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Age- and sex-dependent changes in morphometric and metabolic variables in the long-lived freshwater mussel *Diplodon chilensis*

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Abstract. Markers of oxidative stress and biochemical composition were investigated in digestive gland and gonad tissues in the freshwater mussel *Diplodon chilensis*, as well as morphometric variables in relation to chronological age and sex. Individual growth followed a von Bertalanffy growth model (VBGM). Superoxide dismutase activity, glutathione level and oxidative damage to proteins remain constant through the life of both tissues, whereas catalase and glutathione-S-transferase activities and lipid peroxidation decrease until 24–27 years of age, to remain fairly stable (mostly in the gonads) or increase slowly (mostly in the digestive gland) afterwards. The timing of these age-related changes is coincident with the age estimated (28 years) from the lower confidence interval for L_∞ (the asymptotic length, 69.97 mm), at which the bivalves would reach their minimum growth rate. *D. chilensis* qualifies as an environmental mitigator for water and sediment clearance. Individuals near the age of minimum growth (20–30 years) would be better suited for bioremediation strategies compared with younger individuals (more sensitive) or to older ones, which are less active and show increasing lipid peroxidation with age. Utilising *D. chilensis* of this age class in sewage-polluted lake shores, in parallel with efforts to improve sewage treatment plants, would ensure an enhancement of the water and sediment cleansing for several decades.

Additional keywords: biochemical variables, freshwater bivalves, growth.

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Introduction

Aerobic organisms obtain energy from cellular reduction of oxygen. Although very efficient, aerobic respiration produces toxic reactive oxygen species (ROS) as by-products (Murphy 2009). The cellular antioxidant system (non-enzymatic radical scavengers and antioxidant enzymes) counterbalances the ROS production, preventing oxidative damage to macromolecules. Changes in the levels of both antioxidants and oxidative damage products are useful indicators of the intensity of oxidative stress (Monaghan *et al.* 2009). The damage to cellular components may cause cell death, aging and, finally, the death of the organism (Abele *et al.* 2009). According to theories of cellular aging by free radicals, the effects of ROS on cellular components are directly related to aging and there is an inverse relationship between ROS production and Maximum Life Span Potential (MLSP) (Pearl 1928; Harman 1956; Bodnar 2009; Buttemer *et al.* 2010). In recent years, the bivalve mollusc model has been proposed as a novel approach for aging studies (Bodnar 2009; Philipp and Abele 2010; Abele and Philipp 2013). The diversity of species, lifestyles and life expectancy are reasons for choosing bivalves for aging studies. In addition, the age of an individual can be precisely determined by counting internal shell growth lines, which periodicity can be calibrated by measuring stable oxygen and carbon isotope ratios ($\delta^{18}O$, $\delta^{13}C$) (Forester *et al.* 1973; Krantz *et al.* 1987; Wefer and Berger 1991). This enables the reconstruction of individual and population life histories (Abele *et al.* 2009).

The relationship between the physiological aging process and free radicals in bivalve molluscs has been studied by several authors (Philipp *et al.* 2005; Ivanina *et al.* 2008; Philipp and Abele 2010; Buttemer *et al.* 2010; Guerra *et al.* 2012). Bivalves with different lifestyles show different strategies for minimising

oxidative damage and the environmental conditions play an important role for longevity. All the data on long-lived bivalves reported so far suggest that low water temperatures and food limitation during winter at high latitudes favour longevity (Buick and Ivany 2004). Philipp and Abele (2010) summarised the current knowledge on cellular aging in bivalves with a focus on antioxidant systems, as well as on tissue repair and metabolic capacities of extremely long-lived species, such as the ocean quahog (*Arctica islandica*). This species, the longest-lived non-colonial animal species (MLSP of 507 years: Butler *et al.* 2013), has a slow growth rate and low aerobic scope, together with superoxide dismutase (SOD) activity that remains constant for many years (Abele *et al.* 2008).

In contrast to their marine counterparts, the aging process of freshwater invertebrates is far less well known. *Margaritifera margaritifera*, the freshwater pearl shell clam with MLSP of 217 years (Schöne *et al.* 2004) has been studied in a few works with the focus on both reproduction and growth (Österling *et al.* 2008; Addy *et al.* 2012). Due to its alarming population decline, *M. margaritifera* has become a threatened species (Bauer 1983) and therefore its use for population growth curves and aging studies, which require sampling many individuals, is not recommended.

The freshwater mussel *Diplodon chilensis* (d'Orbigny, 1835) is the most abundant bivalve mollusc in lakes and rivers of Patagonia, Argentina, and it is widely distributed in Chile. In Argentina, the distribution of *D. chilensis* ranges from Mendoza province (32°52′S, 68°51′W) to La Balsa Lagoon (43°45′S, 71°48′W) in Chubut province (Castellanos 1959; Bonetto 1973; Semenas *et al.* 1994). These autochthonous mussels can colonise sandy or muddy bottoms from 1 m to 50 m of water depth, where they can form banks of considerable densities (Lara and Parada 1988; Rocchetta *et al.* 2014*a*). This characteristic makes this species a suitable model for population analysis and for those studies that involve the use of fairly high numbers of individuals.

Due to its high filter-feeding capacity and abundance, *D. chilensis* is a key species in the maintenance of the oligotrophic conditions of lakes and rivers (Valdovinos and Pedreros 2007). This species has been proposed as a bioremediation tool for water and sediments affected by sewage discharges and by fish farming (Soto and Mena 1999; Lara *et al.* 2002; Bianchi *et al.* 2014*a*). It is also a promising sentinel for pollution monitoring (Ribeiro Guevara *et al.* 2004; Sabatini *et al.* 2011*a*, 2011*b*; Bianchi *et al.* 2014*a*, 2015; Rocchetta *et al.* 2014*a*, 2014*b*).

D. chilensis has a slow shell growth rate, which reaches its maximum in winter and minimum in summer, during the reproductive period (Soldati *et al.* 2009). Growth rates, and therefore, maximum life spans can be influenced in this species, as in other bivalves, by environmental conditions (Valdovinos and Pedreros 2007; Begum *et al.* 2009; Basova *et al.* 2012). Seasonal periodicity of shell growth and composition of *D. chilensis* reveals that this species can reach ~90 years (Soldati *et al.* 2009), being among the five longest-lived freshwater mussel species reported so far (Haag and Rypel 2011). A recent study shows differences in the maximum ages reached, and age distribution among three neighbouring populations from Lakes Lacar and Nonthue (Patagonia, Argentina), in relation to environmental organic matter quality and quantity (Rocchetta *et al.* 2014*a*). The same study shows differences in some oxidative stress biomarkers among individuals of the same age class from these three populations. Aging in long-lived bivalves could affect metabolic variables, which have frequently been used as biomarkers of environmental stress (Abele *et al.* 2009). The study of specific life-history parameters, together with age-related metabolic variables, in *D. chilensis* would provide important information for water quality biomonitoring of highly valuable fresh water bodies of southern Argentina and Chile and for understanding of the aging processes. Also, specific life-history parameters could be used as proxies in themselves.

The aim of this study was to analyse possible changes in metabolic and morphometric variables over the lifetime in the freshwater mussel *Diplodon chilensis* and their implications for environmental purposes. We studied biomarkers of oxidative stress, antioxidant defence system and biochemical composition in different tissues, along with morphometric variables, in relation to chronological age and sex.

Materials and methods

Study area and sampling

Individuals of *D. chilensis* were collected from Nonthue Lake (40°08′S, 71°38′W) at 10 m water depth by SCUBA diving in August of 2009 and 2010, during winter. This lake is located ~50 km west of the city of San Martin de los Andes and it is connected to Lacar Lake, Argentina. We recorded the shell length (*L*, anterior–posterior axis) at a precision of 0.1 mm and used the right shell for age determination (see below). Tissue dissection was carried out in the cold and sex was recorded (analysing gonad with a light microscope). Sex proportion was analysed by Chi-Square test. Both tissues, digestive gland and gonad, were weighed to obtain wet tissue mass (DGM and GM respectively), and then homogenised for biochemical analysis.

Age estimation

The individual age of 83 bivalves was estimated from internal shell growth bands, following the procedure of Rocchetta *et al.* (2014*a*). Examination of polished shell cuts showed a pattern of alternating dark and light bands. These light–dark band pairs were previously tested as annual growth marks for *D. chilensis* by means of stable oxygen isotope ratio ($\delta^{18}O$) shell profiles (Soldati *et al.* 2009). Additionally, we incorporated 73 age and morphometric data obtained from the same population in a previous study (Rocchetta *et al.* 2014*a*) in order to increase the sample number to 153 individuals and improve the fit to the growth model.

The dark bands of each individual were counted and the relationship between age and length was fitted to the general von Bertalanffy growth model, using the non-linear iterative Newton algorithm.

$$Lt = L_{\infty}\left(1 - e^{-k(t-t_0)}\right)$$

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

Table 1. Biochemical variables related to age in *Diplodon chilensis* from Nonthué as described by linear and non-linear regression
Differences between sexes in gonad tissue were assessed by ANCOVA. S, significant differences at $\alpha = 0.05$; NS, no significant differences. F, females; M, males. y, year

Tissue	Variable	ANCOVA	Function	R	R^2	N	P
Digestive	Glycogen	NS	$\text{Glycogen} = -34.57 + 6.05y - 0.10y^2$	–	0.34	42	<0.0001
Gland	Protein	–	NS	–	–	82	–
	Carbonyl	–	NS	–	–	–	–
	Lipid	–	NS	–	–	–	–
	TBARS	NS	$\text{TBARS} = 71.202 - 3497y + 0.065y^2$	–	0.35	73	<0.0001
	GSH	–	NS	–	–	–	–
	GST	NS	$\text{GST} = 13.381 - 0.732y + 0.015y^2$	–	0.29	59	<0.05
	CAT	–	NS	–	–	–	–
	SOD	–	NS	–	–	–	–
Gonad	Glycogen	–	NS	–	–	–	–
	Protein	NS	$\text{Protein} = 14.800 - 0.399y$	−0.49	0.30	82	<0.0001
	Lipid	NS	$\text{Lipid} = 1.099 + 0.340y$	0.38	0.48	32	<0.0001
	TBARS	NS	$\text{TBARS} = 22.020\ e^{-0.08y}$	–	0.49	80	<0.0001
	GSH	–	NS	–	–	–	–
	GST	S	F: $\text{GST} = 3.012\ e^{-0.05y}$	–	0.32	24	<0.05
			M: $\text{GST} = 2.402\ e^{-0.07y}$	–	0.41	38	<0.001
	CAT	NS	$\text{CAT} = 1878.962 - 130.734y + 2.396y^2$	–	0.34	55	<0.001
	SOD	–	NS	–	–	–	–

Biochemical analyses

For biochemical measurement, digestive gland and gonad tissue from 83 individuals were homogenised in the cold with phosphate buffer, 0.134 M (pH 7), containing protease inhibitors (benzamidine 10 mM and phenylmethylsulfonil fluoride, PMSF, 0.5 mM).The homogenate was centrifuged for 15 min at 11 000 *g* and the resulting supernatant was used for glutathione (GSH) content and for enzyme activity measurements.

We did not measure all the biochemical variables in each sample as the amount of tissue was not sufficient, especially in the smallest animals (see the number of replicates in Table 1).

Glycogen, lipid and protein content

Glycogen was determined in the homogenates by a spectrophotometric method using anthrone as a reagent and using SIGMA standard glycogen for the standard curve (Van Handel 1965). Total lipid content was extracted from the homogenate with a chloroform–methanol mixture (2 : 1) and was quantified according to Bligh and Dyer (1959). Data were expressed as micrograms of glycogen or lipids relative to grams of wet tissue mass (g WTM). Total soluble protein was measured using the method of Bradford (1976) and was expressed as micrograms of protein relative to grams of WTM.

Oxidative damage

Protein oxidative damage was evaluated as the carbonyl content following the method of Reznick and Packer (1994). Briefly, the homogenate samples were incubated with acidic DNP (2,4-dinitrophenylhydrazine in 2.5 M HCl) for 1 h at room temperature and then the proteins were precipitated with 20% TCA (trichloroacetic acid). The pellet was washed once with 10% TCA and three times with ethanol–ethylacetate (1 : 1) and dissolved in guanidine hydrochloride (6 M in potassium phosphate buffer, pH 2.3). The absorbance was measured at 370 nm (molar extinction coefficient $\varepsilon = 22.000\ M^{-1}\,cm^{-1}$) and the carbonyl content was estimated. Data were expressed as nanomoles per milligram of protein.

Lipid peroxidation levels were determined in total homogenate samples by the thiobarbituric acid reactive substances (TBARS) method (Buege and Aust 1978, modified). Samples were incubated with reaction mixture (15% TCA, BHT, 0.25 N HCl and 0.37% TBA) at 100°C for 15 min and then centrifuged. The supernatant was used for absorbance measurements at 535 nm. Data were expressed as micromoles per gram of WTM.

Antioxidant defenses

Reduced GSH concentration was determined following the method of Anderson (1985). GSH content was oxidised by 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and the formation of 2-nitrobenzoic acid was quantified. Briefly, the samples were deproteinised with 10% sulfosalicylic acid. After centrifugation the supernatant was incubated with 6 mM DTNB in reaction buffer (0.134 M Na-phosphate buffer, pH 7.5, 6.3 mM EDTA). Absorbance was measured at 412 nm and GSH content was quantified using a calibration curve. Data were expressed as nanomoles per gram of WTM.

Glutathione-S-transferase (GST) activity was measured by the technique of Habig *et al.* (1974). GST catalyses the formation of GS-dinitrobenzene as a result of the reaction between GSH (glutathione) and CDNB (1-chloro-2,4-dinitrobenzene, the GST substrate). The sample was mixed with 100 M K-phosphate buffer (pH 6.5) and GSH, and 100 mM CDNB in ethanol was added. Changes in absorbance were recorded at 340 nm. One GST Unit was defined as the amount of enzyme needed to catalyse the formation of 1 μmol 2,4-dinitrophenyl-S-glutathione

(DNP-SG) min^{-1} at 25°C. Data were expressed as units per gram of WTM.

Catalase (CAT) activity was estimated using the methodology described by Chance (1954). The sample was mixed with 50 mM K-phosphate buffer (pH 7.4) and then 0.3 M H_2O_2 was added to start the reaction. CAT activity was determined by recording the rate of H_2O_2 decomposition, resulting in a decrease of absorption at 240 nm. Data were expressed as micromoles of H_2O_2 per gram of WTM.

Superoxide dismutase (SOD) activity was estimated by the Beauchamp and Fridovich (1971) technique, based on inhibition of the reduction of nitroblue tetrazolium. This compound is reduced by the superoxide anion (O_2^-) generated by riboflavin and light in the presence of methionine. Absorbance was measured at 560 nm. One SOD Unit was defined as the amount of sample necessary for 50% inhibition. Data were expressed as SOD units per gram of WTM.

Statistical analyses

The relationship of each biochemical variable with age was fitted to linear or non-linear regressions and the best-fit function was chosen for further tests. When the best-fit function was not linear, it was linearised by logarithmic transformation of the data.

For cases in which the relationship was statistically significant, the fitted function is shown as well as Pearson's correlation coefficient (r, for linear functions), coefficient of determination (R^2). The age range analysed was from 6 to 39 years old.

Then, differences between sexes were studied by analysis of covariance (ANCOVA). Only data corresponding to mussels from an age interval in which males and females were similarly represented (10–30 years old) were used for ANCOVA. Assumptions of normality, homoscedasticity and parallelism were tested (Zar 1999). Graph Pad Prism 5 and Statistica 7 were used.

Results

Sex proportion and age estimation

All the mussels analysed were reproductively mature, with a sex ratio of 1 : 0.86 (male : female) ($\chi^2 = 3.83$, $n = 82$, $P = 0.0503$). Individual growth was best described by the general von Bertalanffy growth model, with $L_\infty = 72.01$ mm (confidence interval at $\alpha = 0.05$, CI = 69.97–74.06), k = 0.14 $year^{-1}$ (CI = 0.09–0.18), $t_0 = 2.08$ years (CI = 0.12–4.04), $R^2 = 0.69$, $n = 156$. The maximum age found was 73 years old (Fig. 1).

Tissue mass

Fig. 2*a* shows the relationship between DGM and chronological age. No significant differences between the sexes were found (ANCOVA, $P > 0.05$). DGM increased significantly with age and its growth pattern could be described as a power equation model:

$$DGM = 0.043\, Age^{1.08}$$

where $R^2 = 0.64$ and $n = 83$.

GM tended to increase with age, with significant differences between sexes (ANCOVA, $P < 0.05$). GM in females showed the same growth pattern as DGM. A significant increase with age could be described as a power equation function (Fig. 2*b*):

$$GM(female) = 0.061\, Age^{1.05}$$

where $R^2 = 0.75$ and $n = 30$.

In contrast, the growth pattern of GM of males fitted a linear function:

$$GM(males) = 0.113\, Age + 0.148$$

where $R^2 = 0.53$ and $n = 53$.

Differences between sexes were observed when animals within the same age range (10–30 years old) were compared (ANCOVA, $P < 0.001$). GM values were 214% higher in males than in females.

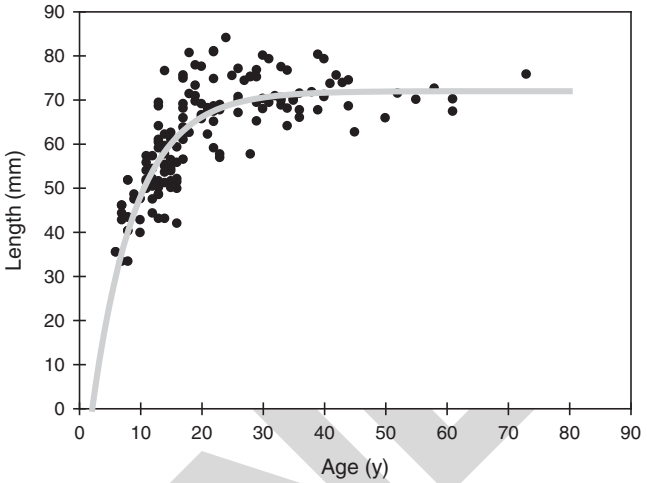

Fig. 1. Length (mm) *v.* age (years) data in *Diplodon chilensis* from the Nonthué population fitted with a von Bertalanffy growth curve ($n = 156$).

Biochemical analysis

The relationship between each biochemical variable with age for digestive gland and gonad tissue is shown in Table 1.

In the digestive gland we did not detect significant differences between the sexes for any of the analysed variables, considering individuals between 10 and 30 years old (ANCOVA, $P > 0.05$). There was a significant second-degree polynomial regression between digestive gland glycogen content and age ($P < 0.05$). This relationship was characterised by an initial fast glycogen content increase up to 30 years of age, followed by a decreasing trend in older individuals (Fig. 3*a*). There were no significant changes in digestive gland protein content in relation to age, although this variable showed high individual variability ($P > 0.05$) (Fig. 3*b*). Hence we have chosen to measure antioxidants (enzymatic and non-enzymatic) in relation to grams of wet tissue mass. Lipid content remained unchanged within the analysed age range ($P > 0.05$) (Table 1), whereas the level of TBARS showed a significant decline until 27 years old ($P < 0.0001$). A slight increase in TBARS was recorded after this age (Fig. 3*c*). Carbonyl content in digestive gland tissue showed no any functional relationship with age ($P > 0.05$) (Table 1). The only

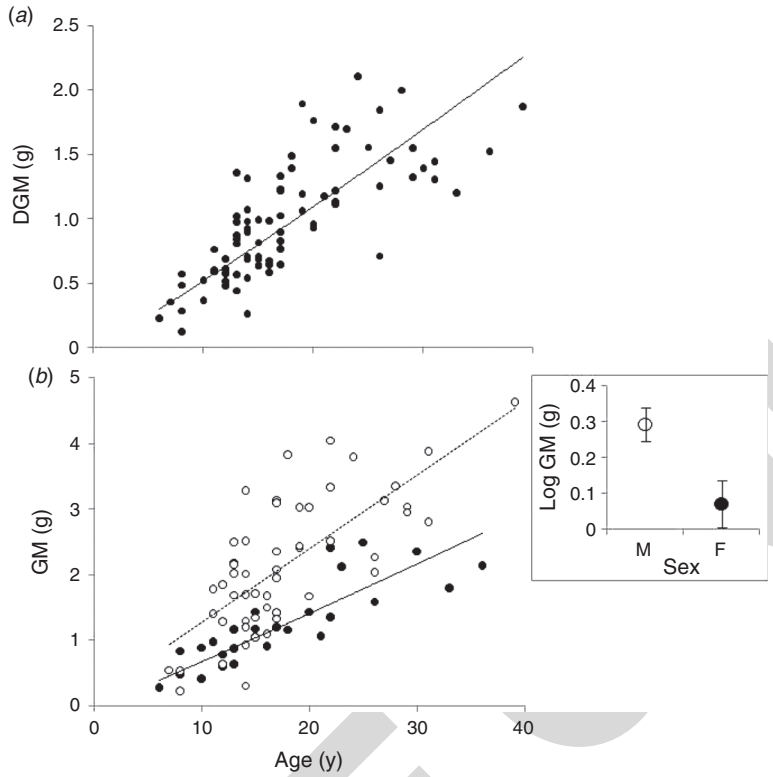

Fig. 2. (*a*) Digestive gland mass (DGM, g) related to age (years) of *Diplodon chilensis* from the Nonthue population ($n = 83$), (*b*) gonad mass (GM, g) related to age (years). Full circles and continuous line, females (F), ($n = 30$); open circles and dashed line, males (M), ($n = 53$). Inset shows the differences between the sexes of log(GM), with age range = 10–30 years old; results are expressed as adjusted mean ± s.d. ($n_{females} = 24$, $n_{males} = 47$).

enzyme for which activity was significantly related to age in this organ was GST ($P < 0.0001$). The second-degree polynomial function between GST activity and age showed an initial decline up to 24 years of age and a posterior increase between 24 years and the maximum age analysed (Fig. 3*d*).

Protein content in gonad tissue decreased linearly with age and was not significantly different between males and females within the same age range (ANCOVA, $P > 0.05$) (Fig. 4*a*). There was an increase in lipid content of gonad tissue with age, which fitted a linear function (Fig. 4*b*), whereas the level of lipid peroxidation fitted an exponential function with a significant decline with age (Fig. 4*c*). No significant differences between sexes were observed in these two variables (ANCOVA, $P > 0.05$). In contrast, GST activity was different between males and females within the same age range (10–30 years old) (ANCOVA, $P < 0.0001$), with 60% higher activity in females than in males, in spite of similar covariation with age in both sexes. GST activity of males and females fitted exponential functions, which showed lower activity in older animals (Fig. 4*d*). CAT activity level was similar in both sexes (ANCOVA, $P > 0.05$). This enzyme's activity fitted a polynomial function (Degree 2) with age, decreasing steeply until 27 years old, and increasing slightly from this age upward (Fig. 4*e*).

Discussion

Our results show that several morphometric and biochemical variables in the digestive gland and gonad of the freshwater mussel *Diplodon chilensis* are correlated with age. A higher number of biochemical variables are age-dependent in the gonad than in the digestive gland. In contrast, most of the variables analysed in both organs are sex-independent.

Individual age in Diplodon chilensis

According to the results obtained from the combination of both datasets analysed in this study and previously described for the same population by Rocchetta *et al.* (2014*a*), bivalves would reach their minimum growth rate at ~28 years old, as estimated from the lower CI for L_∞(69.97 mm). The growth pattern described by the general von Bertalanffy model in both works showed overlapping confidence intervals for L_∞ and k parameters. However, it is important to notice that growth curve parameters of *D. chilensis* (L_∞, k and maximum age) can vary depending on environmental conditions. It was previously observed that these parameters can be affected by anthropic activity (Rocchetta *et al.* 2014*a*), which could recommend them as suitable proxies for water quality assessment.

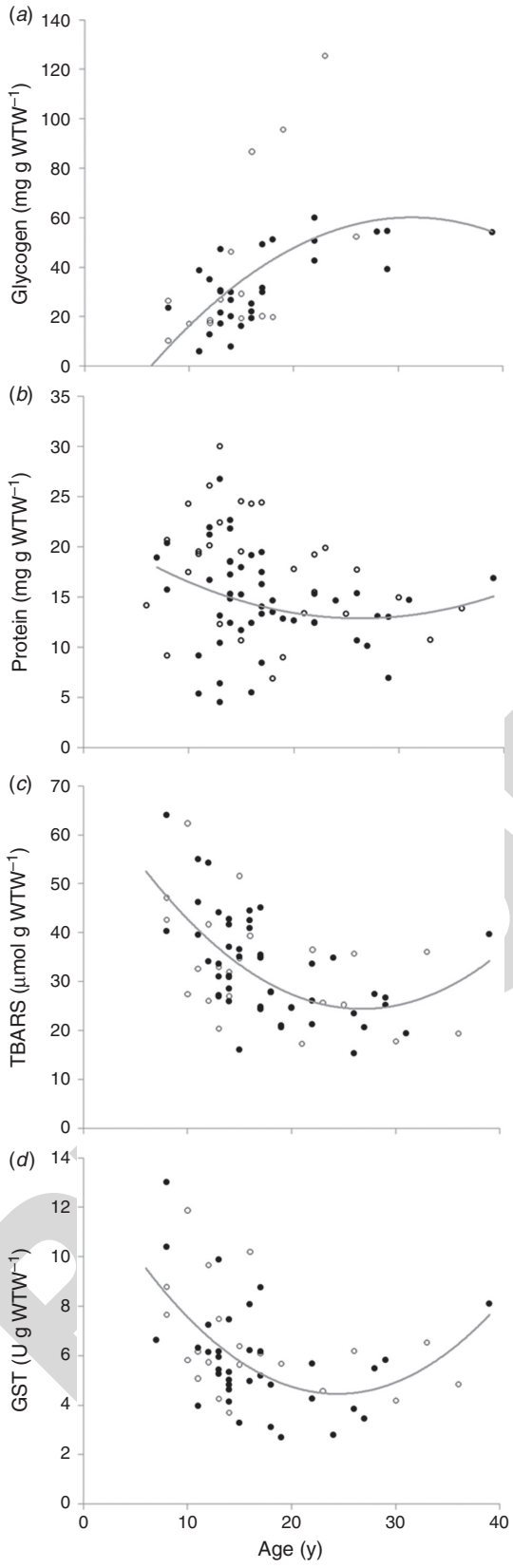

Fig. 3. Biochemical variables measured in digestive gland tissue of *Diplodon chilensis* from Nonthue related to age: (*a*) glycogen (mg glycogen g^{-1} wet tissue mass, WTM), (*b*) protein (mg protein g^{-1} WTM), (*c*) TBARS (µmol TBARS g^{-1} WTM), and (*d*) GST activity (GST Units (U) g^{-1} WTM).

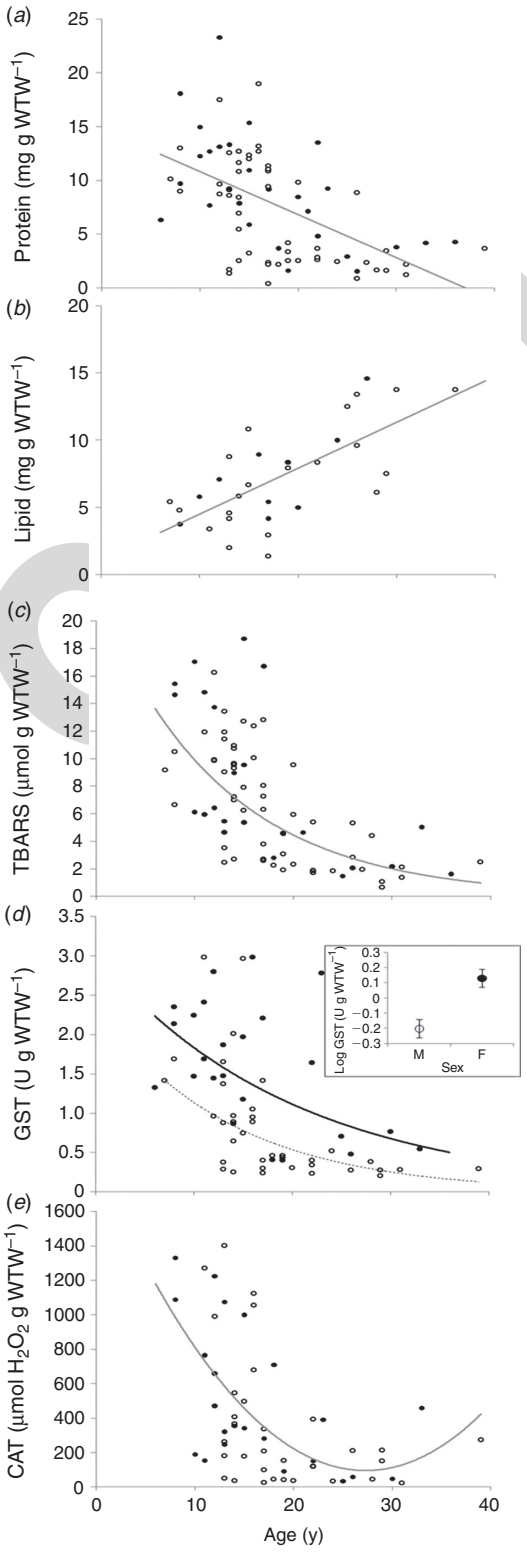

Fig. 4. Biochemical variables measured in gonad tissue of *Diplodon chilensis* from Nonthue related to age: (*a*) protein (mg protein g^{-1} WTM), (*b*) lipid (mg lipid g^{-1} WTM), (*c*) TBARS (µmol TBARS g^{-1} WTM), (*d*) GST activity (GST Units (U) g^{-1} WTM), and (*e*) CAT activity (µmol H_2O_2 g^{-1} WTM). The inset figure in (*d*) shows the differences between the sexes of log(GST) with age range = 10–30 years old; results are expressed as adjusted mean ± s.d. ($n_{females} = 20$, $n_{males} = 34$).

Age-related metabolic changes

The age-related changes in DGM and glycogen content per gram of digestive gland are initially coincident. Low levels in young individuals are followed by an increment with age. However, although DGM increment follows a power equation model, the glycogen content per gram of this tissue follows a polynomial function, which predicts that glycogen content stops increasing at ~30 years of age and then decreases. The decreasing phase of this function starts near the age estimated from the lower Cl for the L_∞, as mentioned above. It has been reported that younger scallops could have lower glycogen concentration than older ones under the same exercise conditions (see Philipp and Abele 2010 for review), which suggests reserve accumulation with age. It is known that glycogen is the principal energy reserve of adult bivalves and could be important for gamete development and during nutritional stress (Beninger and Lucas 1984). As *D. chilensis* is a long-lived species that remains reproductively active for at least four decades beyond the age at which minimum growth rate would be reached, the decrease of glycogen content in the digestive gland after this age could be related to allocation of reserves to fuel reproductive functions. This could be important for individuals with declining filtration activity and thus reduced energy intake (Harman 1998). The lack of change in protein and lipid concentration in this tissue suggests that digestive gland activity remains stable throughout the age interval studied.

We detected no signs of reproductive senescence in *D. chilensis*. Besides, gonad mass of *D. chilensis* increases throughout the entire age range studied herein. In this species gonad mass is higher in males than in females. This could be related to the fact that males invest reproductive energy only in gonad development, whereas female *D. chilensis* also have to allocate energy to the development of the gill marsupium, where glochidium larvae are incubated (Semenas and Brugni 2002). Regardless of the sex, this continuous gonadal growth seems to be mostly related to lipid accumulation, because lipid content per gram of tissue increases with age, whereas protein content decreases. In spite of the lack of reproductive senescence, these changes in tissue composition suggest a decline in protein synthesis rates, associated with lower metabolic activity (Philipp *et al.* 2005). An alternative explanation is that accumulated lipids could constitute an energy store to fuel reproductive activity in old animals with low filtration activity. Besides lipids, bivalve gonads accumulate glycogen, which can be consumed during the mature phase or during the reproductive season (Román 1992; Ojea *et al.* 2002). Although we have found large glycogen stores in the gonad of *D. chilensis*, this content remains unchanged with age. As the individuals used in our study were collected before the reproductive season, we cannot attribute this lack of change to differential use of glycogen for reproductive demands by individuals of different age classes.

In general, young bivalves show high antioxidant levels in different tissues, associated with high metabolic and growth rates, part of which decrease rapidly with age (Abele *et al.* 2008). Particularly for the digestive gland, Canesi and Viarengo (1997) and Viarengo *et al.* (1990) have reported, for the short-lived mussel *Mytilus edulis*, decreasing CAT activity and GSH content, a tendency for the TBARS levels to increase, and constant GST and SOD activities with age. In the digestive gland of *D. chilensis*, only TBARS and GST change with age. In this species, both TBARS level and GST activity show a declining trend in young bivalves until 25–27 years of age, followed by a slight recovery in older individuals. This recovery of TBARS level and GST activity could be related to metabolic reactions involved in glycogen mobilisation, as suggested above. Additionally, lower feeding activity during the low growth phase could involve dietary changes, which may affect the activity of detoxification enzymes, such as GST (Harman 1998). However, filtration rates, reserve mobilisation and enzyme activity should be studied within a wider age interval, to further support these ideas. In *D. chilensis*, CAT, SOD, GSH and protein carbonyl groups remain constant through life, in concordance with the age-independent digestive gland SOD and CAT activities reported by Fernández *et al.* (2009) for 21–45-year-old *M. margaritifera*.

At variance with the behaviour described above for the digestive gland, gonad TBARS and GST show declining trends throughout the entire age range analysed, although at very low rates during the slow growth period. In contrast, CAT decreases in young individuals and starts to recover at 27 years of age. The slow reduction of lipid peroxidation and the maintenance of stable concentration of protein carbonyls could result from the combination of the decreasing gonad activity and increasing proportion of storage lipids (less sensitive to oxidation), both suggested by the increasing lipid proportion in the gonad. Increased CAT activity in older individuals, together with constant SOD activity and GSH levels would also contribute to the maintenance of gonad redox homeostasis.

Growth, reproduction and longevity

Since we have analysed metabolic variables in different tissues, the life strategy of *D. chilensis* can be only partially compared with that of other bivalves. *D. chilensis* has a low growth rate, with growth constants (k) calculated by the von Bertalanffy model between 0.064 and 0.14, for Lacar lake and Nonthue lake populations, whose maximum ages found were 62 and 73 years respectively (Rocchetta *et al.* 2014*a*; present paper). Haag and Rypel (2011) have summarised growth and longevity data for 57 freshwater mussel species from 146 North American and European populations. These authors observed important differences among populations of the same species and obtained a strong negative relationship between log(maximum age) and log(k) (VBGM), with long-lived species having low k values. Accordingly, among marine bivalves, the Icelandic population of extremely long-lived species *A. islandica* has an extremely low growth constant (k = 0.057, VBGM), compared with other marine species (Strahl *et al.* 2007), which is similar to those obtained for long-lived freshwater mussels (Haag and Rypel 2011; Rocchetta *et al.* 2014*a*; present paper). *A. islandica* has been shown to live and reproduce for at least 150 years after the age of minimum growth rate, keeping low and stable levels of protein carbonyls in the gills and mantle (Strahl *et al.* 2007; Abele *et al.* 2008). In concurrence, our data suggest that *D. chilensis* lives and reproduces for more than 40 years beyond the age at which minimum growth rate would be reached and keeps stable carbonyls levels in the digestive gland and gonad.

Bauer (1987) has also reported the absence of reproductive senescence in the longest-lived freshwater mussel, *M. margaritifera*, for a population with maximum age of 75 years.

On the other hand, the late onset of reproductive activity reported for the longest-lived bivalve, *A. islandica* (10–32 years) has been highlighted as a feature related to longevity (Haag and Staton 2003; Abele and Philipp 2013). Among long-lived freshwater mussels, the reproductive onset reported for Scottish populations of *M. margaritifera* (maximum age: 48–123 years) is between 12 and 13 years old (Young and Williams 1984*a*, 1984*b*). In contrast, the age of reproductive maturity of *D. chilensis*, estimated from a length at sexual maturity of 20–23 mm (Semenas and Brugni 2002) and the growth curve presented in present paper, is ~5 years. However, the age at which all bivalves become reproductive and its correlation with longevity should be further studied in different populations of *D. chilensis.*

Relevance to the aquatic system

Oligotrophic Patagonian freshwater bodies are recognised as valuable aquatic resources with great social, economic and ecological relevance. During the last decades the water quality in different parts of this region has been changing as a consequence of increasing urban impact (Valdovinos and Pedreros 2007). Consequently, *D. chilensis* was proposed as a suitable sentinel species and recent model for bioremediation purposes. Thus, we could also observe that life-history parameters, like growth pattern and oxidative stress parameters, change with environmental conditions (Rocchetta *et al.* 2014*a*), making them a useful tool for water quality studies. Our results suggest that young bivalves, which are the most metabolically active and, thus, more active filter feeders than older individuals, would also be more sensitive to oxidative stress caused by sewage pollution. Therefore, individuals near the age of minimum growth, between 20 and 30 years would be better suited for bioremediation strategies than would younger individuals (more sensitive) or older ones, which are less active and show increasing lipid peroxidation with age. Utilising *D. chilensis* of this age class in sewage-polluted lake shores, in parallel with efforts to improve sewage treatment plants, would ensure an enhancement of the water and sediment cleansing for several decades.

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