaka, Y., 2015. Differential inimical effects of *Alexandrium* spp. And *Karenia* spp. On cleavage, hatching and two larval stages of Japanese Pearl oyster *Pinctada fucata martensii*. *Harmful algae* 43, 1–12.

Bayne, B.L., Brown, D.A., Burns, K., Dixon, D.R., Ivanovici, A., Livingstone, D.R., Lowe, D.M., Moore, M.N., Stebbing, A., Widdows, J., 1985. The Effects of Stress and Pollution on Marine Animals. Praeger, Greenwood Press, pp. 384.

Bricelj, V.M., Lee, J.H., Cembella, A.D., Anderson, D.M., 1990. Uptake kinetics of paralytic shellfish toxins from the dinoflagellate *Alexandrium fundyense* in the mussel *Mytilus edulis*. Mar. Ecol. Prog. Ser 63, 117–188.

Bricelj, V.M., Cembella, A.D., Laby, D., Shumway, S.E., Cucci, T.L., 1996. Comparative physiological and behavioral responses to PSP toxins in two bivalve molluscs, the softshell clam, Mya arenaria, and surfclam, Spisula solidissima. In: Yasumoto, T., Oshima, Y., Fukuyo, Y. (Eds.), Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO, Paris.

Bricelj, V.M., Ward, J.E., Cembella, A.D., Mcdonald, B.A., 1998. In: Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T. (Eds.), Application of Video-Endoscopy to the Study of bivalve Feeding on Toxic Dinoflagellates. Xunta de Galicia and Intergovernmental Oceanographic Commission (UNESCO), Santiago de Compostela, Spain.

Bricelj, V.M., Connell, L., Konoki, K., MacQuarrie, S., Scheuer, T., Catterall, W.A., Trainer, V.L., 2005. Sodium cannel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature 434 (7034), 763–767.

Bricelj, V.M., MacQuarrie, S.P., Doane, J.A.E., Connell, N.B., 2010. Evidence of selection for resistance to paralytic shellfish toxins during the early life history of soft-shell clam (*Mya arenaria*) populations. Limnol. Oceanogr 55 (6), 2463–2475.

Büchner-Miranda, J.A., Thompson, R.J., Pardo, L.M., Matthews-Cascon, H., Salas-Yanquin, L.P., Andrade-Villagrán, P.V., Chaparro, O.R., 2018. Enveloping walls, encapsulated embryos and intracapsular fluid: changes during the early development stages in the gastropod *Acanthina monodon* (Muricidae). J. Mollus Stud. 84, 469–479.

Buschmann, A., Farías, L., Tapia, F., Varela, D., Vásquez, M., 2016. Informe final. Comisión Marea Roja.

Choi, M.C., Peter, K.N., Hsieh, D.P., Lam, P.K., 2006. Trophic transfer of paralytic shellfish toxins from clams (*Ruditapes philippinarum*) to gastropods (*Nassarius festivus*). Chemosphere 64 (10), 1642–1649.

Clement, A., Aguilera, A., Fuentes, C., 2002. Análisis de marea roja en Archipiélago de Chiloé, contingencia verano 2002. XXII Congreso de ciencias del Mar. 28-30 de mayo de 2002, Valdivia, Chile. .

Compagnon, D., Lembeye, G., Marcos, N., Ruiz-Tagle, N., Lagos, N., 1998. Accumulation of paralityc shellfish poisoning toxins in the bivalve *Aulocomya ater* an two carnivotous gastropods *Concholpeas concholepas* and *Argobuccinum ranalliformis* during *Alexandrium catenella* loom in Southern Chile. J. Shell. Res. 67–73 17, N°1.

Conover, R.J., 1966. Assimilation of organic matter by zooplankton. Limnol.Oceanogr 11, 338–345.

Costa, P.R., Rosa, R., Pereira, J., Sampayo, M.A.M., 2005. Detection of domoic acid, the amnesic shellfish toxin, in the digestive gland of *Eledone cirrhosa* and *E. moschata* (Cephalopoda, Octopoda) from the Portugal coast. Aquat. Living Resour. 18, 395–400.

Deeds, J.R., Landsberg, J.H., Etheridge, S.M., Pitcher, G.C., Longan, S.W., 2008. Non-traditional vectors for paralytic shellfish poisoning. Mar. Drugs 6, 308–348.

Elliot, M., Davison, W., 1975. Energy equivalents of oxygen consumption in animal energetics. Oecologia. 19, 195–2011.

Fernández-Reiriz, M.J., Navarro, J.M., Contreras, A.M., Labarta, U., 2008. Trophic interactions between the toxic dinoflagellate *Alexandrium catenella* and *Mytilus chilensis*: Feeding and digestive behaviour to long-term exposure. Aquat. toxicol 87 (4), 245–251.

Fernández-Reiriz, M.J., Navarro, J.M., Cisternas, B.A., Babarro, J.M.F., Labarta, U., 2013. Enzymatic digestive activity and absorption efficiency in *Tagelus dombeii* upon *Alexandrium catenella* exposure. Helgol. Mar. Res. 67, 653–661.

Franco, J.M., Fernandez-Vila, P., 1993. Separation of paralytic shellfish toxins by reversed phase high performance liquid chromatography, with postcolumn reaction and fluorimetric detection. Chromatographia 35 (9-12), 613–620.

Gallardo, C.S., 1979. Develompmental pattern and adaptions for reproduction in *Nucella crassilabrum* and other Muricacean gastropods. Biol. Bulletin-Us 157, 453–463.

Garrido, C., 2016. Exposición al Veneno Paralizante de Moluscos (VPM): Conducta y fisiología de la ostra chilena (*Ostrea chilensis*, Philippi 1845) durante el proceso de incubación. Tesis de pregrado. Instituto de Ciencias Marinas y Limnológicas. Facultad de Ciencias. Universidad Austral de Chile, Valdivia. Chile.

Guillard, R.R., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum Press, NY, pp. 29–60.

Guillard, R.R., 1995. Culture methods. In: Hallegraeff, G.M., Anderson, D.M., Cemebella, A.D. (Eds.), Manual on Harmful Marine Microalgae, IOC Manuals and Guides N°. 33. UNESCO, Paris, pp. 45–62.

Haberkorn, H., Lambert, C., Le Goïc, N., Moal, J., Suquet, M., Gueguen, M., Sunila, I., Soudant, P., 2010. Effects of *Alexandrium minutum* exposure on nutrition-related processes and reproductive output in oysters *Crassostrea gigas*. Harmful algae 9, 427–439.

Harding, J.M., Mann, R., Moeller, P., Hsia, M.S., 2009. Mortality of the veined rapa whelk, rapana venosa, in relation to a bloom of alexandrium monilatum in the york river, united states. J. Shellfish Res. 28, 363–367.

Ito, K., Asakawa, M., Beppu, R., Takayama, H., Miyazawa, K., 2004. PSP toxicification of the carnivorous gastropod *Rapana venosa* inhabiting the estuary of Nikoh River, Hiroshima Bay, Hiroshima Prefecture, Japan. Mar. Pollut. Bull. 48, 1116–1121.

Lance, E., Paty, C., Bormans, M., Brient, L., Gérard, C., 2007. Interactions between cyanobacteria and gastropods: II. Impact of toxic *Planktothrix agardhii* on the life-history traits of *Lymnaea stagnalis*. Aquatic Toxicology 81 (4), 389–396.

Lardies, M.A., Fernandez, M., 2002. Effect of oxygen availability in determining clutch size in Acanthina monodon. Mar. Ecol. Prog. Ser 239, 139–146.

Le Goïc, N., Hégaret, H., Fabioux, C., Miner, P., Suquet, M., Lambert, C., Soudant, P., 2013. Impact of the toxic dinoflagellate *Alexandrium catenella* on Pacific oyster reproductive output: application of flow cytometry assays on spermatozoa. Aquat. Living Resour 26, 221–228.

Lesser, M.P., Shumway, S.E., 1993. Effects of toxic dinoflagellates on clearance rates and survival in juvenile bivalve molluscs. J. Shellfish Res. 12, 377–381.

Leverone, J.R., Shumway, S.E., Blake, N.J., 2007. Comparative effects of the toxic dinoflagellate CKarenia brevis on clearance rates in juveniles of four bivalve molluscs

from Florida, USA. Toxicon 49 (5), 634–645.
Li, S.-Ch., Wang, W.-X., Hsieh, D.P.H., 2001. Feeding and absorption of the toxic dinoflagellate *Alexandrium tamarense* by two marine bivalves from a the South China Sea. Mar. Biol. 139, 617–624.
Li, S.-Ch., Wang, W.-X., Hsieh, D.P.H., 2002. Effect of dinoflagellate *Alexandrium tamarense* on the energy budgets and growth of two marine bivalves. Mar. Environ. Res. 53, 145–160.
Lopes, V.M., Lopes, A.R., Costa, P., Rosa, R., 2013. Cephalopods as vector of harmful algal bloom toxins in marine food web. Mar. Drugs 11, 3381–3409.
Marsden, I.D., Shumway, S.E., 1992. Effects of dinoflagellate *Alexandrium tamarense* on the greenshell mussel *Perna canaliculus*. New Zeal. J. Mar. Fresh 26, 371–378.
Marsden, I.D., Shumway, S.E., 1993. The effect of a toxic dinoflagellate (*Alexandrium tamarense*) on the oxygen uptake of juvenile filter-feeding bivalve molluscs. Comparative Biochemistry and Physiology Part A: Physiology 106, 769–773.
Maturana, M., 2005. Fisiología energética de *Xantochorus cassidiformis* (Gastropoda: Muricidae) (Blainville, 1832) alimentado con el bivalvo *Mulina edulis* (King y Broderip, 1832) conteniendo veneno paralizante de los mariscos (VPM). Tesis de grado para optar al título de Licenciado en Biología marina. Escuela de Biología Marina. Facultad de Ciencias, Universidad Austral de Chile, pp. 64.
Navarro, J.M., Contreras, A.M., 2010. An integrative response by *Mytilus chilensis* to the toxic dinoflagellate *Alexandrium catenella*. Mar. Biol. 157, 1967–1974.
Navarro, J.M., Urrutia, G.X., Carrasco, C., 2006a. Scope for growth versus actual growth in the juvenile predatory gastropod Chorus giganteus. J. Mar. Biol. Ass. U.K 86, 1423–1428.
Navarro, J.M., Muñoz, M.G., Contreras, A.M., 2006b. Temperature as a factor regulating growth and toxin content in the dinofllagelate *Alexandrium catenella*. Harmful Algae 5, 762–769.
Navarro, J.M., Aguila, B.L., Machmar, F., Chaparro, O.R., Contreras, A.M., 2011. Dynamic of intoxication and detoxification in juveniles of *Mytilus chilensis* (Bivalvia: Mytilidae) exposed to paralytic shellfish toxins. Aquat. Living Resour 24, 93–98.
Navarro, J.M., Gonzales, K., Cisternas, B., López, J.A., Chaparro, O.R., Segura, C.J., 2014. Contrasting physiological responses of Two populations of the razor *Tagelus dombeii* with different histories of exposure to paralytic shellfish poisoning (PSP). Plos One 9 (8), e105794.
Navarro, J.M., Labraña, W., Chaparro, O.R., Cisternas, B., Ortíz, A., 2016. Physiological constraints in juvenile *Ostrea chilensis* fed the Toxic Dinoflagellate *Alexandrium catenella*. Estuaries and Coasts 39, 1133–1141.
Osorio, C., 1979. Moluscos marinos de importancia económica en Chile. Biol. Pesq. Chile 11, 3–47.
Oyarzun, C.T., 2015. Histología de gónada y glándula digestiva durante la gametogénesis de *Ostrea chilensis*, Philippi, 1845, (Bivalvia, Ostreidae) expuesta a la toxina paralizante de molusco. *Tesis de grado para optar al título de Licenciado en Biología marina*. Escuela de Biología Marina, Facultad de Ciencias, Universidad Austral de Chil, pp. 86.
Pérez, M., 1998. Efecto de las distintas concentraciones y fuentes de nitrógeno sobre el crecimiento y toxicidad de *Alexandrium catenella* (Whedon & Kofoid) Balech 1985. Thesis Escuela de Biología Marina, Facultad de Ciencias, UACh.
Poblete, T., Toledo, H., Arteaga, R., Cárdenas, R., Toledo, M., 1987. Estimación de la estructura por clases anuales de tamaño en una población de *Nucella crassilabrum* (Gastropoda, Murcidae) 3. pp. 9–11 *Biota* (Chile).
Rolton, A., Soudant, P., Vignier, J., Pierce, R., Henry, M., Shumway, S.E., Bricelj, V.M., Volety, A.K., 2015. Susceptibility of gametes and embryos of the eastern oyster, *Crassostrea virginica,* to *Karenia brevis* and its toxins. Toxicon 99, 6–15.
Rolton, A., Vignier, J., Volety, A.K., Pierce, R.H., Henry, M., Shumway, S.E., Bricelj, V.M., Hegaret, H., Soudant, P., 2016. Effects of field and laboratory exposure to the toxic dinoflagellate *Karenia brevis* on the reproduction of the eastern oyster, *Crassostrea virginica*, and subsequent development of offspring. Harmful Algae 57, 13–26.
Rolton, A., Vignier, J., Volety, A., Shumway, S., Bricelj, M., Soudant, P., 2018. Impacts of exposure to the toxic dinoflagellate *Karenia brevis* on reproduction of the northern quahog, *Mercenaria mercenaria*. Aquatic Toxicology. 202, 153–162.
Scholin, C.A., Gulland, F., Doucette, G.J., et al., 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403, 80–84.
Sephton, D.H., Haya, K., Martin, J.L., LeGresley, M.M., Page, F.H., 2007. Paralytic shellfish toxins in zooplankton, mussels, lobsters and caged Atlantic salmon, *Salmo salar*, during a bloom of *Alexandrium fundyense* off Grand Manan Island in the Bay of Fundy. Harmful Algae 6, 745–758.
Setälä, O., Lehtinen, S., Kremp, A., Hakanen, P., Kankaanpää, H., Erler, K., Suikkanen, S., 2014. Bioaccumulation of PSTs produced by *Alexandrium ostenfeldii* in the northern Baltic Sea. Hydrobiologia 726, 143–154.
Sierra-Beltran, A., Palafox-Uribe, M., Grajales-Montiel, J., Cruz-Villacorta, A., Ochoa, J.L., 1997. Sea bird mortality at Cabo San Lucas, Mexico: evidence that toxic diatom blooms are spreading. Toxicon 35, 447–453.
Solórzano, L., 1969. Determination ammonia in natural wáter by the Henolhypoclorite methods. Limnol.Oceanogr 14, 799–801.
Wang, L., Yan, T., Zhou, M., 2006. Impacts of HAB species *Heterosigma akashiwo* on early development of the scallop *Argopecten irradians* Lamark. Aquaculture 255, 374–383.
Widdows, J., 1985. Physiological Response to Pollution. Marine Pollution Bulletin 16 (4), 129–134.