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ORIGINAL ARTICLE

Enzymatic digestive activity and absorption efficiency in *Tagelus dombeii* upon *Alexandrium catenella* exposure

M. J. Fernández-Reiriz · J. M. Navarro · B. A. Cisternas · J. M. F. Babarro · U. Labarta

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Abstract We analyzed absorption efficiency (AE) and digestive enzyme activity (amylase, cellulase complex, and laminarinase) of the infaunal bivalve *Tagelus dombeii* originating from two geographic sites, Corral-Valdivia and Melinka-Aysén, which have different long-term paralytic shellfish poisoning (PSP) exposure rates. We report the effects of past feeding history (origin) on *T. dombeii* exposed to a mixed diet containing the toxic dinoflagellate *Alexandrium catenella* and another dinoflagellate-free control diet over a 12-day period in the laboratory. Absorption efficiency values of *T. dombeii* individuals that experienced PSP exposure in their habitat (Melinka-Aysén) remained unchanged during exposure to toxic food in the laboratory. In contrast, *T. dombeii* from a non-PSP exposure field site (Corral-Valdivia) showed a significant reduction in AE with toxic exposure time. This study established that the amylase and cellulase complexes were the most important enzymes in the digestive glands of *Tagelus* from both sites. The temporal evolution of enzymatic activity under toxic diet was fitted to exponential (amylase and cellulase) and to a logarithmic (laminarinase) models. In all fits, we found significant effect of origin in the model parameters. At the beginning of the experiment, higher enzymatic activity was observed for clams from Corral-Valdivia. The amylase activity decreased with time exposure for individuals from Corral and increased for individuals from Melinka. Cellulase activity did not vary over time for clams from Corral, but increased for individuals from Melinka and laminarinase activity decreased over time for individuals from Corral and remained unchanged over time for Melinka. A feeding history of exposure to the dinoflagellate *A. catenella* was reflected in the digestive responses of both *T. dombeii* populations.

Keywords *Alexandrium* · *Tagelus dombeii* · Absorption efficiency · Enzymatic activity · Trophic regimen

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M. J. Fernández-Reiriz (✉) · J. M. F. Babarro · U. Labarta
Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Eduardo Cabello 6, 36208 Vigo, Spain
e-mail: mjreiriz@iim.csic.es

J. M. Navarro · B. A. Cisternas
Facultad de Ciencias, Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

Introduction

Tagelus dombeii (razor clam) is an infaunal species that inhabits soft sediments at a water depth of 1–13 m between the Tumbes region of northwestern Perú and Aysén in the southern Chile. Dense razor clam populations are farmed by traditional fisheries, and total landings have decreased from 7,294 t in 1995 to 3,248 t in 2010 (SERNAPESCA 2010). Razor clam farming (*T. dombeii*) represents about 5 % of benthic resources utilized in Chile. The two razor clam populations investigated here inhabit the subtidal shallow water, characterized by a sandy bottom. The *T. dombeii* population of Melinka-Aysén has been exposed to frequent PSP events in recent decades, while no PSP events have been recorded for Corral-Valdivia (Guzman and Campodonico 1978; Molinet et al. 2003).

Navarro et al. (2008) studied the feeding behavior of *T. dombeii* from intertidal and subtidal communities in Chile looking for a dual feeding strategy (suspension/detritivoral). They concluded that *T. dombeii* is mainly a

suspension feeder. *Alexandrium catenella* are harmful algae that produce paralytic shellfish poisoning (PSP) toxin, which can be fatal to humans and may be present at different concentrations in the diet of bivalves, zooplankton, crustaceans, and gastropods (Huss 2003). Shumway and Cucci (1987) studied the effects of the dinoflagellate *Protogonyaulax tamarensis* on the feeding behavior of bivalve mollusks. They noted that the animals exhibited valve closure and/or siphon retraction (*Mya arenaria*, *Mytilus edulis*, *Geukensia demisa*) as well as a reduced (*M. arenaria*, *G. demisa*) or increased (*Ostrea edulis*) clearance rates. In contrast, the latter authors also observed that two species, *Modiolus modiolus* and *Spisula solidissima*, were not affected by the toxic dinoflagellates.

Lassus et al. (2004) reported that the Pacific oyster *Crassostrea gigas* and the *M. arenaria* clam reduced their filtration activity when feeding on microalgae containing PSP toxins. *Alexandrium catenella* exposure is also known to have a negative impact on initial filtration and ingestion in *Mytilus chilensis* (Navarro and Contreras 2010). Exposure of bivalves to harmful microalgae can also affect their digestion and energy allocation. Li et al. (2002) assessed the effects of *Alexandrium tamarense* on the energy budget, quantified as scope for growth (SFG), of the mussel *Perna viridis* and the manila clam *Ruditapes philippinarum* and demonstrated that an increase in PSP burden was associated with a significant reduction in SFG in both clams and mussels, primarily because of decreases in absorption efficiency (AE). This effect can hypothetically be linked to modification of the digestive process as observed by Fernández-Reiriz et al. (2008) who studied trophic interactions between *M. chilensis* and the toxic dinoflagellate *A. catenella* over a 21-day period to determine the absorption kinetics of biochemical components and the enzymatic activity of the digestive gland (amylase, cellulase complex, laminarinase, and protease). Their study showed that *M. chilensis* was able to develop mechanisms that enable the use of toxic microalgae as a food source, despite the fact that its feeding and digestive processes were affected in the early days of contamination.

The digestive gland in bivalves plays an important role in the digestion and assimilation of nutrients. Fernández-Reiriz et al. (2001) and Labarta et al. (2002) established that investment in enzyme resources is one of the mechanisms used by bivalve mollusks to optimize energy gains when variations in food supplies occur. According to Galimany et al. (2008a, b, c) and Hégaret et al. (2009), the digestive gland is generally the organ most affected by PSP exposure. Thus, it appears pertinent to study the digestive process in the digestive gland of the razor clam *T. dombeii*.

The objective of this study was to determine the AE as well as the enzymatic activity of the digestive gland (amylase, cellulase complex, and laminarinase) in two razor clam *T. dombeii* populations with differing historical exposures to PSP toxins, when exposed to the toxic dinoflagellate *A. catenella*.

Materials and methods

Experimental design

Adult specimens of the razor clam *T. dombeii* ranging in shell length from 50 to 70 mm were collected from natural banks at Corral-Valdivia and Melinka-Aysén in 2010 (see Fig. 1). They were transported to the laboratory, where they were acclimated for 1 week at a temperature of 14 °C and a salinity of 30 practical salinity units (PSU). During this period, the clams were buried in aquarium tanks and continuously fed a diet containing 60 % microalgae clone T-ISO and 40 % inorganic sediment (1.5 mg/L) using a peristaltic pump. The laboratory conditions (i.e., temperature, salinity, food supply, and organic content of the diet) were set to follow the conditions of the animals' natural habitat. The tanks were constantly aerated, and the seawater was changed every 48 h. Following the acclimation period, one group of clams was exposed to a mixed diet containing the toxic dinoflagellate *A. catenella* (proportions by weight: 50% *A. catenella*, 10% T-ISO, and 40% sediment) and another group was exposed to a dinoflagellate-free control diet (60 % T-ISO, 40 % sediment) for 12 days. Given the large amount of laboratory analysis and large individual variability in the physiological responses of bivalves exposed to PSP (Navarro and Contreras 2010), four replicates (8-L tanks, temperature 14 °C, salinity 30 PSU) containing 25 clams each were supplied with the toxic diet, whereas three replicates containing the same number of clams were supplied with the control diet (less variable responses). The forth toxic tank was included in the experiment as an extra to be used if necessary, but all the statistical analysis was conducted with three toxic and control aquaria. The clams were permanently buried in sediment collected from their natural habitats and given a daily allotment of food equal to 2 % (measured, ca. 14 mg/day) of the dry body weight of the experimental clams. We observed no significant differences (p value > 0.05) in the daily amounts of food reaching each population, and the concentration of both diets was below the threshold of pseudofeces production. The diets were delivered continuously using a peristaltic pump. The monoclonal non-axenic *A. catenella* (strain ACC02) and clone T-ISO were cultivated following the method of Fernández-Reiriz et al. (2008). Toxicity was quantified using an electrophysiological test as described by Vélez et al. (2001), and a mean value was calculated from the test results of 15 samples. The toxic diet was prepared by mixing laboratory cultured *A. catenella*, T-ISO, and washed and ashed sediment. Samples of this diet were collected on

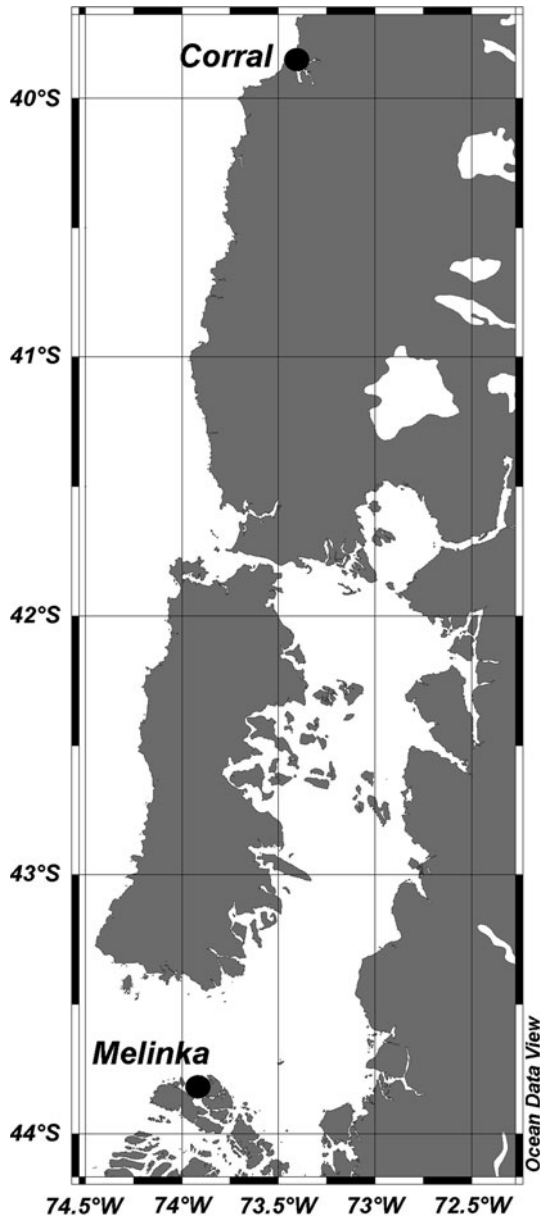

Fig. 1 Map of southern Chile showing habitat origin sites

pre-washed, ashed, and weighed Whatman GF/C glass fiber filters and rinsed with 0.5 M ammonium formate. Subsequently, triplicate samples were taken for total matter (following oven-drying to a constant weight at 100 °C) and organic matter (following ashing in a muffle furnace to a constant weight at 450 °C). The digestive behavior, AE, and enzymatic activity in the control samples and the individual exposed to the contaminated diet were monitored throughout the experiment (0, 2, 5, 8, and 12 day).

Absorption efficiency

We estimated AE by determining the organic and inorganic content of the food and feces following the ratio method of Conover (1966). Representative samples of each diet mixture (ca. 15 mg; three replicates) were collected during the experiment, and the AE was calculated for each specimen by collecting feces (4–10 mg) from the top of the sediment using glass pipettes. Fecal pellets were separated from the sediment by careful observation under a stereoscope, and they were filtered through pre-ashed, pre-weighed Whatman GF/C filters in a manner similar to the diet mixture. Filters were rinsed with isotonic ammonium formate, dried to a constant weight at 100 °C (24 h), and then weighed and combusted at 450 °C to a constant weight. The filters were weighed again to estimate the organic and inorganic fraction contained in the food and feces.

In vitro enzyme assays

Freeze-dried digestive glands dissected from the experimental animals were cold-homogenized in 0.01 M phosphate–citrate buffer, about 10 mg/mL (containing 20 mM NaCl) at pH 6.9. The enzyme extracts were immediately centrifuged at $15{,}938 \times g$ for 20 min, and the supernatant was used for in vitro enzyme assays following the methodology described by Ibarrola et al. (1996). The protein content within the clams' digestive extracts was determined as described by Lowry et al. (1951) using bovine serum albumin as the standard.

The substrate solutions (starch 1 %, carboxymethylcellulose 1 %, laminarin 0.4 %) were prepared with a 0.2 M citrate–phosphate buffer containing 20 mM NaCl to the corresponding pre-established optimal pH of 6.5 for amylase, laminarinase, and cellulase complex. To follow the reaction, 0.5 mL of digestive extract and 0.5 mL of substrate solution were mixed and incubated at ambient temperature (14 °C) in a circulating water bath for 30 min. The concentration of the digestive extract and time interval for lineal release of end-product were assessed relative to body mass (frozen and freeze-dried) and confirmed to be optimal according to preliminary studies (Fernández-Reiriz et al. 2001, 2008).

Carbohydrase activity (amylase, cellulase complex, and laminarinase) was determined using the Nelson–Somogyi method (Nelson 1944; Somogyi 1952) with a calibration curve established using maltose. In this work, the enzyme assays are expressed in terms of specific activity (sp) expressed as (mg end-product released)/(mg protein h). To preclude digestive organ variability caused by weight differences, these weights were corrected to a standard-sized individual with a dry weight of 1,000 mg, following the formula

$$Y_s = (1{,}000/W_e)^b Y_e$$

where Y_s = dry weight of the standardized organ; Y_e = experimental dry weight of the organ; W_e = total dry weight of the experimental clam; b = weight-specific

exponent relating digestive organ weight to complete specimen weight ($b = 0.7256$ and 0.8900 for the digestive gland of *T. dombeii* from Melinka and Corral, respectively).

Statistical analysis

In our experimental design, AE and enzymatic activity are measured repeatedly on each tank over time, that is, we have longitudinal data. Therefore, we should apply repeated measures analysis of variance (Quinn and Keough 2002), which, on contrast with classical ANOVA models, considers the temporal dependence among samples from the same tank. Three-way repeated-measures ANOVA (tank as random factor) was conducted to analyze the effects of diet (control and toxic), origin (Melinka and Corral), and time of exposure (TE) on the AE and enzymatic activity. When the temporal change in AE and enzymatic activity depended on the diet, that is, in case of significant interaction between diet and time and/or three-way interaction, the temporal pattern of individuals under control and toxic diets were analyzed separately. For each diet, we applied two-way repeated-measures ANOVA (tank as random factor) to test the effect of origin and TE on AE and enzymatic activity. When the effect of time was significant, we fitted the temporal change in AE and enzymatic activity and applied covariance analysis (ANCOVA) for comparison between origins. For each ANOVA conducted, normality and homoscedasticity were previously tested by Shapiro–Wilk and Levene tests. Statistical analysis was conducted using R 2.14.2 software (R Development Core Team 2011).

Results

Experimental diets

Total concentration of particulate material (TPM) did not differ significantly between the toxic (1.99 ± 0.06 mg/L) and control (1.95 ± 0.19 mg/L) diet groups (p value > 0.05). Moreover, organic content also did not differ significantly (p value > 0.05) between the toxic diet group (1.21 ± 0.05 mg/L, 61 %) and the control diet group (1.08 ± 0.09 mg/L, 56 %). The mean toxic content of *A. catenella* cells (strain ACCO2) was 10.31 ± 0.91 fmol STXeq cell.

Absorption efficiency

Table 1 illustrates the AE in *T. dombeii* for each diet, origin, and TE. Table 2 shows the three-way repeated-measures ANOVA for AE, the between-group test indicates that the effects of diet, origin, and interaction between them were significant. The within- tank test shows a significant TE effect, interaction between TE and both group factors (diet and origin), and three-way interaction.

Table 1 Descriptive analysis (mean, SD) of AE by diet, origin, and TE

TE	Corral-Valdivia				Melinka-Aysén			
	Control		Toxic		Control		Toxic	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AE								
0	75.89	2.88	75.03	10.36	78.34	0.13	76.31	1.41
2	75.60	1.97	74.01	8.73	78.04	2.06	77.99	0.79
5	73.72	2.14	66.36	9.02	79.85	2.43	78.45	3.23
8	72.00	3.00	52.07	5.01	77.12	3.65	74.18	3.62
12	73.67	4.51	40.64	8.96	77.56	6.76	74.25	1.09

Table 2 Three-way repeated-measures ANOVA for AE

	df	Sum sq	Mean sq	*F*	*p* value
Error: tank					
Diet	1	788.2	788.2	50.2	0.0001***
Origin	1	1,300.2	1,300.2	82.82	1.7×10^{-05}***
Diet:origin	1	422	422	26.88	0.0008***
Residuals	8	125.6	15.7		
Error: within					
TE	4	1,006.8	251.71	8.951	5.7×10^{-05}***
TE:diet	4	660.1	165.03	5.869	0.0012**
TE:origin	4	601.1	150.28	5.344	0.0021**
TE:diet:origin	4	486.6	121.66	4.327	0.0066**
Residuals	32	899.8	28.12		

** $p < 0.01$

*** $p < 0.001$

For the control diet, we only found significant differences between origins (two-way repeated-measures ANOVA, p value $= 0.0139$). Thus, AE for *T. dombeii* under control diet did not vary over time and was lower for individuals from Corral (74.18 %) than for individuals from Melinka (78.18 %).

For individuals subjected to toxic diet, significant effects of time, origin, and interaction between them (two-way repeated-measures ANOVA, p value < 0.01) were found. We fitted the temporal evolution of AE by a linear model (Fig. 2). Comparison between origins (ANCOVA, Table 7) did not find differences at the beginning of the experiment (t test intercept, p value $= 0.903$), but found differences between slopes: While toxic exposure caused a decrease in AE for clams from Corral ($AE = 78.19 - 3.068TE$; Adj $R^2 = 0.738$; p value $= 2.5e^{-5}$), AE did not vary over time ($AE = 77.76 - 0.282TE$; Adj $R^2 = 0.1504$; p value $= 0.0849$) for clams from Melinka.

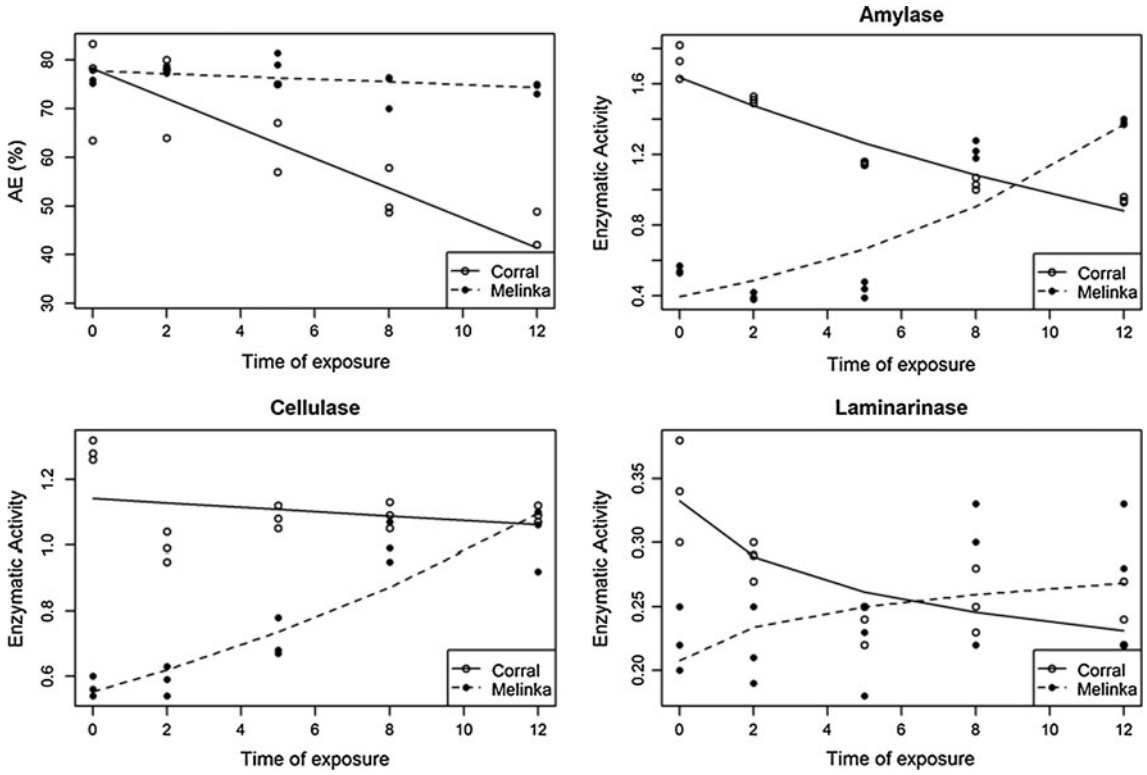

Fig. 2 Fist of temporal evolution of AE and carbohydrase activities for *T. dombeii* individuals subjected to toxic diet

Table 3 Descriptive analysis (mean, SD) of specific enzymatic activity by diet, origin, and TE

TE	Corral-Valdivia				Melinka-Aysén			
	Control		Toxic		Control		Toxic	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Amylase								
0	1.16	0.04	1.73	0.10	0.63	0.11	0.55	0.02
2	1.17	0.02	1.51	0.02	0.67	0.02	0.40	0.02
5	1.19	0.04	1.15	0.01	0.64	0.06	0.44	0.05
8	1.16	0.04	1.03	0.04	0.66	0.03	1.23	0.05
12	1.16	0.03	0.94	0.02	0.64	0.05	1.38	0.02
Cellulase								
0	1.02	0.04	1.29	0.03	0.54	0.03	0.57	0.03
2	1.04	0.03	0.99	0.05	0.54	0.06	0.59	0.05
5	1.00	0.06	1.08	0.04	0.53	0.06	0.71	0.06
8	1.01	0.04	1.09	0.04	0.53	0.07	1.00	0.06
12	1.00	0.03	1.09	0.03	0.58	0.01	1.03	0.09
Laminarinase								
0	0.25	0.03	0.34	0.04	0.25	0.05	0.22	0.03
2	0.24	0.03	0.29	0.02	0.20	0.05	0.22	0.03
5	0.24	0.02	0.24	0.02	0.21	0.02	0.22	0.04
8	0.27	0.01	0.25	0.03	0.26	0.03	0.28	0.06
12	0.29	0.03	0.24	0.03	0.26	0.01	0.28	0.06

Enzymatic activity: amylase, cellulase, and laminarinase

Dry weight was higher (p value < 0.05) in *T. dombeii* individuals from Melinka-Aysén ($\sim$850 mg) compared to those from Corral-Valdivia ($\sim$600 mg). Protein levels did not differ between the diet groups (p value > 0.05), with dry weight values close to 20 % for both the toxic and control groups.

Table 3 illustrates the specific enzymatic activity (sp) in *T. dombeii* for each diet, origin, and TE. Tables 4, 5, and 6 show the three-way repeated-measures ANOVA for the carbohydrase activities. Between-group comparison shows significant effects of diet, origin, and interaction between them for amylase and cellulase activity, while for laminarinase we only found significant effect of origin. The within tanks test shows significant TE effect, interaction between TE and both group factors, and three-way interaction for amylase and cellulase, while for laminarinase there was no interaction between TE and diet (p value $= 0.3463$).

For *T. dombeii* individuals under control diet, the two-way repeated-measures ANOVA found that enzymatic activity of amylase and cellulase was higher for clams from Corral (p value $= 4.23 \times 10^{-7}$ and 1.01×10^{-5}, respectively) and did not vary over time (p values > 0.1 for TE effect and interaction between time and origin). On the

Table 4 Three-way repeated-measures ANOVA for amylase

	df	Sum sq	Mean sq	*F*	*p* value
Error: tank					
Diet	1	0.248	0.248	418.53	3.4×10^{-08}***
Origin	1	3.69	3.69	6,219.51	7.5×10^{-13}***
Diet:origin	1	0.007	0.007	11.51	0.0095**
Residuals	8	0.005	0.001		
Error: within					
TE	4	0.273	0.0683	28.03	4.7×10^{-10}***
TE:diet	4	0.3353	0.0838	34.42	3.6×10^{-11}***
TE:origin	4	1.7074	0.4269	175.3	$<2 \times 10^{-05}$***
TE:diet:origin	4	1.6626	0.4156	170.69	$<2 \times 10^{-16}$***
Residuals	32	0.0779	0.0024		

** $p < 0.01$

*** $p < 0.001$

Table 5 Three-way repeated-measures ANOVA for cellulase

	df	Sum sq	Mean sq	*F*	*p* value
Error: tank					
Diet	1	0.4067	0.4067	245.76	2.7×10^{-07}***
Origin	1	2.408	2.408	1,454.99	2.5×10^{-10}***
Diet:origin	1	0.0735	0.0735	44.41	0.0002***
Residuals	8	0.0132	0.0017		
Error: within					
TE	4	0.1484	0.0371	15.11	4.9×10^{-07}***
TE:diet	4	0.1556	0.0389	15.84	3.0×10^{-07}***
TE:origin	4	0.2379	0.0595	24.22	2.7×10^{-09}***
TE:diet:origin	4	0.1994	0.0499	20.3	2.1×10^{-08}***
Residuals	32	0.0786	0.0025		

*** $p < 0.001$

other hand, we found significant effect of TE on laminarinase activity (p value = 0.0108), which increased over time (Melinka: sp = 0.2353 + 0.0042TE, Adj $R^2 = 0.2113$, p value = 0.0495; Corral: sp = 0.2209 + 0.0029TE, Adj $R^2 = 0.343$, p value = 0.0128).

For *T. dombeii* individuals under toxic diet, the two-way repeated-measures ANOVA found significant effects of time, origin, and interaction between them (p value < 0.001) for amylase and cellulose activity, as well as significant effect of time and interaction between time and origin for laminarinase (p value < 0.05). The temporal evolution of specific enzymatic activity (sp) was fitted by exponential models for amylase and cellulase and by a logarithmic model for laminarinase. In all three fits, we found significant effect of origin in the model parameters (ANCOVA, Table 7, p value < 0.001). At the beginning of the experiment, higher enzymatic activity was observed for clams from Corral (Table 3; Fig. 2). The amylase activity decreased with TE for individuals from Corral ($b = -0.0516$) and increased for

Table 6 Three-way repeated-measures ANOVA for laminarinase

	df	Sum sq	Mean sq	*F*	*p* value
Error: tank					
Diet	1	0.0017	0.0017	1.903	0.205
Origin	1	0.0091	0.0091	10.178	0.0128*
Diet:origin	1	0.0002	0.0002	0.186	0.6778
Residuals	8	0.0072	0.0009		
Error: within					
TE	4	0.0185	0.0046	4.321	0.0066**
TE:diet	4	0.0050	0.0012	1.161	0.3463
TE:origin	4	0.0113	0.0028	2.634	0.0523
TE:diet:origin	4	0.0159	0.0040	3.713	0.0136*
Residuals	32	0.0343	0.0011		

* $p < 0.05$

** $p < 0.01$

Melinka ($b = 0.1032$). Cellulase activity did not vary over time for clams from Corral (Adj $R^2 = 0.0167$, p value = 0.28) and increased for individuals from Melinka ($b = 0.0569$). Finally, laminarinase activity decreased over time for individuals from Corral ($b = -0.0393$) and did not vary over time for Melinka (Adj $R^2 = 0.1597$, p value = 0.078).

Amylase sp (toxic diet):

$$\text{Melinka: sp} = 0.3968e^{0.1032TE},$$
$$\text{Adj } R^2 = 0.6708, p \text{ value} = 0.0001.$$
$$\text{Corral: sp} = 1.6366e^{-0.0516TE},$$
$$\text{Adj } R^2 = 0.9093, p \text{ value} = 2.3 \times 10^{-08}.$$

Cellulase sp (toxic diet):

$$\text{Melinka: sp} = 0.5532e^{0.0569TE},$$
$$\text{Adj } R^2 = 0.8495, p \text{ value} = 6.4 \times 10^{-07}.$$
$$\text{Corral: sp} = 1.1419e^{-0.0061TE},$$
$$\text{Adj } R^2 = 0.0167, p \text{ value} = 0.28.$$

Laminarinase sp (control diet):

$$\text{Melinka: sp} = 0.2353 + 0.0042TE,$$
$$\text{Adj } R^2 = 0.2113, p \text{ value} = 0.0495.$$
$$\text{Corral: sp} = 0.2209 + 0.0029TE,$$
$$\text{Adj } R^2 = 0.343, p \text{ value} = 0.0128.$$

Laminarinase sp (toxic diet):

$$\text{Melinka: sp} = 0.2078 + 0.0236\ln(TE + 1),$$
$$\text{Adj } R^2 = 0.1597, p \text{ value} = 0.078.$$
$$\text{Corral: sp} = 0.3322 - 0.0393\ln(TE + 1),$$
$$\text{Adj } R^2 = 0.6458, p \text{ value} = 0.0002.$$

Discussion

Bivalve mollusks have developed great flexibility in their feeding regimen in response to changes in quantity and quality of seston, enabling them to optimize their energy gains (Velasco and Navarro 2002; Labarta et al. 2002). Bayne et al. (1989) suggested that changes in enzymatic activity can also be a key factor governing short-term digestive responses. However, few studies have established the relationship between digestive enzyme activity and the nutritional conditions in which bivalves live (Ibarrola et al. 2012). Fernández-Reiriz et al. (2001) and Labarta et al. (2002) showed that investment in enzymatic resources is one of the mechanisms used by *M. chilensis* and *Mulinia edulis* to optimize energy gain concurrent with variations in feeding.

The physiological processes of bivalves can be affected by consumption of toxic algae (Gainey and Shumway 1988). Li et al. (2002) studied the effect of *A. tamarense* (PSP toxins) on the energy budget (SFG) and growth of the clam *R. philippinarum* and the mussel *P. viridis*. They found that changes in PSP toxin levels affected clearance rate in clams, but not in mussels, and attributed this dissociation to poisoning of cilia in clams. Meanwhile, they observed that AE decreased in both species with diets containing PSP toxins, which reduced SFG. According to Li et al. (2002), bivalves fed with *A. tamarense* produced abnormal feces mixed with white mucus, suggesting the presence of a digestive system disorder affecting AE. Similar observations have been made by Navarro and Contreras (2010) for *M. chilensis*, for which the lowest AE values occurred during the first day of exposure to a toxic diet, and that nadir coincided with the production of less compact, yellowish-colored feces.

In our study, we observed a drop in the AE of *T. dombeii* upon toxic exposure of the Corral-Valdivia population not previously exposed to PSP in their original site (Molinet et al. 2003). Such a drop in AE could be related to alterations in digestive processes, as described by Wikfors and Smolowitz (1995) for the eastern oyster (*Crassostrea virginica*) fed with a toxic diet. According to Bayne et al. (1989), the digestive process of mussels requires prolonged periods of acclimation in response to variations in diet or feeding versus fasting regimens, as observed in other mollusks (Widdows et al. 1979; Smolowitz and Shumway 1997).

Our study showed a higher level of amylase and cellulase activity in the digestive gland of *T. dombeii* originally from Corral-Valdivia compared with the activities observed for individuals from Melinka-Aysén. This higher carbohydrase activity in *T. dombeii* from Corral-Valdivia might be related to their original habitat which is characterized by a high amount of continental runoff from the Valdivia River carrying high particulate loads. Additionally, there is an abundance of macroalgae (*Lessonia* sp. and *Durvillea* sp.) in the exposed coastal zone where, nonetheless, there are no SP-producing microalgae present (Molinet et al. 2003). Our findings are in agreement with the work of Teo and Sabapathy (1990), who established that the high variability in digestive enzymes in bivalves may be due to differences in diet, since detritus particles originating from vascular plants and green algae contain starch and cellulose, whereas those from brown algae contain laminarin and cellulose. Brock et al. (1986) report that live particles also differ in the amount of carbohydrates that constitute them: Dinoflagellates and green algae contain starch and cellulose whereas cyanobacteria contain starch, and diatoms contain laminarin. Ibarrola et al. (1996) stated that cellulase activity is essential for nutrient acquisition when food is composed of cellulose walls, since the cellulase complex liberates organic compounds that occur naturally in cellulose walls.

According to Brock et al. (1986), the enzyme production can be regulated by external factors such as food availability and composition so the response observed for laminarinase activity of *Tagelus* fed with control diets (see Table 7) can be derived from control diet composition (60 % T-ISO). These algae contain laminarin as the main carbohydrate (Robert et al. 1994). Ibarrola et al. (2012) recently stated that the efficient utilization of the complex mix of carbohydrates present in natural diets requires an appropriate combination of digestive enzymes. Accordingly, reported interspecific differences in the composition of the pool of carbohydrases likely reflect microhabitat variability in the organic composition of suspensions that are available to the organisms' alimentary systems.

Table 7 Covariance analysis (ANCOVA) comparing regression fits for AE and carbohydrase activities between origins

	Model	Intercept		TE	
		T value	*p* value	*T* value	*p* value
AE	AE*a + bTE	−0.123	0.903	5.510	8.8×10^{-06}
Amylase	$sp = ae^{bTE}$	−10.561	6.7×10^{-11}	7.943	2.0×10^{-08}
Cellulase	$sp = ae^{bTE}$	−12.537	1.6×10^{-12}	7.504	5.8×10^{-08}
Laminarinase					
Control	sp = a + bTE	0.785	0.439	0.484	0.633
Toxic	sp = a − blnTE	−4.814	5.5×10^{-05}	4.336	0.0002

Similar to clams fed with the control diet, amylase and cellulase complex were the most active enzymes in the digestive glands of *T. dombeii* fed toxic algae, while laminarinase activity remained lower than the activities of the other enzymes. This finding agrees with Brock and Kennedy (1992) who reported high laminarinase levels in style extracts of *C. virginica*, whereas low laminarinase activity was reported in oyster diverticula extracts (Brock et al. 1986).

Tagelus dombeii from Melinka-Aysén, which are adapted to toxic PSP exposure, increased their enzymatic activity with TE to toxic diet. Similar to what has been shown for mollusks like *M. chilensis*, individuals with a history of toxic food exposure likely developed mechanisms that enabled them to use toxic microalgae as a food source, despite any initial negative feeding and digestive effects (Fernández-Reiriz et al. 2008). This phenomenon can be considered an example of acclimation—a form of ecological memory that enables nutrient extraction from alternative, even toxic, food sources. The observed ability of *T. dombeii* to modify enzymatic expenditures and achieve constant or high AE in the presence of toxic *A. catenella* in the laboratory suggests that there is long-term adaptation to PSP toxins in the field. Moreover, toxin-naïve individuals (i.e., the Corral-Valdivia population) were unable to obtain any benefit from toxin exposure within the experimental time period and suffered a continued AE deficiency that could eventually weaken their energy status.

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