Introduction

Harmful algae blooms (HABs) are cosmopolitan phenomena that cause serious public health problems. HABs are also detrimental to aquatic organisms, with negative effects on their physiological functions and also on aquaculture activities. During recent decades, HABs producing paralytic shellfish poisoning (PSP) have increased worldwide [1,2], and dinoflagellates of the genus *Alexandrium* are the primary producer of the paralytic toxin. This toxin may accumulate in different taxa of the marine food chain, including bivalves, zooplankton, crustaceans, and gastropods [3]. Several physiological and behavioral effects have been described in marine copepods and bivalves exposed to diets containing PSP, such as reductions in ingestion, metabolism and growth rates [4,5,6,7,8] and changes in the burial patterns of infaunal bivalves [9]. However, the responses to PSP may be influenced by the history of exposure to the toxin [9]. The evolution of grazer adaptation to toxic algae, in both the ocean and freshwater, has been well established [8]. Populations of the copepod *Acartia hudsonica* historically exposed to PSP produced by bloom of dinoflagellates of the genus *Alexandrium spp.*, exhibit enhanced feeding and growth rate, as well as fecundity [10,7], compared to populations never exposed to PSP. Hairston et al. [11] showed that the freshwater grazing cladoceran *Daphnia galeata* evolved a selection response to increased abundance of toxic cyanobacteria in its environment. *Mya arenaria* clams from areas frequently exposed to toxic dinoflagellate blooms are less affected by PSP than specimens from areas that have not been previously exposed to PSP [9]. According to these authors, the

Contrasting Physiological Responses of Two Populations of the Razor Clam *Tagelus dombeii* with Different Histories of Exposure to Paralytic Shellfish Poisoning (PSP)

Jorge M. Navarro¹*, Katerina González², Barbara Cisternas¹, Jorge A. López¹, Oscar R. Chaparro¹, Cristian J. Segura¹, Marco Córdova³, Benjamín Suárez-Isla³, María J. Fernandez-Reiriz⁴, Uxio Labarta⁴

1 Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile, 2 Escuela de Acuicultura, Universidad Católica de Temuco, Temuco, Chile, 3 Laboratorio de Toxinas Marinas, Facultad de Medicina, Universidad de Chile, Santiago, Chile, 4 Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Vigo, España

Abstract

This study describes the physiological performance of two populations of the razor clam *Tagelus dombeii* from two geographic areas with different histories of exposure to paralytic shellfish poisoning (PSP) linked to the toxic dinoflagellate *Alexandrium catenella*. Clams from Melinka-Aysén, which are frequently exposed to PSP, were not affected by the presence of toxins in the diet. However, clams from Corral-Valdivia, which have never been exposed to PSP, exhibited significantly reduced filtration activity and absorption, affecting the energy allocated to scope for growth (SFG). Ammonia excretion and oxygen uptake were not affected significantly by the presence of *A. catenella* in the diet. Measurements of energy acquisition and expenditure were performed during a 12-day intoxication period. According to three-way repeated measure ANOVAs, the origin of the clams had a highly significant effect on all physiological variables, and the interaction between diet and origin was significant for the clearance and absorption rates and for the scope for growth. The scope for growth index showed similar positive values for both the toxic and non-toxic individuals from the Melinka-Aysén population. However, it was significantly reduced in individuals from Corral-Valdivia when exposed to the diet containing *A. catenella*. The absence of differences between the physiological response of the toxic and non-toxic clams from Melinka-Aysén may be related to the frequent presence of *A. catenella* in the environment, indicating that this bivalve does not suffer negative consequences from PSP. By contrast, *A. catenella* has a negative effect on the physiological performance, primarily on the energy gained from the environment, on *T. dombeii* from Corral-Valdivia. This study supports the hypothesis that the history of PSP exposure plays an important role in the physiological performance and fitness of filter feeding bivalves.

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* Email: jnavarro@uach.cl
different responses of bivalves to toxins are related to nerve sensitivity, where resistance to the toxin is caused by a mutation of an amino acid that causes a decrease in the affinity of saxitoxin at the sodium channel pore of the cell membrane. Thus, the presence of PSP in the environment can act as an agent of natural selection, leading to increased resistance of the bivalves to the toxin, with a smaller impact on behavioral and physiological responses. This response favors an increased concentration of toxin in the bivalve, thereby increasing the risk to humans. The expansion of toxic algal blooms to geographical areas not previously affected may result in structural changes in the communities and ecosystem because toxins produced by dinoflagellates can cause significant mortalities in bivalve populations with no history of exposure to PSP [12,13]. It is possible to find individuals with different physiological and behavioral responses depending on the history of exposure to toxic events [14,19]. A study that analyzed the digestive enzymatic activity and absorption efficiency in the razor clam *Tagelus dombeii* upon exposure to *Alexandrium catenella* [15] showed that a feeding history of exposure to *A. catenella* was reflected in the digestive responses of *T. dombeii*.

In southern Chile, the dinoflagellate *Alexandrium catenella* has expanded its geographical distribution during the last several decades, with frequent blooms in the Aysén and Magallanes regions and extending north to the center of the Chiloé Island [16,17,18]. This geographical region has numerous species of commercially important bivalves, where the extraction and consumption of bivalves have been significantly reduced by the temporary or indefinite closure of areas where bivalves remain toxic with PSP throughout the year. We used the bivalve *Tagelus dombeii*, an infaunal species with a broad latitudinal distribution and that inhabits soft sediments of the tidal and subtidal zones of south Chile, as a model. Razor clam fishery represents greater than 5% of all commercially important benthic resources of Chile. Navarro et al. [19] studied the feeding behavior of *T. dombeii* and concluded that this bivalve behaves as a suspension-feeder when immersed, which indicates that algal blooms are part of its diet. Because of the wide geographical distribution of this species along the Chilean coast, there are populations in southern Chile exposed frequently to PSP, unlike the majority of other populations located in the north, which do not have a history of PSP exposure.

The present study looks at how historical exposure to a toxic dinoflagellate may affect physiological performance and fitness of specimens of *Tagelus dombeii* from two populations from different geographic areas.

Materials and Methods

Animal Collection and diet preparation

Adult specimens of *Tagelus dombeii* were collected from the natural banks at Corral-Valdivia (39°53’S, 73°25’W; no previous PSP exposition) and Melinka-Aysén (43°52’S, 73°45’W; previous PSP exposition). No specific permissions were required to collect the experimental clams from Corral-Valdivia. However, a special permit from the Regional Health Department was required to collect clams from Melinka-Aysén. Individuals ranging from 50 to 40 mm (mean 53.7 ± 4.5 mm) shell length were maintained for one week before the measurements were initiated in aquaria at 14°C, 30 psu. The clams were buried in fine sediment collected from the same location where specimens were collected and fed continuously with a diet containing (by weight) 60% of the microalgae *Isochrysis galbana* and 40% inorganic sediment (1.5 mg L−1). The monoclonal non-axenic *Alexandrium catenella* (strain ACC02; 32–36 µm spherical diameter) used for the experiments was isolated from the Aysén Region of Chile and was cultivated in 0.45 µm filtered seawater enriched with “L1” algae culture medium [20]. The toxicity of *A. catenella* cells was quantified using the electrophysiological test of Vélez et al. [21], and a mean value from 15 samples was obtained. The microalgae *Isochrysis galbana* was cultivated using 1/2 medium [22]. Both species of algae were harvested during the exponential growth phase. Sediment was added to the diets to emulate the organic/inorganic fractions of the natural suspended particulate matter recorded in the field [23]. This sediment was collected from the upper centimeter of the Yaldad tidal flat in south Chile, passed through a 40-µm mesh sieve, rinsed with distilled water, and ashed in a muffle furnace at 450°C for 12 h to eliminate the organic fraction. After ashing, the sediment was resieved (40-µm sieve) to eliminate the sediment aggregates.

Experimental Design

Three replicates of 25 individuals each were maintained in 8 L aquaria. The clams were permanently buried in the sediment collected from their natural habitat and fed with toxic diet (by weight: 50% *Alexandrium catenella*, 10% *Isochrysis galbana* and 40% inorganic sediment) for a period of 12 days. In parallel, three other similar aquaria were maintained as controls, with the same number of individuals in each group (n = 25) that were fed the non-toxic diet (60% *I. galbana* and 40% inorganic sediment). The diets were continuously supplied with a Masterflex L/S peristaltic pump. The quantity of food provided daily was equivalent to 2% (ca. 14 mg/day/ clam) of the dry weight of the soft tissue of the experimental animals. For each sampling date, all physiological processes were measured on the same clam (one from each aquarium, 3 toxic and 3 non-toxic), beginning with clearance rate; feces produced during that time were used to measure the absorption rate. Once the clearance rate experiments were completed, ammonia excretion and oxygen uptake were determined. All following sections are based on this experimental design. Once all measurements were done, the clams were sacrificed to determine the soft tissue weight. To estimate the total weight and organic content of the diets, a known volume of each was filtered, in triplicate, through Whatman 47-mm-diameter glass fiber GF/C filters, which were previously washed, burnt and weighed. A blank filter and those containing the samples were washed with an isotonic solution of ammonium formate to remove the salt and prevent cell lysis. The filters were dried at 100°C for 24 h, weighed, burnt at 450°C for 3 h and reweighed after cooling in a desiccator.

Physiological measurements

The feeding, absorption, excretion and respiration rates were monitored throughout the experiment on days 0, 1, 2, 3, 5, 8 and 12 in different clams exposed to both the toxic and non-toxic diets. All experiments were performed under controlled temperature (14°C) and salinity (30 psu) conditions.

Clearance rate (CR)

The CR was estimated in a static system homogenized by aeration and using a food concentration ca. 2.0 mg L−1 dry weight (Table 1). Each experimental aquarium (1.0 L volume) contained a single clam, and the reduction in particle concentration in the aquarium was monitored periodically with a Beckman model Z2 particle counter equipped with a 100 µm aperture counting tube. The decrease in particle concentration in the experimental aquarium was maintained between 10 and 40% in relation to the initial concentration and was measured every 30 min for 4 h, with replacement of the consumed food. To test for any growth or cell sedimentation during the feeding
measurements, a control aquarium without clams was maintained. The CR (l h⁻¹ ind⁻¹) was calculated following the method of Coughlan [24].

Absorption rate (AR)
The AR was calculated as the product of the absorption efficiency and the organic ingestion rate. The absorption efficiency data were obtained from Fernández-Reiriz et al. [15], who performed a parallel study using the same experimental specimens on the digestive enzyme activity of Tagelus dombeii.

Ammonia excretion (VNH₄-N)
The clams fed toxic and non-toxic diets were placed individually in glass beakers containing filtered (0.45 μm) seawater. One additional beaker containing filtered seawater but no clams was used as a control. All beakers were maintained at the experimental temperature by submersion in a thermostatic water bath. After 2 h, water samples from each beaker were removed and analyzed for ammonia–nitrogen according to Solórzano [25].

Oxygen uptake (VO₂)
Oxygen uptake was determined individually in 1.0 L chambers sealed for 60 min. Measurements of the oxygen dissolved in the sea water were recorded after this period of time to prevent the oxygen concentration from falling below 70% saturation. A chamber of similar volume without bivalves was used as the control. The initial and final concentrations of oxygen were measured on 50 ml samples using the micro-Winkler method.

Scope for growth (SFG)
The measurements of the energy available for growth and reproduction (SFG) were calculated using the equation given by Widdows [26] after converting all physiological rates to energy equivalents (J h⁻¹):

$$SFG = A - (R + E)$$

Where $A =$ energy absorbed; 1 mg organic matter of food = 21 J [27]; $R =$ oxygen uptake; 1 ml O₂ = 19.9 J [28] and $E =$ ammonia excretion; 1 μg NH₄-N = 0.0249 J [28].

Statistical analysis
The diets and individual physiological rates were compared by a one-way analysis of variance (ANOVA). The different physiological processes were measured in each tank over time; therefore, it was necessary to apply an analysis of variance for repeated measures [29], which considers the temporal dependency between samples from the same aquarium. Three-way repeated measure ANOVAs (tank as random factor) were performed to analyze the effects of diet (toxic and non-toxic), origin (Corral-Valdivia and Melinka-Aysén), and time of exposure (TE) on the clearance, absorption, ammonia excretion and respiration rates, and scope for growth. When the interaction was significant and involved the time of exposure (TE), a two-way ANOVA for repeated measures was used. The normality and homoscedasticity of the data were tested using the Kolmogorov-Smirnov and Bartlett tests, respectively [30]. The statistical analyses were performed using the R 3.0.2 software (R Development Core Team 2011).

Animal Research
This study was performed in the Laboratory of Marine Ecophysiology of the Universidad Austral de Chile and the species involved in this research is not endangered or protected.
Figure 1. *Tagelus dombeii*. Clearance rate measured for a period of 12 days in individuals with different histories of exposure to PSP and exposed to toxic and non-toxic diets (3 replicates per experimental group at each sampling time). A, Melinka, Aysén (with previous PSP exposure); B, Corral, Valdivia (without previous PSP exposure).

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Figure 2. *Tagelus dombeii*. Absorption rate measured for a period of 12 days in individuals with different histories of exposure to PSP and exposed to toxic and non-toxic diets (3 replicates per experimental group at each sampling time). A, Melinka, Aysén (with previous PSP exposure); B, Corral, Valdivia (without previous PSP exposure).

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Figure 3. *Tagelus dombeii*. Ammonia excretion measured for a period of 12 days in individuals with different histories of exposure to PSP and exposed to toxic and non-toxic diets (3 replicates per experimental group at each sampling time). A, Melinka, Aysén (with previous PSP exposure); B, Corral, Valdivia (without previous PSP exposure). doi:10.1371/journal.pone.0105794.g003
Figure 4. *Tagelus dombeii*. Oxygen uptake measured for a period of 12 days in individuals with different histories of exposure to PSP and exposed to toxic and non-toxic diets (3 replicates per experimental group at each sampling time). A, Melinka, Aysén (with previous PSP exposure); B, Corral, Valdivia (without previous PSP exposure).

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Figure 5. *Tagelus dombeii*. Scope for growth measured for a period of 12 days in individuals with different histories of exposure to PSP and exposed to toxic and non-toxic diets (3 replicates per experimental group at each sampling time). A, Melinka, Aysén (with previous PSP exposure); B, Corral, Valdivia (without previous PSP exposure).

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### Table 2. Three-way repeated-measures ANOVA for clearance rate, absorption rate, ammonia excretion, oxygen uptake and scope for growth in the razor clam *Tagelus dombeii*. TE = Time exposure.

| Error: Tank        | Diet                  | Origin                   | Diet:Origin              |
|-------------------|-----------------------|--------------------------|--------------------------|
|                   | df  F  p              | df  F  p                 | df  F  p                 |
| Clearance Rate    | 1  0.483  0.506       | 1  33.476  0.00041 ***   | 1  9.314  0.016 *        |
| Absorption Rate   | 1  0.039  0.848       | 1  37.296  0.00028 ***   | 1  13.175  0.0067 **     |
| Ammonia Excretion | 1  0.026  0.875       | 1  57.970  6.22·10^{-5} *** | 1  2.235  0.173         |
| Oxygen Uptake     | 1  0.304  0.596       | 1  11.708  0.00906 **    | 1  3.175  0.112          |
| Scope for Growth  | 1  0.074  0.792       | 1  28.813  0.000671 ***  | 1  19.026  0.0024 **     |

| Error: Within     | TE         | TE:Diet                | TE:Origin                | TE:Diet:Origin            |
|-------------------|------------|------------------------|--------------------------|---------------------------|
|                   | df  F  p  | df  F  p               | df  F  p                 | df  F  p                  |
| Clearance Rate    | 6  7.339  \(1.33 \times 10^{-5}\) *** | 6  1.203  0.321         | 6  7.014  2.13·10^{-5} *** | 6  1.009  0.430           |
| Absorption Rate   | 6  5.952  0.0001 ***   | 6  1.073  0.392        | 6  8.442  2.87·10^{-6} *** | 6  0.649  0.690           |
| Ammonia Excretion | 6  6.474  \(4.74 \times 10^{-5}\) *** | 6  0.802  0.574        | 6  9.981  3.86·10^{-7} *** | 6  0.801  0.574           |
| Oxygen Uptake     | 6  11.980  \(3.55 \times 10^{-8}\) *** | 6  3.469  0.0063 **     | 6  3.401  0.0071 **      | 6  1.144  0.352           |
| Scope for Growth  | 6  4.011  0.00247 **   | 6  1.267  0.290        | 6  6.150  7.75·10^{-5} *** | 6  1.036  0.414           |

*p* < 0.05, **p** < 0.01, ***p*** < 0.001.
The protocol was approved by the Committee on the Bioethics of Animal Research of the Universidad Austral de Chile (Permit Number: 26-2011).

Results

Experimental diets

The characteristics of the toxic and non-toxic diets are summarized in Table 1. No significant differences ($P > 0.05$) were observed between the total weight of the toxic diet ($1.99\pm 0.06$ mg L$^{-1}$) and the non-toxic diet ($1.95\pm 0.19$ mg L$^{-1}$), nor among their organic fractions (toxic: 60.80% and non-toxic: 56.08%). The mean concentration of toxin in *A. catenella* (strain ACC02) was $10.3\pm 0.91$ fmol STX eq/cell. The concentration of *A. catenella* cells in the experimental diet was $1.98\pm 10^5$ cells L$^{-1}$, resulting in a concentration of saxitoxin equivalent to $2039$ pmol L$^{-1}$ (Table 1).

Physiological responses

Figures 1–5 (see File S1) illustrate the different physiological processes, CR, AR, VNH$_4$-N, VO$_2$, and SFG, measured in the 2 populations of *T. dombeii*, in relation to time of exposure (TE) to the toxin and the two diets. Clams from Melinka, Aysén maintain high levels of filtration and absorption during the experimental period, without significant differences ($p > 0.05$) between the toxic and non-toxic groups (Fig 1A, 2A). On the contrary, the clams from Corral, Valdivia exposed to the toxic diet reduced significantly ($p < 0.05$) their clearance and absorption rates (Fig. 1B; 2B). Ammonia excretion did not show significant differences ($p > 0.05$) between the clams exposed to the toxic diet and those fed on the non-toxic diet in both studied populations (Fig. 3 A and B). Oxygen uptake was similar for both groups of clams; however, significant differences were recorded on a few occasions (Fig. 4 A and B). The scope for growth of clams from Melinka, Aysén ($10.04\pm 1.72$ J h$^{-1}$ ind$^{-1}$), was not affected by diet containing *A. catenella*, accumulating similar or higher amounts of energy than clams from the non-toxic group (Fig. 5 A). Conversely, the scope for growth of the clams of Corral, Valdivia ($-1.09\pm 0.47$ J h$^{-1}$ ind$^{-1}$) exposed to PSP was negative and significantly lower than in the non-toxic group, during the whole experimental period (the Fig 5 B).

When the three factors, origin, time exposure and diet were included in the analyses, the three-way repeated measure ANOVA (Table 2) showed that the diet did not have a significant ($p > 0.05$) effects on the different physiological processes. By contrast, the origin of the clams was significant ($p < 0.05$) for all physiological variables, and interaction between diet and origin was significant ($p < 0.05$) for CR, AR, and SFG. According to the within-tank analyses, TE and the interaction between TE and the factor origin, showed a significant ($P < 0.05$) effect on all of the physiological variables measured. The interaction between the TE and diet showed a significant ($p < 0.05$) effect only for VO$_2$, and the three-way interaction was not significant for all physiological processes measured. The clearance rate, absorption rate and ammonia excretion rate measured in the clams exposed to the toxic diet were significantly affected by the origin of the clams (two-way ANOVA repeated-measured, $p < 0.05$; Table 3), with significantly lower values for the individuals from Corral-Valdivia (Table 4). The physiological index scope for growth for the specimens from Corral-Valdivia was also significantly affected ($p < 0.05$) by the diet containing PSP, resulting in negative values ($-1.09\pm 0.47$ J h$^{-1}$ ind$^{-1}$) compared to the high values ($10.04\pm 1.72$ J h$^{-1}$ ind$^{-1}$) for the specimens from Melinka-Aysén (Table 3).
Figure 6 (see File S1) shows the comparison of the *T. dombeii* clearance rate for *A. catenella* only. The one-way ANOVA showed that the clams from Corral-Valdivia had significantly ($P < 0.05$) lower clearance rates ($0.33 \pm 0.05$ L h$^{-1}$ ind$^{-1}$) than the individuals from Melinka-Ayseñ ($0.62 \pm 0.07$ L h$^{-1}$ ind$^{-1}$).

**Discussion**

The *Tagelus dombeii* clams from Melinka-Ayseñ, which are frequently exposed to PSP, were not affected by the presence of toxin in the diet, unlike the population from Corral-Valdivia, which is not exposed to PSP. The clams from Corral-Valdivia showed significantly reduced filtration activity and absorption, which affected the amount of energy channeled to growth and reproduction (SFG). Previous studies have shown similar results for other species of filter-feeder organisms [7,31,32,33,34].

The effect of PSP on bivalve filter feeders can vary intra and inter specifically, depending on multiple factors, such as toxicity of the algae, differences in digestive functions and the history of exposure to toxic algae blooms [35,9,36]. *Crassostrea gigas* presents a complete inhibition of filtration activity during the first hours of exposure to a diet containing *Alexandrium tamarense* [37]. However, this species requires two weeks to initiate normal feeding activity on a diet containing the dinoflagellate *A. catenella*.

**Table 4.** Physiological variables (mean ± standard error) of specimens of *Tagelus dombeii* from two populations with different history of exposure to PSP.

| Physiological Rate                  | Corral-Valdivia | Melinka-Ayseñ |
|------------------------------------|----------------|--------------|
| Clearance Rate (L h$^{-1}$ ind$^{-1}$) | Toxic 0.31±0.02 | Non-toxic 0.54±0.06 |
|                                   | Toxic 0.23±0.02 | Non-toxic 0.46±0.03 |
| Absorption Rate (mg h$^{-1}$ ind$^{-1}$) | Toxic 17.82±2.83 | Non-toxic 23.43±3.16 |
| Ammonia Excretion (µg NH$_4$-N h$^{-1}$ ind$^{-1}$) | Toxic 0.28±0.02 | Non-toxic 0.24±0.02 |
| Oxygen Uptake (mL h$^{-1}$ ind$^{-1}$) | Toxic 1.09±0.47 | Non-toxic 4.21±0.87 |
| Scope for Growth (J h$^{-1}$ ind$^{-1}$) | Toxic 10.04±1.72 | Non-toxic 5.36±0.93 |

Figure 6. *Tagelus dombeii*. Clearance rate measured on *Alexandrium catenella* cells for a period of 12 days in individuals with different histories of exposure to PSP. A, Melinka, Ayseñ (with previous PSP exposure); B, Corral, Valdivia (without previous PSP exposure).

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According to Marsden and Shumway [46], the mussel *Perna* *Mya arenaria* shown by the bivalves a decrease in oxygen consumption, in contrast to the increase exposed to *canaliculus*. Therefore, from the body to maintain the osmotic balance of the bivalve. *T. dombeii* been described for other species of bivalves. The scallop containing *M. chilensis* to toxic diet, thereby maintaining physiological stability against high to PSP (Yaldad Bay, Chiloe ´). Degradation of the toxin produces blooms on the growth rate of various species of filter feeder consumption.

References

1. Anderson DM (1989) Toxic algal blooms and red tides. In: A global perspective in Red Tides: Biology, Environmental Science and Toxology. Okuchi T, Anderson DM, Nemoto T, editors. Elsevier. pp. 11–16
2. Anderson DM, Kulis DM, Doucette GJ, Gallager JC, Balech E (1994) Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeast United States and Canada as determined by morphology, bioluminescence, toxin composition, and mating compatibility. Mar Biol 120: 467–478 for individuals of *M. edulis* and *M. chilensis*, respectively, which were fed monocultures of non-toxic microalgae. The present study shows similar values for individuals of similar sizes from Melinka-Ayse ´n for both the non-toxic group and the group exposed to PSP (ca.10 J h⁻¹ g⁻¹). However, the scope for growth of *T. dombeii* from Corral-Valdivia was negative (−1.09±0.47 J h⁻¹ g⁻¹) when the specimens were exposed to the toxic diet, similar to that described by Li et al. [5] for the clam *Ruditapes philippinarum* (−6.2±2.8 J h⁻¹ g⁻¹). The non-toxic groups of both populations showed no significant differences between the values of SFG, suggesting that the differences between the two populations exposed to PSP are due to different responses to *A. catenella* associated with the history of exposure to the dinoflagellate. Our results are consistent with those of MacQuarrie [49] and Bricelj et al. [9], who described the different behavioral and physiological response of the clam *M. arenaria* to *A. tamarensis*, depending on their prior history of exposure to PSP. Therefore, *T. dombeii* specimens from populations with no history of exposure to PSP show a greater sensitivity to the presence of STX in the diet by reducing their feeding and growth rates compared to individuals from populations that experience frequent exposure to PSP events. Thus, the presence of PSP in the natural environment may have a potential negative effect on the broodstock of the clam from Corral-Valdivia. In *Mytilus edulis* [50] and *Ostrea chilensis* [51] it has been observed that stress feeding conditions reduce fecundity and quality of the eggs, with a smaller number of larvae being obtained.

According to the present study, clams from the Melinka-Ayse ´n population apparently do not suffer negative consequences from the toxin produced by *A. catenella*; an adaptive response to the frequent blooms of this dinoflagellate that occur in their environment. This contrast with that observed for *T. dombeii* specimens with no history of exposure to *A. catenella*, which were affected by exposure to diets containing PSP, with a large reduction in the energy allocated to growth. The present study suggests that the history of exposure to PSP plays an important role in the physiological performance and fitness of filter feeding bivalves.

Supporting Information

File S1 Data for the different physiological variables measured are included in the file S1. (XLSX)

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Author Contributions

Conceived and designed the experiments: JMN KG BC. Performed the experiments: JMN KG JAL. Analyzed the data: JMN KG BC CS. Contributed reagents/materials/analysis tools: MC BS. Contributed to the writing of the manuscript: JMN MJF ORC.

References

1. Anderson DM (1989) Toxic algal blooms and red tides. In: A global perspective in Red Tides: Biology, Environmental Science and Toxology. Okuchi T, Anderson DM, Nemoto T, editors. Elsevier. pp. 11–16
2. Anderson DM, Kulis DM, Doucette GJ, Gallager JC, Balech E (1994) Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeast United States and Canada as determined by morphology, bioluminescence, toxin composition, and mating compatibility. Mar Biol 120: 467–478
3. Chen CY, Chou HN (1998) Transmission of the paralytic shellfish poisoning toxins, from dinoflagellate to gastropod. Toxicon 36:515–523
4. Shiozawa NE, Ciucci TL, Newell RC, Ventich CM (1985) Particle selection, ingestion and absorption in filter-feeding bivalves. J Exp Mar Biol Ecol 91: 77–92
5. Li SC, Wang WX, Hsieh DPF (2002) Effects of toxic dinoflagellate *Alexandrium tamarense* on the energy budgets and growth of two marine bivalves. Mar Environ Res 55: 145–160
6. Navarro JM, Contreras AM (2010) An integrative response by *Mytilus chilensis* to the toxic dinoflagellate *Alexandrium catenella*. Mar Biol 157: 1967–1974
7. Colin AP, Dam HG (2007) Comparison of the functional and numerical responses of resistant versus non-resistant populations of the copepod *Aristea boudouUPI* fed the toxic dinoflagellate *Alexandrium tamarense*. Harmful Algae 6: 875–882
8. Dam HG (2013) Evolutionary adaptation of marine zooplankton to global change. Annu Rev Mar Sci 5: 549–570
9. Bricelj VM, Connell L, Kousik K, MacQuarrie SP, Schuer C, et al. (2005) Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature 434: 765–767
10. Colin SP, Dam HG (2004) Testing for resistance of pelagic marine copepods to a toxic dinoflagellate. Ecol Evol 18: 355–377
11. Hairson NG Jr, Holmteijn CL, Lampert W, Weider LJ, Post DM, et al. (2001) Natural selection for grazer resistance to toxic cyanobacteria evolution of phenotypic plasticity? Evolution 55: 2203–2214
12. Tangen K (1977) Blooms of *Gyrodinium aureolum* (Dinophyceae) in north European waters, accompanied by mortality in marine organisms. Sarsia 63: 123–133
13. Boalch GT (1979) The dinoflagellate bloom on the coast of south west England, August-September 1978. J Mar Biol Assoc UK 59: 515–517 DOI: 10.1017/ S002531540004281B
14. Bricelj VM, Shumway SE (1998) Paralytic toxin in bivalve molluscs: occurrence, transfer kinetics and biotransformation. Rev Fish Sci 6 (4): 315–393
15. Fernández-Reiriz MJ, Navarro JM, Cineras BA, Labarta U, Baharof JMF (2013) Enzymatic digestive activity and absorption efficiency in *Tagelus dombeii* upon *Alexandrium catenella* exposure. Helgol Mar Res DOI 10.1007/s10152-013-0351-6
16. Clément A, Aguilera A, Fuentes C (2002) Análisis de Marea Roja en el Archipiélago de Chiloé. Contingencia 2002. Resúmenes XXII jornadas de Ciencias del Mar 120 p.
17. Molinet C, Lalou A, Lembeye G, Moreno C (2003) Patrones de distribución espacial y temporal de floraciones de *Alexandrium catenella* (Whedon & Kofoid) Balech 1985, en agua interiores de la patagonia norecidente de Chile. Rev Chil Hist Nat 76: 681–698
18. Navarro JM, Muñoz MG, Contreras AM (2006) Temperature as a factor regulating growth and toxin content in the dinoflagellate *Alexandrium catenella*. Harmful Algae 5: 762–769
19. Navarro JM, Contreras AM, Chaparro O (2008) Short-term feeding response of the mussel *Mytilus chilensis* exposed to diets containing the toxic dinoflagellate *Alexandrium catenella*. Rev Chil Hist Nat 81: 41–49
20. Guillard RRL (1995) Culture Methods. In: Hallegreaff GM, Anderson DM, Cembella AD, editors. Manual on Harmful Marine Microalgae. IOC Manuals and Guides, No.33 UNESCO. pp 45–62
21. Velez P, Sierraltz J, Alcyaca C, Fonseca M, Lolyoa H, et al. (2001) A functional assay for paralytic shellfish toxin that uses recombinant sodium channel. Toxicon 39: 929–937
22. Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Cherley MH, editors. Culture of Marine Invertebrate Animals. Praeger Scientific Publications N.Y. pp 161–178
23. Gainey LF, Shumway SE (1988) A compendium of the responses of bivalve molluscs to toxic dinoflagellates. J Shellfish Res 7: 626–638
24. Bardoul M, Bohec M, Bougier S, Lasaus P, Traupert P (1996) Feeding responses of *Crasostrea gigas* (Thunberg) to inclusion of different proportions of toxic dinoflagellates in their diet. Oceanol Acta 19: 177–182
25. Willifish D, Lasaus P, Martin J, Saudnier A, Bardoul M (1998) Effect of the PSP-causing dinoflagellate, *Alexandrium*, sp. on the initial feeding response of *Crasostrea gigas*. Aquat. Living Resour. 11: 35–43
26. Gainey LF, Shumway SE (1988) A compendium of the responses of bivalve molluscs to toxic dinoflagellates. J Shellfish Res 7: 626–638
27. Gainey LF, Shumway SE (1988) A compendium of the responses of bivalve molluscs to toxic dinoflagellates. J Shellfish Res 7: 626–638
28. Bardoul M, Bohec M, Bougier S, Lasaus P, Traupert P (1996) Feeding responses of *Crasostrea gigas* (Thunberg) to inclusion of different proportions of toxic dinoflagellates in their diet. Oceanol Acta 19: 177–182
29. Wilson JA, Chaparro OR, Thompson RJ (1996) The importance of broodstock nutrition on the viability of larvae and spat in the Chilean oyster *Ostrea chilensis*. Aquaculture 139: 63–75
30. Zar JH (1999) Biostatistical Analysis. 4th ed. Prentice Hall, Upper Saddle River, NJ. 662 p.