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An intertidal limpet species as a bioindicator: Pollution effects reflected by shell characteristics

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A B S T R A C T

Mollusc shells have been widely used for monitoring the bioavailability of contaminants in the aquatic environment. The present work examined malformations among the shells of the limpet *Siphonaria lessoni* from heavily polluted, light polluted and unpolluted sites in Argentina. Data on shell shape, thickness, dry weight, microstructure and semi-quantitative elemental composition was evaluated as well as soft tissue dry weight. Shells from the heavily polluted site were significantly ($p < 0.001$) thicker than those from other areas. SEM (scanning electron microscopy) analysis of thickened shells revealed the presence of globular malformations on inner shell surfaces. On heavily polluted shells, elemental composition analysis by EDS (electron dispersive spectroscopy) of such malformations indicated concentrations three times higher of carbon and four times lower of calcium and oxygen than the control. Light polluted shells presented concentrations two times lower of calcium. In addition, soft tissues were lighter at the heavily polluted site ($p < 0.001$). These data demonstrate the sensitivity of this abundant and widely distributed intertidal limpet to aquatic pollutants, and support the use of this limpet as a potential biomarker.

1. Introduction

Hard structures of marine organisms contain considerable information about their own growth history, including the changing conditions of mineralization and environmental stress or disease (Okoshi and Sato-Okoshi, 1996). Mollusc shells contain not only a record of their life history but also information of environmental changes preserved through structural, morphological, and chemical changes within the shell.

The presence of malformations (Frazier, 1976; Phillips, 1977, 1978) as well as histopathological injuries on molluscs have been linked with environmental chemical pollution (Gold-Bouchot et al., 1995; Najle et al., 2000). Moreover, other contaminants effects, such as shell thickening of bivalves (Almeida et al., 1998a; Waldock and Thain, 1983), differential size in limpets (Espinosa et al., 2007), inhibition of shell growth (Cunningham, 1976) and shell chambering (Bayen et al., 2007) in oysters are well described.

Species with high sensitivity, low motility and wide distribution are preferred for monitoring since they facilitate location of high polluted areas and comparison among wide separated places. This is the case of siphonarids, which are distributed around the world, and inhabit both South American coasts. De Pirro and Marshall (2005) found that siphonarids tolerate polluted environments better than patellogastropod limpets, and suggested them as pollutant indicators. In fact *S. lessoni* is the dominant intertidal mollusc species in two polluted areas of the South West Atlantic coast, Mar del Plata and Quequén ports. Meanwhile in other nearby less polluted intertidal zones *S. lessoni* cohabits with large population of mussels.

Observations of *S. lessoni* anatomy, development and spatial distribution have been reported from natural areas of rocky shore in Mar de1 Plata (38°03′ S, 57°33′ W) (38″03′ S, 57″33′ W) (Olivier and Penchaszadeh, 1968), and its growth and feeding habits were studied in populations growing on rafts within the port of Mar de1 Plata (Bastida et al., 1971). Tablado et al. (1994) and Tablado and López Gappa (2001) reported morphometric differences observed in this limpet from different habitats as a result of differential growth rates in response to environmental pressures: tidal level, wave exposure, food availability, and intraspecific competition. However, sensitivity to pollution has never been evaluated.

The aim of the present study is to determine whether pollution affects *S. lessoni* shell deposition, structure and morphology to evaluate whether this species can be used as a bioindicator. In order to achieve this objective *S. lessoni* population from three differently polluted areas are compared.

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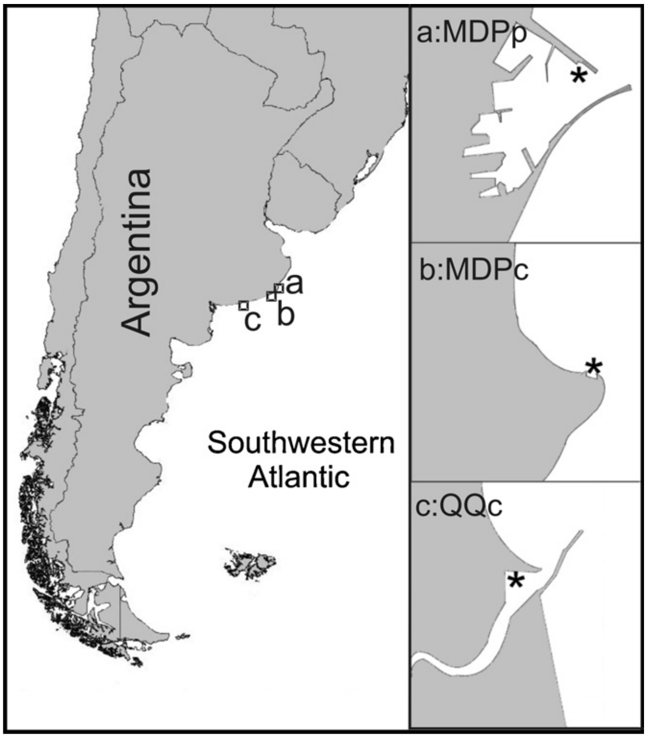

Fig. 1. Sampling sites. Study sites showing location where individuals of *S. lessoni* were collected. Sites codes: high polluted sites MDPp (Mar del Plata port), QQp (Quequen port) and less polluted site MDPc (Waikiki beach).

2. Materials and methods

2.1. Study sites

Siphonaria lessoni was sampled in two areas of the Mar del Plata shore (MDPc and MDPp) and one area in Quequén port (QQp) (Fig. 1). The three chosen areas have similar environmental and ecological characteristics but different contaminant concentrations. The first zone, MDPc (Waikiki beach 38°04′50 S, 57°30′08 W) is located within the southern limit of Mar del Plata city. This zone is protected by a jetty and considered to be slightly polluted (Laitano, unpublished data). The second zone, MDPp, was set within the MDP port, 5 km northwards to the first zone (38°02′10 S, 57°31′28 W). It is considered to be highly polluted: 728.3 ng/g of Tributyltin (Bigatti et al., 2009; Cledón et al., 2006), 6.78 ng/g of PCB's, 8.75 μg/g of hydrocarbons and 30.59 ng/g of organochlorine pesticides (Colombo et al., 2005). The third zone QQp (38°29′23 S; 58°48′59 W) is more contaminated than MDPc, but less than MDPp: 1.4 ng/g TBT, 5.77 μg/g of hydrocarbons, 2.51 ng/g of PCB's and 4.91 ng/g of organochlorine pesticides.

2.2. Morphometric

During April 2008, 30 individuals of *S. lessoni* were collected at each site. Only adult specimens (>6 cm) were included in all morphometric analyses. The shells were photographed from the right side and were always positioned in the same orientation. Digital images were taken against a white illuminated background in order to maximize the contrast of shell outlines. All the images were binarized and then processed using the SHAPE software (Iwata and Ukai, 2002).

The shape of the shell of these limpets is rather simple with very few homologous points that can be used as landmarks; moreover they are difficult to localize because they correspond to landmarks type 2 (maximum of curvature along the boundary or outline of a specimen) (Bookstein, 1991). Therefore, the shell shape variation between *S. lessoni* specimens from MDPp, MDPc and QQp was measured using outline analyses based on the Elliptic Fourier analysis (EFA) on the outline coordinates (Rohlf and Archie, 1984).

Elliptic Fourier coefficients were mathematically normalized in order to avoid biases in results caused by different size, location, rotation and starting position of shells (Rohlf and Archie, 1984). The closed curve of each shell was decomposed into a sum of 15 harmonically related ellipses. These 15 harmonics represent 99.99% of the total Fourier power spectrum (Crampton, 1995). With the PrinComp module, we performed the principal components analyses (PCA) on the variance–covariance matrix of the normalized Elliptic Fourier coefficients. PCA is effective in summarizing the information regarding the variation contained in these coefficients (Rohlf and Archie, 1984), which were estimated using PAST v.1.77 (Hammer et al., 2001). Finally, multivariate analyses of variance (MANOVA) were performed with PAST in order to evaluate the importance of between-group differentiation relative to within-group variation. A test to detect if there are any significant morphological differences (Wilk's Lambda test) was performed and *post hoc* Hotelling pairwise comparisons (Bonferroni corrected and uncorrected) were also performed using PAST to detect the significant differences.

2.3. Morphological variables

A pool of 100 individuals of *S. lessoni* was collected at each of the three sites around a randomly determined point. Shell length, was measured to the nearest 0.1 mm with a micrometer eyepiece under a binocular microscope and individual shell thickness was measured under stereomicroscope to the nearest 0.01 mm. To determine the dry weight of shells and soft tissues, limpets were frozen at −20 °C for 24 h prior to dissection. Then, they were thawed and whole tissue was removed from shell using a scalpel. They were placed into pre-weighted aluminium pans, dried for 12 h at 75 °C, and weighed to the nearest 0.001 g.

Data normality (Shapiro–Wilk test) and homogeneity of variances (Cochran's test) were tested and when necessary transformed (Underwood, 1997; Zar, 1999). The slopes and elevations of the regressions of the shell thickness, dry weight of shells and soft tissues (dependent variables) were tested with analysis of covariance (ANCOVAs) to assess the pollution effects (difference between zones) using limpet shell length as the covariate. ANCOVA can be used to compare elevations of regression lines if their slopes are not statistically different (Zar, 1984). When slopes were heterogeneous, we used Tukey multiple comparison tests (Zar, 1984) to determine which combinations of slopes differ. In these cases, we applied the Johnson–Neyman test (Huitema, 1980) to identify the depth range over which elevations are not significantly different.

2.4. Microstructure and shell elemental composition

A detailed observation of the inner surface of the shell of three individuals of each place was done through Scanning Electron Microscopy (SEM). Samples were metallized with Ag/Pd in a Denton Vacuum Desk II metallizer. The analyses were carried out with a Jeol JSM 6460LV microscope in the Laboratorio de Microscopía Electrónica at the Universidad Nacional de Mar del Plata, Argentina.

Simultaneously, Energy Dispersive Spectroscopy analysis was performed with an EDAX GENESIS V5.11 connected to the microscope to determine the elemental composition of the shells. Elements analysed were C, O, Na, Mg, Al, Si, Cl and Ca. Energy