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Pre-ingestive selection efficiency in two populations of the razor clam *Tagelus dombeii* with different histories of exposure to paralytic shellfish poisoning (PSP)

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ABSTRACT

We studied toxic effects of the dinoflagellate *Alexandrium catenella* on filtration activity and pre-ingestive selection efficiency in *Tagelus dombeii* (razor clam). Samples came from two populations with different histories of exposure to paralytic shellfish poisoning (PSP): Melinka, Aysén (with frequent exposure to PSP) and Corral, Valdivia (without previous exposure to PSP). Feeding activity of *T. dombeii* was affected by a diet containing *A. catenella*, showing a reduction in individuals from Corral, Valdivia and Melinka, Aysén. Furthermore, pre-ingestive selection efficiency was significantly higher in specimens from the population of Melinka, than those from Corral. Significantly higher values of clearance rate and pre-ingestive selection efficiency from the Melinka samples may reflect adaptation to specific environmental conditions where PSP events frequently occur.

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Introduction

Harmful algal blooms (HABs) are a natural phenomena caused by microalgae, and can develop in high concentrations when certain favourable environmental conditions are present (e.g. increase in brightness, stratification of water, high temperature, increased nutrients) (Guzmán et al. 1975; Vila et al. 2001; Amorim et al. 2002). Some of these blooms can produce high concentrations of paralytic shellfish poisoning (PSP) in marine bivalves, such as those produced by dinoflagellates of the genus *Alexandrium*, *Gymnodinium* and *Pyrodinium* (Lehanne 2000). Harmful algae blooms cause serious public health problems and are detrimental to aquatic organisms, negatively effecting physiological functions and aquaculture activity (Bricelj et al. 2005; Fernández-Reiriz et al. 2013; Navarro et al. 2014). The main vectors that transmit this toxin to higher levels of the food chain are bivalve filter feeders that feed on suspended particulate matter in the water column (Hayashi et al. 1982; Shumway 1995). The presence of PSP causes numerous effects on bivalve feeding, metabolism and growth (Shumway & Cucci 1987; Li et al. 2002; Navarro & Contreras 2010). Different responses have been described for filter feeding bivalves fed with diets containing

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PSP toxins; mussels are highly resistant and in most cases do not exhibit an adverse reaction when feeding on PSP-producing dinoflagellates (Cucci et al. 1985; Mardsen & Shumway 1992). Conversely, oysters, clams and scallops have been described as highly sensitive to PSP toxins (Lassus et al. 1989; Bardouil et al. 1993; Navarro et al. 2016), showing a reduction in filtration activity and growth. The genus *Mytilus* is described as highly insensitive to dinoflagellate producers of PSP (Bricelj & Shumway 1998; Ichimi et al. 2001; Navarro & Contreras 2010), showing a reduction in filtration activity only during the first hours of exposure. This behaviour is accompanied by a rapid increase in the toxin concentration in the tissue, eliminating toxins in a short period of time (Shumway 1990; Navarro et al. 2008). Shumway and Cucci (1987) studied the effects of the dinoflagellate *Protogonyaulax tamarensis* on feeding behaviour of *Modiolus modiolus* and *Spisula solidissima*, and reported these species were not affected by the toxic dinoflagellates, concluding that continuous exposure to PSP would have allowed development of mechanisms to use toxic phytoplankton as a food source. In contrast, species like *Crassostrea gigas* and *Mya arenaria* are highly sensitive to PSP, resulting in a low accumulation of toxins in tissues, due to development of responses and physiological mechanisms for avoiding ingestion of toxic dinoflagellate cells (Gainey & Shumway 1988; Bricelj et al. 2005; MacQuarrie & Bricelj 2008).

Bivalves feed on small suspended particles, which are retained by the gills and transported to the mouth palps, where selection of particles may occur depending on their concentration and characteristics (Shumway et al. 1985; Newell et al. 1989; Navarro & Widdows 1997). Shumway and Cucci (1987) found that the clam *M. arenaria* selectively removed toxic *Alexandrium tamarense* cells in pseudofaeces, resulting in a greater proportion of toxic cells in pseudofaeces than in the food offer. The bivalve *Mytilus edulis* and the scallop *Argopecten irradians*, when exposed to the dinoflagellate *Porocentrum minimum*, produce large quantities of pseudofaeces, containing numerous non-digested cells of this dinoflagellate (Hégaret et al. 2007).

Since 1972, there have been many events of HABs in southern Chile, extending from Castro, Chiloé (42°29′S, 73°48′W) to the Beagle Channel (55°07′S, 68°36′ W). These blooms have been caused by the dinoflagellate *Alexandrium catenella*, with the largest event having occurred during the summer through autumn of 2002, reaching a maximum concentration of 7.79×10^5 cells L^{-1}, and toxicity with values exceeding 20.000 μg STX eq. 100 g^{-1} tissue of the bivalve *Mytilus chilensis* (Clement et al. 2002). Southern Chile is characterized by great diversity of bivalve species having ecological and commercial importance, where extraction and consumption have been significantly reduced by temporary or indefinite closure of areas where bivalves remain toxic with PSP throughout the year. We used the razor clam *Tagelus dombeii* as a model; an infaunal bivalve with a broad latitudinal distribution, inhabiting soft sediments within tidal and subtidal zones of southern Chile. Razor clam fishery represents more than 5% of all commercially important benthic resources of Chile. Navarro et al. (2008) studied the feeding behaviour of *T. dombeii* and concluded that this bivalve behaves as a suspension-feeder when immersed, indicating 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 frequently exposed to PSP, unlike the majority of other populations located in the north, having no history of PSP exposure. It is therefore important to determine feeding behaviour in bivalves exposed to diets containing dinoflagellate producers of PSP. However, it is currently unknown whether selection of toxic cells occurs with the same intensity in bivalve populations with different histories of exposure to HABs. Our study evaluates effects of the dinoflagellate *A. catenella* on feeding activity of two populations of the razor clam *T. dombeii*, with different histories of exposure to PSP; and determines the ability of this species to select and reject toxic *A. catenella* cells in the pseudofaeces.

Materials and methods

Animal collection and acclimation

Adult specimens of *T. dombeii* (razor clam) were collected from subtidal populations of Melinka, Aysén (44º01′S; 74º19′W; with previous PSP exposition) and Corral, Valdivia (39º51′S; 73º26′W; no previous PSP exposure). Experimental clams (5–7 cm shell length) were acclimated in a static system for 2 weeks at 14 °C, 30 psu, buried in fine sediment collected from the same location where specimens live, and fed continuously with a diet containing (by weight) 60% of the microalga *Isochrysis galbana* and 40% inorganic sediment, at a concentration of 20 mg L^{-1}. The seawater was changed every 48 h.

Experimental design

Experiments to determine clearance rate (CR) and pre-ingestive selection efficiency were carried out on four occasions. Each experiment was replicated four times for each treatment (contaminated and control) at each population and clams held individually in acrylic chambers containing 1.5 L of filtered seawater. The experiment lasted for six hours, and the medium was homogenized by constant aeration.

Preparation of diets

A. catenella (strain ACC02) used during the experiments was isolated from the Aysén Region (Chile) and cultured in filtered seawater (0.45 μm) enriched with “L1” growth medium (Guillard 1995). *I. galbana* was cultured with “f/2” medium (Guillard 1975). Both species of algae were harvested for experimental use when they were in their exponential growth phase. Inorganic sediment was added to the diets to emulate organic/inorganic fractions of natural suspended particulate matter. This sediment was collected from the upper centimetre of the tidal flat at Yaldad Bay (Chiloe), passed through a 40 μm sieve, rinsed with distilled water, and ashed in a muffle furnace at 500 °C for 12 h to eliminate the organic fraction. After ashing, sediment was sieved again (40 μm sieve) to eliminate aggregations. Both diets were prepared with a concentration of 20 mg L^{-1}. The contaminated diet consisted of 50% of *A. catenella*, 10% *I. galbana* and 40% sediment. Composition of the control diet was 60% *I. galbana* and 40% of sediment. The organic fraction of the contaminated diet was 62.93 ± 1.94 and 61.06 ± 5.79% for the control diet. Both control and contaminated diets were obtained during the logarithmic growth of both cultures. The toxic content of *A. catenella* cells was measured throughout the experiments, and a mean value was obtained from 15 samples. Average saxitoxin concentration was 10.3 ± 0.91 fmoles STX eq. $cell^{-1}$.

Clearance rate

The CR was determined on clams exposed to control and contaminated diets, using a particle concentration of 20 mg L^{-1}. CR experiments were carried out in a static system homogenized by aeration, in which decreases in particle concentration were periodically monitored in each experimental aquarium using a particle counter Elzone 180 XY equipped with a counting tube of 120 µm aperture. These tests were carried out over a period of six hours, with measurements made every 60 min, replacing the food consumed in every case. To test any sedimentation of cells during feeding measurements, a control aquarium without clams was operated to correct CR measurements. CR (L h^{-1}) was calculated following Coughlan (1969).

Pre-ingestive selection efficiency

Clams from Melinka, Aysén and Corral, Valdivia were exposed to a contaminated diet (mixture of *A. catenella* + *I. galbana* + sediment) at a concentration of 20 mg L^{-1}, which is over the threshold of pseudofaeces production (Bayne & Hawkins 1990). Having identified and collected with Pasteur pipettes the pseudofaeces, they were disintegrated using slow speed vortex and resuspended in filtered seawater. This was performed in order to calculate cell count using the Utermöhl method (inverted microscope), determining the proportion of *Isochrysis/Alexandrium* cells contained in the material. Proportion of *Isochrysis/Alexandrium* algae cells contained within the diet was counted using the same method. Particle selection efficiency was calculated according to the proportion of *Isochrysis/Alexandrium* cells existing in the food (f) and the pseudofaeces (p) according to the formula given by Bayne and Hawkins (1990):

$$SE = 1 - (p/f)$$

When SE = 0, there is no selection ($f = p$) and when SE = 1, there is a complete selection and ingestion of only *Isochrysis* cells.

Statistical analyses

A two-way blocked ANOVA was conducted to analyse effects of diet (control and toxic) and origin (Melinka and Corral) on physiological measurements (experiments as random factor). Selection efficiency between both populations was compared with a one-way blocked ANOVA (experiments as random factor). Before analyses, normality and homoscedasticity of the data were tested using Kolmogorov-Smirnof and Bartlett tests, respectively. All analyses were performed using RStudio, GAD library.

Results and discussion

Diet characteristics

Cell size of *A. catenella* ranged between 28 and 35 µm, and *I. galbana* between 5 and 6 µm. Previous studies on filter feeding behaviour have confirmed that selective removal does not occur when mussels feed on particles ranging between 2 and 100 µm (Bayne et al. 1976).

Similarly, Vahl (1972) demonstrated 80–100% retention by the mussel *M. edulis* of all particles larger than 2 μm diameter.

The organic fraction of both diets was not significantly different ($p > 0.01$), being 62.93 ± 1.94% for the contaminated diet and 61.06 ± 5.79% for the non-toxic diet.

Clearance rate

CR measured from individuals of two populations of the razor clam *T. dombeii* (with different histories of PSP exposure) exposed to the toxic and non-toxic diet is shown in Figures 1 and 2. Feeding activity of *T. dombeii* was affected by the diet containing toxic dinoflagellate *A. catenella*, being reduced in 26.3% (mean = 0.14 ± 0.01 L h^{-1}) in individuals from Corral, Valdivia (without previous PSP exposure) when compared to clams fed with the non-toxic diet (mean = 0.19 ± 0.01 L h^{-1}) (Figure 1). Specimens from Melinka, Aysén (with previous PSP exposure) also showed a reduction (47%) of the CR (mean = 0.18 ± 0.02 L h^{-1}), when exposed to the diet containing toxic dinoflagellate, in comparison with clams fed with the non-toxic diet (mean = 0.34 ± 0.03 L h^{-1}) (Figure 2). Two-way blocked ANOVA analysis showed a significant effect from factors such as diet ($F_{1,57} = 16.69$, $p < 0.001$) and origin ($F_{1,57} = 13.36$, $p < 0.001$), as well as the block ($F_{3,57} = 4.81$, $p < 0.05$). Whereas the interaction between diet and origin ($F_{1,57} = 2.05$, $p > 0.001$) was not significant for CR.

The significant effect of factor origin on CR likely reflects adaptation to specific environmental conditions (e.g. different food supply). Reduced CR observed in specimens of *T. dombeii* exposed to a diet containing *A. catenella* was consistent with the response described for several species of bivalves exposed to similar diets (Wildish et al. 1998; Navarro & Contreras 2010; Navarro et al. 2014). The highest CR of clams from Melinka, Aysén population, in comparison with the clam of Corral, Valdivia, when fed *A. catenella*, appears to be associated with frequent exposure events to PSP, indicating a physiological adaptation to food supply containing toxic *A. catenella*. Similar results were described by Bricelj et al.

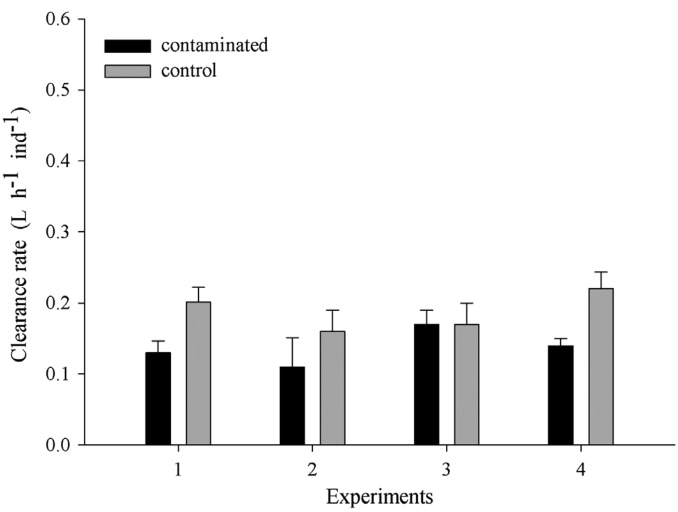

Figure 1. *Tagelus dombeii*. Clearance rate of individuals from Corral, Valdivia exposed to a contaminated and control diet. Values represent means ± SE.

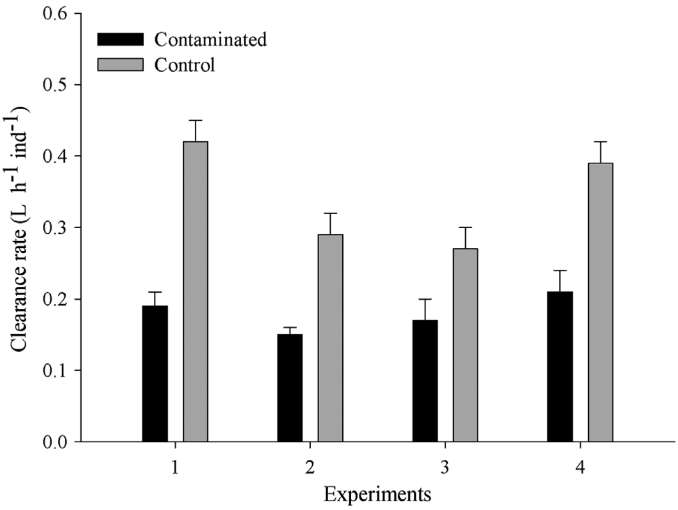

Figure 2. *Tagelus dombeii*. Clearance rate of individuals from Melinka, Aysén exposed to a contaminated and control diet. Values represent means ± SE.

(2005) for two populations of the clam *M. arenaria* with different PSP histories, and fed with the toxic dinoflagellate *A. tamarense*. Navarro et al. (2014), working with the same two populations of *T. dombeii*, observed similar results by exposing the clams to a diet with the same proportion of *A. catenella – I. galbana* (1:1), however with a food concentration below the threshold of pseudofaeces production (2.0 mg L^{-1}). Similar responses are described for the mussel *M. edulis* fed with the dinoflagellate *Gyrodinium aureolum* (Widdows et al. 1979) and *Perna viridis* fed with *Alexandrium monilatum* (May et al. 2010). Conversely, mussels *Perna canaliculus* (Contreras et al. 2012) and *M. modiolus* (Shumway & Cucci 1987) have high insensitivity to PSP, without reducing feeding activity, when exposed to the toxic dinoflagellate *A. tamarense*. This suggests that feeding responses of marine bivalves to toxic dinoflagellates are species-specific, being inhibited or unaffected (Leverone et al. 2007; Contreras et al. 2012; Navarro et al. 2014). The decrease in CR observed in clams fed with the toxic diet and the significant higher feeding activity in clams from Melinka, Aysén is highlighted by the statistical significance of the interaction between diet and origin.

Pre-ingestive selection efficiency

Rejection of particulate matter in the form of pseudofaeces has been described as a secondary regulatory mechanism of ingestion rate in marine bivalves, and also as a mechanism for pre-ingestive particle selection, allowing selective ingestion and the elimination of unwanted particles in the form of pseudofaeces (Shumway & Cucci 1987; Navarro & Widdows 1997; Wildish et al. 1998). Selection efficiency of suspended particles represents a mechanism that depends on various factors such as size, concentration and chemical composition of particles (Ward 1996; Navarro & Widdows 1997; Beninger et al. 1999; Ward & Shumway 2004). Differences were observed between the proportion of *Isochrysis*/*Alexandrium* cells in food and pseudofaeces produced by the clams from the two populations studied. Thus, at concentrations of food above the pseudofaeces production, clams from Corral, Valdivia and from Melinka, Aysén have the ability to select particles at the pre-ingestive level, eliminating a larger proportion of the toxic dinoflagellate *A. catenella* cells in the form of pseudofaeces. One-way blocked ANOVA showed a significant effect from the factor origin ($F_{1,27} = 5.04$, $p < 0.03$) and block ($F_{3,27} = 3.95$, $p < 0.01$) on the pre-ingestive selection efficiency. Higher values were registered in clams from the population of Melinka, Aysén (mean = 68.0 ± 1.8%) than those from Corral, Valdivia (mean = 54.3 ± 9.0%) (Figure 3). The significant effect of block represents the high individual variability described for bivalves when exposed to PSP (Shumway & Cucci 1987).

The present results demonstrate that pre-ingestive selection efficiency was a very effective mechanism in the two populations of the clam *T. dombeii*, in order to reduce ingestion of toxic cells. This feeding behaviour has been described for different filter feeding bivalves Kiørboe and Møhlenberg (1981) using the chlorophyll *a* ratio in food and pseudofaeces. These authors found that the cockle *Cerastoderma edule* was able to select phytoplankton cells with selection efficiencies as high as 77%. Bayne et al. (1989), modified this index using the organic fraction in food and pseudofaeces, and reported values of selection efficiency around 50% for *M. edulis*.

Twarog and Yamaguchi (1974) suggest that bivalve species periodically exposed to harmful algae blooms can develop mechanisms to exploit toxic cells as a food resource. Similarly, Shumway and Cucci (1987) found that specimens of *M. edulis*, from populations with different exposure to PSP, significantly differ in their CR when facing the presence of toxic cells from *P. tamarensis*. Significantly higher values of CR and pre-ingestive selection efficiency of clams from Melinka, Aysén suggest the presence of an adaptive response to the frequent blooms *A. catenella* occurring in their environment; in contrast with that observed for *T. dombeii* from Corral, Valdivia, with no history of exposure to *A. catenella*.

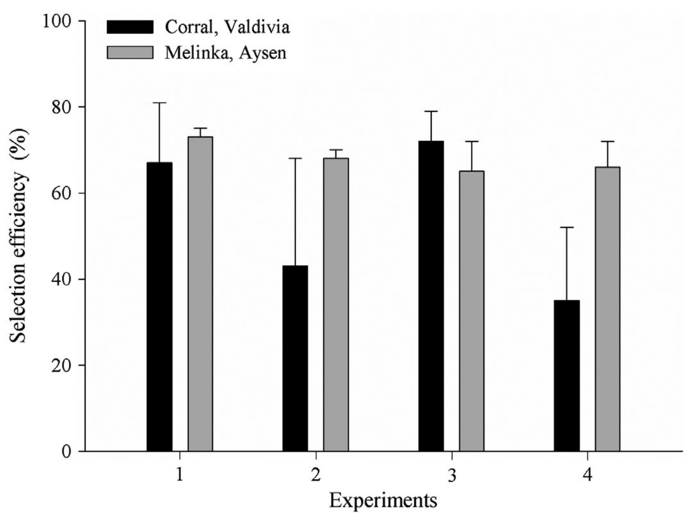

Figure 3. *Tagelus dombeii*. Selection efficiency in individuals from Corral, Valdivia and Melinka, Aysén exposed to a contaminated diet. Values represent means ± SE.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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