



Growth, abundance, morphometric and metabolic parameters of three populations of *Diplodon chilensis* subject to different levels of natural and anthropogenic organic matter input in a glacial lake of North Patagonia

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ABSTRACT

Three populations of *Diplodon chilensis* (Hiridae, Bivalvia) from North Patagonia (Lacar lake, Argentina) have been studied to determine how organic matter (OM) influence their growth, density, morphometric and metabolic parameters in two pristine sites (Yuco and Nonthué) and in a growing touristic locality (San Martín de los Andes Bay, site SMA) affected by urban discharges. In Nonthué (chemical and biological oxygen demand ratio COD/BOD ratio of 4.7), a dense neighboring forest provides higher quantities of vegetal detritus compared to Yuco, while in SMA the OM input increase is related to anthropogenic impact, mainly sewage discharges, which is more biodegradable (COD/BOD ratio of 1.7). Our results show that population's size distribution and growth rates are affected positively by increased OM, independently of its natural or anthropogenic origin. The modal shell length interval for SMA and Nonthué is two-fold higher (70 mm), in agreement to the growth rate increase ($k=0.079$), compared to Yuco (35 mm, $k=0.045$). The morphometric relationships between size–size and size–mass show a higher slope for SMA and Nonthué, which underline allometric differences between these two populations and the Yuco's one. The lower population densities in both sites (SMA 33 ind./m² and Nonthué 76 ind./m²) compare to Yuco (176 ind./m²) and the absence of individuals younger than 7 and 5 years old, respectively, in SMA and Nonthué could be related to the higher allochthonous OM content in the sediments and total suspended solids in water. Increased OM due to urban pollution in SMA bivalves leads to higher oxidative damage to lipids, which is not counterbalanced by the higher detoxification enzyme glutathione-S-transferase activity. Hence, we can conclude that pollution would explain the drastic reduction in population density, probably related to a high impair in the juvenile's survival/recruitment, the higher observed mortality and the lower population longevity. When increased OM is supply by the forest, like in Nonthué, this has less negative effect on population density and no effect on longevity at all. However, a negative effect of oxygen depletion due to increased OM (either anthropogenic or natural) on juvenile survival cannot be discarded, but further studies should be carried out to support this idea.

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Introduction

Growth, production and physiological activity of bivalve mollusks can be affected by environmental conditions such as temperature, salinity, substrate characteristics, depth and food availability (Kube, 1996; Morsan and Orensanz, 2004). Moreover, density-dependence and community composition could also affect growth rate of populations inhabiting different latitudes (Abele

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et al., 2009). Environmental conditions affect the survival of many invertebrates, especially at juvenile stages (Baldwin et al., 2002; Osterling, 2006). Particularly, pollution has been shown to affect the survival rates, reproductive success, growth, and behavior of aquatic organisms (Hedouin et al., 2010; King-Heiden et al., 2012).

Natural and anthropogenic factors produce oxidative stress in the aquatic biota (Winston and Di Giulio, 1991; Lushchak, 2011). Agricultural and urban products may stimulate the generation of reactive oxygen species (ROS): O_2^- , H_2O_2 , $\bullet OH$, etc. (Halliwell and Gutteridge, 1999). As a response, antioxidant defenses are often enhanced in the presence of increased ROS levels. Over-production of ROS in response to environmental stress can lead to increased lipid peroxidation (LPO) and may affect cell viability by causing membrane damage and enzyme inactivity (Nordberg and Arnér, 2001). Defense enzymes such as superoxide dismutase (SOD), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and catalase (CAT) usually act in a coordinated manner to ensure the optimal protection against oxidative stress (Sies, 1993). Metals and organic chemicals discharged into aquatic environments are readily transported to the sediments resulting in concentrations that are often several orders of magnitude greater than those in the overlying water column (Williams et al., 1994). Some studies have demonstrated that sediment ingestion is an important way of pollutant accumulation for benthic aquatic animals (Ruus et al., 2005; Fukunaga and Anderson, 2011).

In natural freshwaters dissolved organic carbon (DOC) and dissolved organic matter (DOM) could contribute a significant amount of bivalve's carbon demand, especially where large mussel colonies exist and phytoplankton densities are low. It has been shown that zebra mussels can filter a range of particle sizes from the water column and, under certain food limiting conditions; they can even utilize DOC and DOM as carbon source (Horgan and Mills, 1997; Roditi et al., 2000).

Diplodon chilensis (Hyriidae) is one of the most abundant bivalves in water bodies of the South-West of Argentina and Southern Chile (Bonetto, 1973; Parada et al., 2007). In most lakes, these mussels colonize sandy or muddy bottoms between 2 m and 50 m depth, where they form beds of considerable densities (Lara and Parada, 1988). Through their filter-feeding activity, these mussels can reach high clearance rates not only for microalgae and bacteria but also for nutrients (Soto and Mena, 1999; Lara et al., 2002a; Parada et al., 2008; Sabatini et al., 2011a). These characteristics suggest that *D. chilensis* plays an important role in the nutrient recycling system and in the maintenance of oligotrophic conditions of Patagonian lakes and rivers (Lara et al., 2002b). Besides basic characteristics of growth, and age reported by Parada et al. (1989) and by Semenas and Brugni (2002), Soldati et al. (2009) have reported differences in initial growth rates and in maximum age under different limnological conditions. Valdovinos and Pedreros (2007) have studied the abundance of this species in relation to water quality deterioration in several Chilean lakes, warning about a dramatic population decrease associated to eutrophication processes. These authors have shown that, in lakes with highly increased turbidity, *D. chilensis* populations were severely endangered. Sabatini et al. (2011a), have recorded higher digestive gland mass/shell length ratio and, increased antioxidant defenses and lipid damage in bivalves collected from an urban discharge polluted area of Lacar lake (Patagonia, Argentina), than in those collected from a clean area of the same lake. This study suggests adult *D. chilensis* can tolerate the moderate oxidative stress induced by chronic exposure to wastewater pollution. However, the sensitivity of juveniles and possible effects on population dynamics remain to be studied.

The East–West orientation of Lacar lake determines a marked precipitation gradient with maximum precipitation rates at the Western extreme. This, in term, determines a gradient in the organic matter provided by the nearby forest to the lake. The

forest-born organic matter constitutes the main carbon source for this aquatic ecosystem. At the Eastern extreme of the lake (SMA) additional organic matter is provided by sewage water discharges.

Given the different support of organic matter in the area, we propose to assess the impact of natural and anthropogenic organic matter provided either by the forest or by urban discharges on density, growth, and morphometric and metabolic parameters of *D. chilensis*. Our study comprises the comparison of these parameters among three populations which live in different parts of the Lacar lake and are exposed to different levels of natural (forest-born) organic matter (Yuco and Nonthué) and to increased organic matter associated to urban discharges (SMA).

Materials and methods

Sample sites and water analysis

Three sampling sites located at the Lacar Lake ($40^{\circ}27'S$, $71^{\circ}84'W$) (Fig. 1), a glacial origin lake with oligotrophic characteristics (Diaz et al., 2007) were used for this study. The first site was established at the Nonthué area ($40^{\circ}08'S$, $71^{\circ}38'W$), 50 km from the city of San Martín de los Andes, in the Western part of the Lacar lake, named Nonthué. The second site, Yuco ($40^{\circ}16'S$, $71^{\circ}32'W$), is situated 20 km West from the city of San Martín de los Andes. The third site (SMA) was established on the Eastern Bay shore, approximately 50 meters from the discharge of San Martín de los Andes city sewage treatment plant ($40^{\circ}10'S$, $71^{\circ}20'W$). All the sites studied have sandy bottoms with organic matter supply from the neighboring forest.

The precipitation rate gradient among the selected sites ranges from about 1500 mm/year in SMA to 2000 mm/year in Yuco and 2500 mm/year in Nonthué. The richness and density of the autochthonous forest is increased from East to West according to this gradient, increasing the natural organic matter supply to the lake (Funes et al., 2006). However, the organic matter input is increased in SMA by sewage discharges from the treatment plant.

Mussels for population parameters were sampled in the three sites during austral winter 2007. Mussels for studying metabolic parameters, together with water and sediment samples, were obtained in winter 2008. The samples were collected at the three sites by SCUBA diving, from mussel beds located at a depth of 8–10 m. Water samples were collected per triplicates immediately above the bottom, in sterile plastic containers and cold preserved, for bacteriological and physicochemical analysis. Sediment was collected also per triplicates, from the upper 5 cm using a 40 cm \times 40 cm corer and transported to the laboratory at 4 °C, until the analysis.

The concentration of coliform bacteria in water was measured by the most probable number (MPN) method per 100 mL of water (APHA, 1992). The following variables were measured in situ with portable electronic meters (Hanna, Hach): temperature (°C), pH and dissolved oxygen (mg/L). Total phosphorus (TP) and nitrogen (TN), as $\mu g/L$, were measured according to Koroleff (1983). Chlorophyll a ($\mu g/L$) concentrations were calculated through the equations given by Marker et al. (1980). For the organic matter content (OM), expressed as mg/g sediment, the sediment was previously dried until constant weight at $90 \pm 5^{\circ}C$ during 24 h. A known mass of sediment was burned at $550^{\circ}C$ for 5 h and then the decrease in mass until reaching constant weight was recorded (Dean, 1974). Total suspended solids (TSS) (mg/L), biological oxygen demand (BOD₅) and chemical oxygen demand (COD), expressed as mg/L, were estimated according to Standard Methods (APHA, 1992). Data for water and sediment analysis were presented as mean values \pm standard deviation (SD). Results for each variable were compared between sites of sampling by ANOVA. Differences were considered significant with $p < 0.05$.

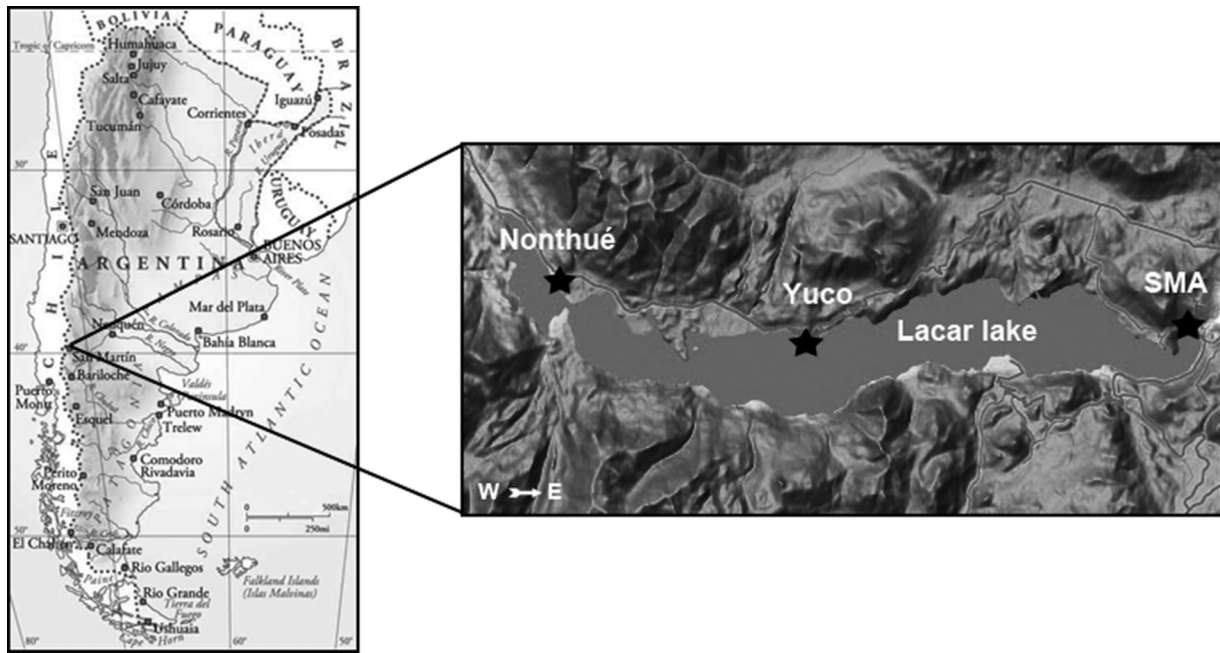


Fig. 1. Geographical locations of the three sampling sites, San Martín de los Andes Bay (SMA), Nonthué and Yuco, Neuquén Province, Argentina.

Normality and homogeneity of variances were tested by Lilliefors and Bartlett tests, respectively (Sokal and Rohlf, 1984).

Density, morphometrics, shell growth, and age

Bivalves were collected using 40 cm × 40 cm metal corers (at least 10 corers per bed), to estimate the relative length frequency distribution and density of Nonthué, Yuco and SMA populations. The beds were previously identified and the corers were randomly distributed. The number of bivalves in each corer was counted and density was compared among beds by ANOVA. Type of spatial distribution was studied by the variance/mean index according to Lara and Parada (1988) and significance was analyzed by Student's *t* test ($p < 0.05$). For every individual, we measured shell length (*L*, anterior–posterior axis), shell height (*H*, umbo to the ventral margin along the axis of maximum growth) and shell width (*W*) at a precision of 0.1 mm, and the shell mass (SM) and total mass (TM) were determined. Then, the soft parts were dried at 70 °C to constant mass in order to obtain the shell free dry mass (SFDM) (precision ± 0.001 g). To compare the morphometric relationships among beds, size–size (*W* vs. *L*, *H* vs. *L*) and mass–size relationships (SM vs. *L*, TM vs. *L*, SFDM vs. *L*) were obtained for each bed and a linear regression analysis was carried out on the log-transformed data to size–mass relationships. Tukey's multiple comparison test (Zar, 1999) was used to compare the slopes of the mass–size and size–size relationships among beds. To enhance overall data set homogeneity, we restricted the statistical analysis to a shell length size range between 37 and 86 mm. Assumptions of normality, homoscedasticity and parallelism were tested. ANCOVA was not performed due to significant differences between bed slopes (see 'Results' section; Zar, 1999).

Individual age was inferred from internal shell growth bands of 125 specimens from each site. Internal growth bands can be identified as translucent and opaque bands (Jones et al., 1990), where translucent bands are narrow and dark and opaque bands are wide and white (Fig. 2). Stable oxygen and carbon isotope ratio analysis demonstrate that the translucent shell growth bands in *D. chilensis* from a close area (41°8'S, 71°17'W, Bariloche, Río Negro, Argentina) are formed annually (Soldati et al., 2009); thus, the number of

translucent growth bands of each individual was considered as its age. Each right valve was embedded in epoxy resin and sectioned along the axis of maximum growth in height (*H*). The cross-sections were polished on lapidary wheels using grits of 400, 600, 1200 and 2400 grade and etched with DE-CAL agent at 0.5%. Polished shell cuts were examined under a stereo microscope using reflecting light and acetate peels were made to confirm the pattern found (Rhoads and Lutz, 1980).

The von Bertalanffy growth model was fitted to the shell length-at-age data using the nonlinear iterative Newton algorithm.

$$L_t = L_\infty (1 - e^{-k(t-t_0)}) \text{ [mm, y]}$$

Where L_∞ is the asymptotic length, k is the growth constant, t the age and t_0 the age at zero length.

To fit the model to the data for each bed, the maximum likelihood method was used (following Edwards, 1992; Hilborn and Mangel, 1997). The von Bertalanffy k , L_∞ and t_0 parameters of the three beds were compared by pair-wise comparisons using the Likelihood ratio test (Cerrato, 1990; Aubone and Wöhler, 2000), and bivariate confidence limits (CI) for k , L_∞ and t_0 were built using profile likelihood (see Hilborn and Mangel, 1997; Aubone and Wöhler, 2000). Additionally, the growth index, $\phi' = 2\log_{10} L_\infty + \log_{10} k$ (Pauly and Munro, 1984) was calculated and used to assess growth performance.

Metabolic parameters

Fourteen animals, between 15 and 25 years old (mean size ± standard deviation, 61.3 ± 14.9 mm shell length), from each site were immediately processed after collection. They were measured as was described in the above section and anesthetized by placing on ice before they were killed. The shell was used to determine the age as was previous explained. Digestive glands were dissected and weighed, and then homogenized (1 g/5 ml) for studying oxidative stress parameters. Total protein content was measured using Bradford method (1976). The ratio between digestive gland mass and shell length (DGM/SL) was calculated as an indicator of metabolic activity/condition (Langston et al., 2007; Sabatini et al., 2011b). The parameters detailed below

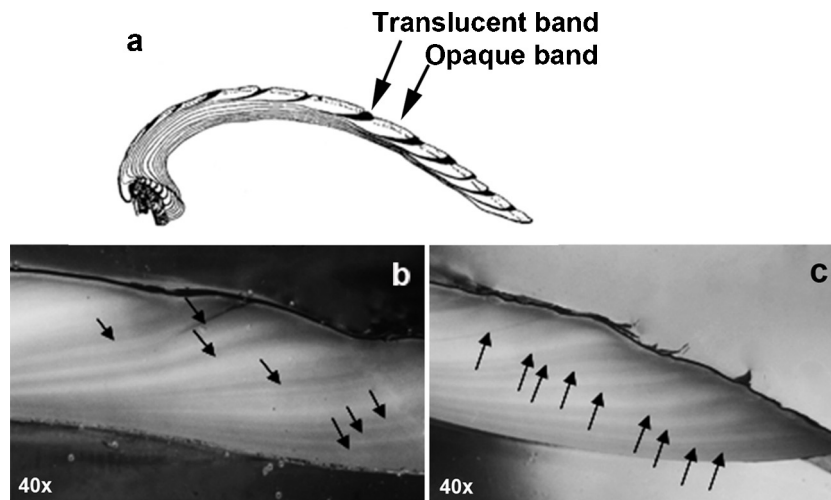


Fig. 2. (a) Scheme of a shell section along growth axis. The translucent and opaque bands correspond to the annual increment. (b and c) Photographs of a polished shell section along the maximal growth axis with optimized contrast and illumination: black (translucent) and white (opaque) parts correspond to annual increments showed by the black arrows.

were measured in order to evaluate the oxidative stress status of similar age bivalves from the three sites. Results from metabolic determinations were analyzed by one way ANOVA followed by Tukey's post hoc test. Differences were considered significant with $p < 0.05$. Normality and homogeneity of variances were tested by Lilliefors and Bartlett tests, respectively (Sokal and Rohlf, 1984).

Defense system enzymes and lipid peroxidation

Digestive gland tissue was homogenized in cold 50 mM phosphate buffer (pH 7) with protease inhibitors (phenylmethylsulfonyl fluoride, PMSF, 0.5 mM and benzamidine, 10 mM). The homogenate was centrifuged for 15 min at $7000 \times g$; and the resulting supernatant was used for the assays. Results were expressed relative to protein amount (mg).

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by the technique of Fridovich (1997). The inhibition of the reduction of nitrobluetetrazolium (NBT) was monitored at 560 nm. One SOD unit was defined as the enzyme amount necessary to inhibit 50% of the reaction rate. Glutathione-S-transferase (GST, EC1.11.1.9) activity was measured by the technique of Habig et al. (1974). The change in absorbance at 340 nm was followed during 90 s. One GST Unit was defined as the amount of enzyme needed to catalyze the

formation of 1 μmol of GS-DNB per minute at 25°C . Both enzyme activity results were expressed as Unit per mg protein.

The level of lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) using the Fraga et al. (1988) method. The $11,000 \times g$ supernatant from total homogenate was mixed with thiobarbituric acid (TBA) solution and incubated at $95\text{--}100^\circ\text{C}$ for 45 min. The absorbance was read at 535 nm in the supernatant, after the reaction mixture centrifugation.

Results

Water analysis

There were significant differences among sites in the COD/BOD ratio and in the chlorophyll *a* content ($p < 0.05$). Nonthué showed a higher COD/BOD ratio, due to three-fold higher COD levels, and lower Chlorophyll *a* concentration than in Yuco and SMA (Table 1). Total suspended solids (TSS) were significantly higher in Nonthué and SMA compared to Yuco ($p < 0.01$). Organic matter content was significantly higher in SMA and Nonthué than in Yuco ($p < 0.05$ and $p < 0.01$, respectively). No fecal coliform bacteria and low concentrations of total coliform bacteria were detected in Nonthué and Yuco while, SMA samples showed important concentrations of fecal bacteria (Table 1).

Table 1

Water physic-chemical variables and sediment organic matter content in samples, collected from the sites SMA, Nonthué and Yuco.

Parameter	SMA	Nonthué	Yuco
T ($^\circ\text{C}$)	7.9 ± 0.3	8.2 ± 0.5	7.7 ± 0.5
pH	7.1 ± 0.4	6.9 ± 0.5	7.0 ± 0.5
DO (mg/l)	9.0 ± 0.7	11.9 ± 1.2	10 ± 0.6
TSS (mg/l)	$6.7 \pm 2.1^*$	$6.3 \pm 0.2^*$	2.7 ± 0.1
Total N ($\mu\text{g/l}$)	$314 \pm 25.4^*$	145 ± 10.1	126 ± 9.9
Total P ($\mu\text{g/l}$)	$4.0 \pm 0.28^*$	1.8 ± 0.1	1.9 ± 0.1
Organic matter mg/g sediment)	$2.8 \pm 0.2^*$	$2.4 \pm 0.2^*$	1.5 ± 0.2
Chl <i>a</i> ($\mu\text{g/l}$)	2.9 ± 0.4	$1.7 \pm 0.4^*$	2.7 ± 0.1
COD (mg/l)	3.2 ± 0.1	$9.0 \pm 0.5^*$	2.9 ± 0.2
BOD ₅ (mg/l)	1.9 ± 0.1	1.9 ± 0.1	1.7 ± 0.1
COD/BOD	1.7 ± 0.2	$4.7 \pm 0.1^*$	1.7 ± 0.2
Total coliform bacteria (NMP/100 ml)	$1730 \pm 56^*$	196 ± 97	74 ± 29
Fecal coliform bacteria (NMP/100 ml)	400 ± 96	–	–

Samples were obtained during winter. Data are expressed as means \pm SD ($n = 3$).

* Significant differences, between the Nonthué and SMA population's respect to the Yuco, are indicated by asterisks with $p < 0.05$.

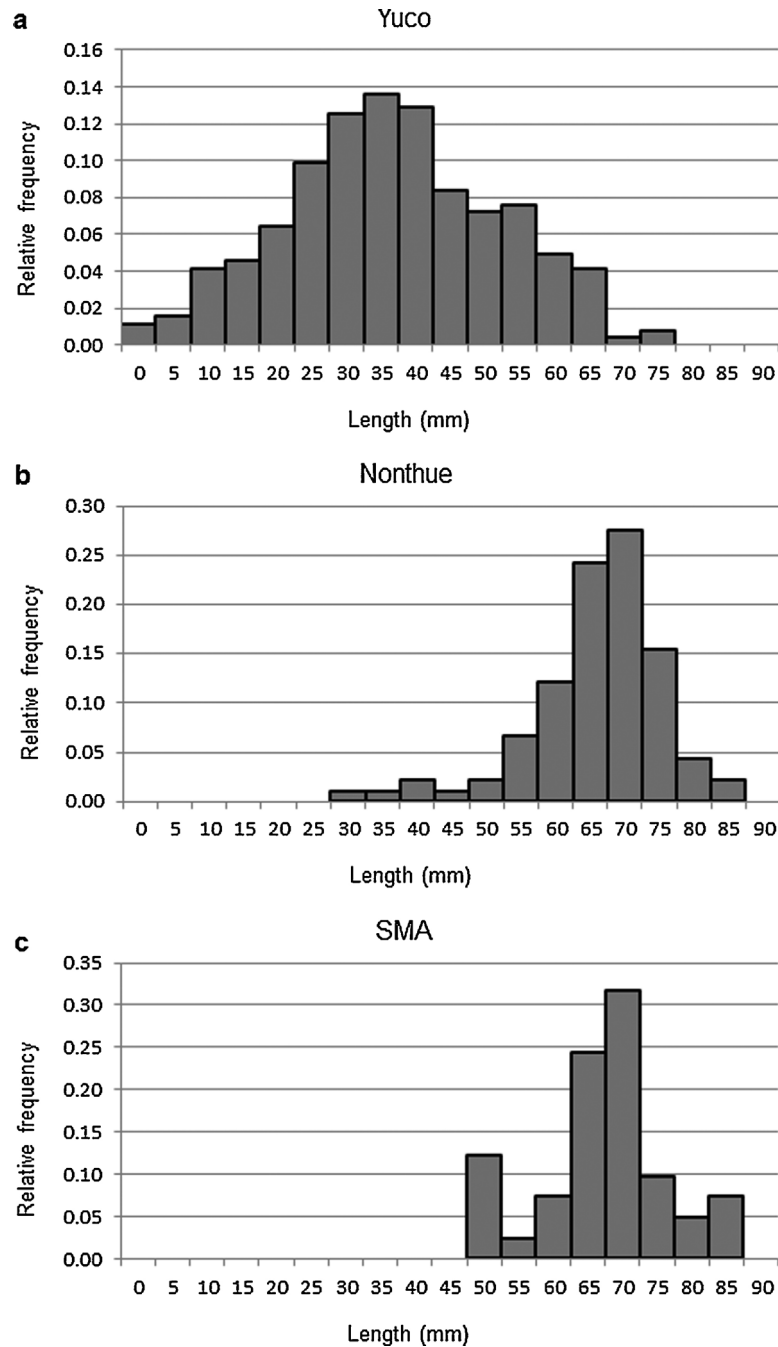


Fig. 3. Length-frequency distribution of different populations of *D. chilensis*: (a) Yuco, (b) Nonthué and (c) SMA ($n > 100$).

Density, morphometrics, shell growth, and age

The Yuco population showed a unimodal distribution at 35 mm *L* with individuals ranging between 2 and 80 mm *L* (Fig. 3a). Nonthué population showed a distribution with a dominant size at 67 mm, among the small individuals, the most frequent length was 37 mm *L* (Fig. 3b). In SMA, the distribution was predominantly unimodal with a modal size of 71 mm *L* and among the small individuals, the most frequent length observed was 50 mm (Fig. 3c). It is remarkable that no individuals smaller than 50 mm *L* were found in this site. Population density was 176 ± 90 individuals/m² in Yuco, 76 ± 10 individuals/m² in Nonthué and, 33 ± 8 individuals/m² in SMA, with significant differences between SMA and the other two populations ($p < 0.05$). Both at Yuco and Nonthué, the bivalves were forming beds placed in disperse way on the substrate. Meanwhile

the individuals in the SMA population were often seen grouped with some dead individuals. According to the analysis of population distribution patterns, the three populations presented an aggregated spatial distribution. Active reproductive individuals were detected by visual inspection of gonads in the three populations.

Morphometric regressions width, total mass, shell mass and SFDM vs. length showed higher slopes in SMA and Nonthué respect to Yuco ($p < 0.05$) (Table 2). The height vs. length showed higher values in Yuco than in the rest.

von Bertalanffy parameters showed higher values of L_{∞} in SMA and Yuco than in Nonthué. SMA and Yuco did not differ significantly for this variable. The growth rate was higher in SMA and Nonthué than in Yuco while no difference was detected between SMA and Nonthué (Fig. 4; Tables 3 and 4).

Table 2

Morphometric relationships in *Diplodon chilensis* from, San Martin de los Andes (SMA), Nonthué and Yuco beds as described by linear regression $y = a + b \times$ (all significant relationships, $p < 0.05$). Differences among beds were assessed by Tukey's test, restricting the statistical analysis to a shell length range of 37–86 mm to enhance overall data set homogeneity. L: shell length, H: shell height, W: width; TM: total body mass; SM: shell mass; SFWM: shell free dry mass. Equal letters indicate no significant differences at $\alpha = 0.05$. The average ratio of dependent variable to covariate decreases in alphabetical sequence.

Variable X	Variable Y	Bed	a	b	R ²	n	Tukey test
L	H	SMA	-3.42	0.91	0.96	134	c
		Nonthué	-0.03	0.88	0.97	101	a
		Yuco	-0.99	0.88	0.97	132	b
L	W	SMA	2.51	0.27	0.76	134	b
		Nonthué	5.83	0.43	0.86	101	a
		Yuco	-0.35	0.30	0.87	133	c
Ln (L)	Ln (TM)	SMA	-8.93	2.90	0.92	104	a
		Nonthué	-8.99	2.92	0.96	98	b
		Yuco	-8.63	2.83	0.97	103	c
Ln (L)	Ln (SM)	SMA	-15.10	4.34	0.84	134	a
		Nonthué	-15.49	4.41	0.91	101	b
		Yuco	-12.83	3.81	0.92	133	c
Ln (L)	Ln (SFDM)	SMA	-13.81	3.33	0.70	56	b
		Nonthué	-8.62	2.15	0.53	50	a
		Yuco	-17.15	4.07	0.88	61	c

Table 3

Parameters of the general von Bertalanffy growth function with confidence intervals (CI) at alpha 0.05 in *Diplodon chilensis* and the growth performance index ϕ' estimated for each bed.

Beds	L_{∞} (mm) (CI)	k (y^{-1}) (CI)	t_0 (y)	n	L_{max} (mm)	Age_{max}	ϕ'
SMA	77.88 (75.48; 80.29)	0.137 (0.079; 0.195)	-0.358 (-4.769; 4.052)	96	85.78	51	2.91
Nonthué	71.36 (69.47; 73.24)	0.113 (0.079; 0.146)	0.484 (-2.445; 3.113)	73	85.70	73	2.63
Yuco	81.34 (73.47; 89.22)	0.064 (0.045; 0.082)	-0.731 (-0.852; 3.113)	103	85.60	62	2.64

The maximum age found was lower in SMA (51 years) followed by Yuco (62 years) and Nonthué (73 years). The growth performance index ϕ' showed higher values in SMA than in Nonthué and Yuco.

Metabolic parameters: defense system enzymes and lipid peroxidation

DGM/SL ratio and biochemical variables were compared between individuals within an age interval of 15–25 years old. Significant differences were observed among groups, with higher DGM/SL ratio in SMA than in the other sites and in Nonthué respect to Yuco (Fig. 5). Total protein content was significantly higher in Nonthué than in the other sites (2.48 ± 0.07 , 2.64 ± 0.07 and 4.27 ± 0.30 mg protein/ml homogenate, for Yuco, SMA and Nonthué, respectively), ($p < 0.001$).

The GST activity (Fig. 6) was increased by 22% in the digestive gland of SMA animals respect to those of Yuco and by 40% respect to those of Nonthué (SMA 0.73 ± 0.04 , Yuco 0.57 ± 0.04 and Nonthué

Table 4

Results of likelihood ratio test for pair-wise comparison for k , L_{∞} and t_0 parameters between different beds.

Beds	H_0	Likelihood ratio test	
		χ^2 (df = 2)	p-Value
SMA – Nonthué	$L_{\infty 1} = L_{\infty 2}$	16.99	0.00004*
	$k_1 = k_2$	0.626	0.4289
	$t_{01} = t_{02}$	0.082	0.7735
SMA – Yuco	$L_{\infty 1} = L_{\infty 2}$	1.11	0.2932
	$k_1 = k_2$	5.62	0.0177*
	$t_{01} = t_{02}$	-0.003	-
Nonthué – Yuco	$L_{\infty 1} = L_{\infty 2}$	1.55	0.00068*
	$k_1 = k_2$	6.94	0.00842*
	$t_{01} = t_{02}$	-0.12	-

* Significance $p < 0.05$.

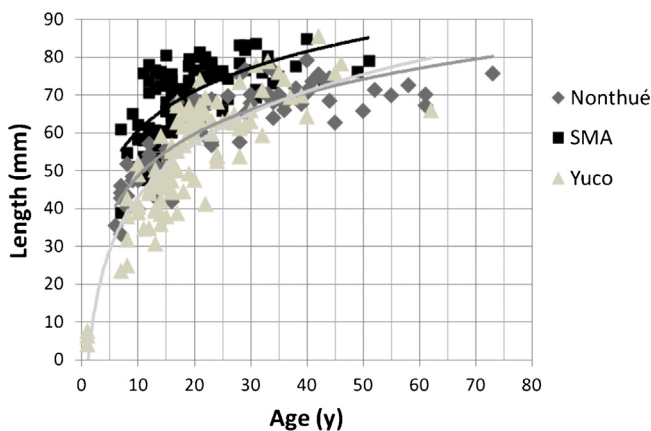


Fig. 4. von Bertalanffy growth curves and corresponding size-at-age data in *D. chilensis* from Yuco, Nonthué and SMA.

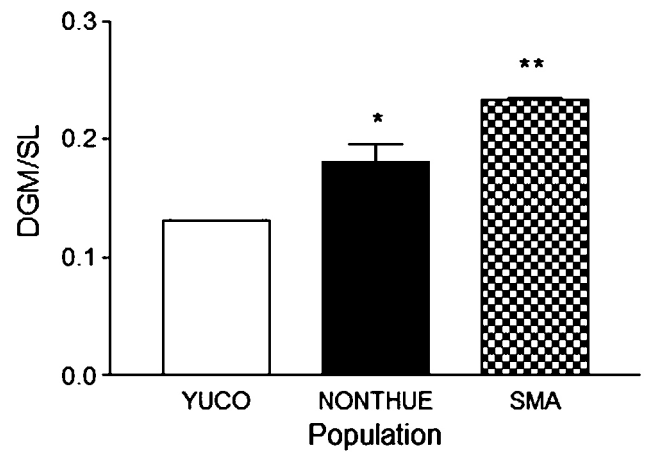


Fig. 5. The ratio between digestive gland mass and shell length (DGM/SL) in samples of *D. chilensis* digestive tissue collected from the different studies' sites, Yuco, Nonthué and SMA. Data are expressed as means \pm SD ($n = 14$). * The asterisk denotes significant differences between Nonthué population and the other two populations with $p < 0.05$ for Yuco and SMA. **Significant differences between SMA population and the other two populations with $p < 0.05$ for Nonthué and $p < 0.001$ for Yuco.

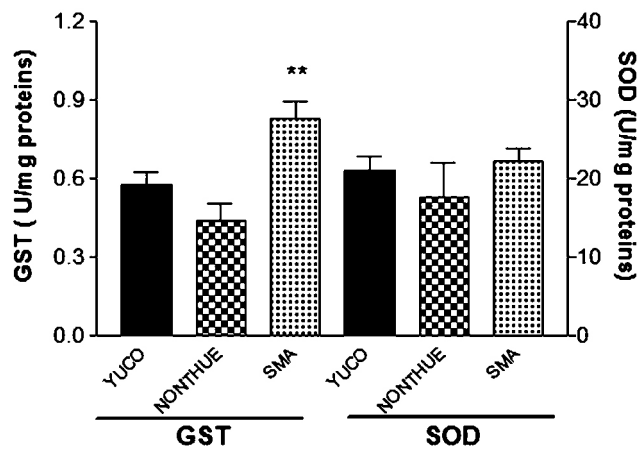


Fig. 6. Glutathione-S-transferase (GST) and superoxide dismutase (SOD) activity determined in samples of *D. chilensis* digestive tissue collected from the different studies' sites. Results are expressed as enzyme Units/mg protein. Data are expressed as means \pm SD ($n = 14$). **Significant differences between SMA population and the other two populations, Yuco and Nonthué, with $p < 0.05$.

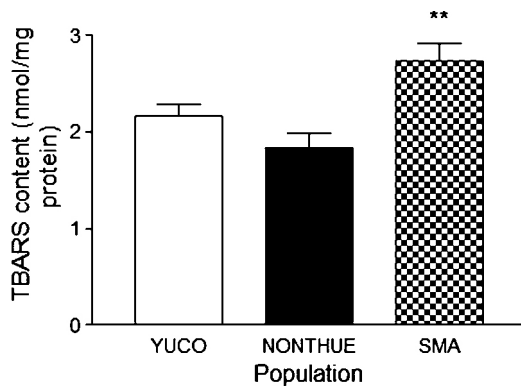


Fig. 7. TBARS content ($\mu\text{mol MDA/mg protein}$), as lipid peroxidation index, determined in samples of *D. chilensis* digestive tissue collected from the different studies' sites. Results are expressed as enzyme Units/mg prot. Data are expressed as means \pm SD ($n = 14$). **Significant differences between SMA population and the other two populations, Yuco and Nonthué, with $p < 0.05$ for Y and $p < 0.001$ for N.

0.44 ± 0.06 U/mg protein, $p < 0.01$). In contrast, SOD activity was not different among groups. TBARS content was significantly higher in SMA compared to the other two sites ($p < 0.001$) (Fig. 7).

Discussion

Our results show that the input of allochthonous organic matter (OM) to the lake affects growth, abundance and morphometric parameters of *D. chilensis*. SMA and Nonthué sites are richer in OM and present higher most frequent size and higher growth rate than Yuco. But only SMA shows lower maximum age and density than the other sites. Additionally, lipid peroxidation levels and GST activity are higher in SMA than in the other two sites.

Under natural conditions, the abundance and persistence of freshwater mussel populations are likely regulated by environmental variables such as food availability, competition, predation pressure and larvae supply (Henry and Simao, 1985; Lara and Moreno, 1995; Osterling et al., 2008). In our study growth, abundance and morphometric parameters vary among sites according to the quantity and quality of organic matter available in the sediment and in the water column. Both, SMA and Nonthué show significantly higher contents of sediment OM and total suspended solids in water (TSS) than Yuco. Although SMA is located in the low end of the Lacar lake precipitation rate gradient, this site receives an

extra OM supply from sewage discharges, which makes the total sediment OM and water TSS similar to those in Nonthué. On the other hand, Nonthué is near the highest precipitation rate end of the lake and, thus, receives an important supply of natural OM from a dense and rich neighboring forest.

The high chemical oxygen demand to biological oxygen demand ratio (COD/BOD = 4.7) calculated for water samples from Nonthué reveals a different kind of organic matter, less degradable (see reference values by Romero Rojas, 1999) compared to those in the other sites (COD/BOD = 1.7). This could be related to the higher forest richness in Nonthué, where the abundant vegetal material is originated not only from the trees, which mostly belong to the same autochthonous forest species as in the other sites, but also from dense understory strata. This vegetal detritus is colonized and partially degraded by fungi and then transported to the lake by the runoff. An increased proportion of disintegrated tree trunks with high lignin content could also explain the low degradability of OM in this site.

These processes also result in increased TSS and low chlorophyll *a* (Chl-*a*) levels. In contrast, the high OM amount found in SMA was measured as very degradable, which is explained by its urban discharge origin, as was observed by Hidalgo et al. (2003) in urban polluted rivers from Tucumán province, Argentina.

The size distribution and the growth rate seem to be affected by increased OM quantity, independently of its natural or anthropogenic origin, showing an effect of bottom up control on these systems, in which an increase in nutrients availability has cascade effects on higher trophic levels (Mozeti et al., 2012). The modal shell length interval for both SMA and Nonthué was around 70 mm, two-fold higher than that of Yuco (35 mm). This result is in concordance with the higher growth rates observed for SMA and Nonthué, which were almost two-fold increased compared to Yuco. The morphometric relationships between size-size and size-mass show a higher slope for SMA and Nonthué than for Yuco. For a mean covariable of shell length, the former two beds have higher values of shell width, total mass, shell mass and shell free dry mass, showing allometric differences between beds. Accordingly, Sara et al. (2012) have reported that mussels exposed to fish farm organic waste or under direct organic emission, reach higher total length, weight and biomass than their controls.

Lara and Parada (1988) have previously shown that *D. chilensis* reaches larger size and higher population density with higher availability of organic matter. This species has been reported to select substrates with adequate organic matter quantity and quality (Lara and Parada, 2009). On the other hand, Valdovinos and Pedreros (2007) have reported a reduction in population density of *D. chilensis* in environments with increased turbidity due to eutrophic processes. It has been reported for unionids from North Bavaria, that juvenile recruitment decreases and mortality is higher under eutrophic processes, mainly derived from increased nutrients (Bauer 1983) (phosphorous, nitrogen). This has also been observed and discussed for *D. chilensis* populations in Southern Chile (Parada et al., 1990).

Excessive organic matter accumulation from phytoplankton and macrophytes with increased microbial activity, could lead to anoxic or hypoxic conditions near the bottom (Borsuk et al., 2002). In this sense, freshwater mussels can be sensitive to oxygen depletion (Chen et al., 2001; Alexander and McMahon, 2004). Since early juvenile unionid bivalves live burrowed within the sediment (Balfour and Smock, 1995), the absence of individuals of *D. chilensis* younger than 7 years old in SMA and 5 years old in Nonthué samples could be related to exposure to excessive OM derived-hypoxia. The lower population densities in both sites respect to Yuco could also be related with high OM and TSS contents. However, it should be taken into account that adult individuals of this species tolerate hypoxia by performing anaerobic metabolism (Grandón et al., 2008).

SMA mussels show lower longevity compared to Nonthué and Yuco (Table 3). For the bivalve *Artica islandica*, which can reach almost 400 years old (Schöne et al., 2005), a combination between metabolic rate depression (Abele et al., 2008) and a very low growth rate appear as main factors for its extended life span. Accordingly, the same species exposed to stressing environmental factors has shown higher growth rate and a drastic decrease in the maximum life span (40 years) in relation to increased energetic cost under such conditions (Begum et al., 2010). In our study, the increased oxidative damage to lipids detected in SMA mussels, which is not counterbalanced by the higher detoxification enzyme glutathione-S-transferase activity, is consistent with previous reports for bivalves exposed to domestic pollutants or to highly eutrophic environments (Bainy et al., 2000; Box et al., 2007; Almeida et al., 2007; Sabatini et al., 2011b).

This eutrophication evidence, caused by anthropic activity observed in the SMA area, could be associated not only to faster growth but also to reduced longevity, indications of high mortality and impaired juvenile recruitment in this population (Bauer, 1983).

Bivalves living in Nonthué, with high levels of natural OM and TSS but with no report on eutrophication processes or increase of human activity (Table 1), show no biochemical signs of damage and grow faster than those of Yuco, reaching similar longevity. The possible effects of hypoxic conditions on early juveniles and larvae recruitment in this area deserve further field and laboratory studies to test the hypothesis of OM-derived hypoxia during the last five years.

The digestive gland mass to shell length ratio (DGM/SL) seems to respond also to OM availability, since it is higher in SMA and Nonthué than in Yuco. In the present work, the higher slope in the morphometric relationships and DGM/SL in Nonthué, which is an unpolluted site as Yuco but with higher OM and TSS and lower chlorophyll a, indicates the importance of the energy input from allochthonous OM. In a previous work of our group (Sabatini et al., 2011b), a higher DGM/SL in SMA samples with respect to Yuco's has been proposed as an effect of pollution in SMA. However, in that study, feeding the mussels in the laboratory either with *Escherichia coli* or with green microalgae have increased equally this ratio, which suggests that the extra energy input and not its urban origin is the main cause of increased DGM/SL. As filter feeders, mussels are consumers of OM from several sources (autochthonous, natural allochthonous or anthropogenic) as a function of its availability (Riera and Richard, 1997; Lindahl et al., 2005). Labile organic matter flux composition was studied in the Cretan Sea, showing a significant correlation between the readily available labile OM and the bacterial biomass found in sediment samples (Danovaro et al., 2000). This suggests that the high degradable organic matter availability found in SMA is related to the presence of high bacterial concentrations.

We can conclude that the increase in allochthonous organic matter available in the sediments and suspended in water strongly enhances the growth patterns of *D. chilensis*. However, increased OM due to urban pollution leads to a drastic reduction in population density through lowering longevity, increasing mortality and, probably, through impairing recruitment of juveniles. In contrast, increased OM supplied by the forest has a lower negative effect on population density and no effect on longevity without mortality indicators. An effect of oxygen depletion due to increased OM (either anthropogenic or natural) on juvenile survival cannot be discarded.

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