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Shell damage in the Tehuelche scallop *Aequipecten tehuelchus* caused by *Polydora rickettsi* (Polychaeta: Spionidae) infestation

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ABSTRACT

The different types of shell damage caused to the commercially valuable Tehuelche scallop (*Aequipecten tehuelchus*) by the polychaete *Polydora rickettsi* are described. X-rays, computerized tomography, shell sections, scanning electron microscopy, Energy Dispersive X-ray analysis (EDAX), mineralogical analyses and geometric morphometrics were applied to that end. Scallop shells presented three types of damage: (1) spots, (2) calcareous alterations, and (3) mud blisters. Microstructural alterations consisted of a simple conchiolin membranous layer in the case of spots, a series of interleaved layers of different degree of calcifications in calcareous alterations, and two different surface morphologies (muddy and mucous layers) in mud blisters. Damage was localized mainly along concentric growth rings, coincidentally with the location of most burrows, as shown by X-ray. Mineralogical analysis showed that in all cases (including non-infested shells) calcite was the calcium carbonate polymorph present. Geometric morphometrics showed that only 5% of shape variation was explained by infestation with *P. rickettsi*, irrespective of the type of damage. Number of worms per infested shell varied significantly among four beds. Left shells (upward-oriented) were significantly more affected than right shells, which are in closer contact with the bottom.

1. Introduction

Infestation by spionid polychaetes, particularly by members of the *Polydora*-complex, constitutes a serious problem for mollusc fisheries and aquaculture around the world (Lauckner, 1983; Cremonte, 2011). When the intensity of infestation is high, it is zfrequently associated with poor condition (Silina and Zhukova, 2009), shell damage, reduced growth rate (Silina, 2006), and increased mortality (Bergman et al., 1982). The worst type of damage is the formation of mud blisters which, because contained anaerobic metabolites like hydrogen sulphide, cause bad odour, turning molluscs unsuitable for the market (Galtsoff, 1964; Blake and Evans, 1973; Handley and Bergquist, 1997).

Tehuelche scallops, and other bivalves to a lesser extent, support a regionally significant artisanal fishery in San José Gulf, Argentine Patagonia (Ciocco et al., 2005). During recent years infestation of landed bivalves became a subject of concern to fishers, processing plants, the media and consumers. A previous study (Diez et al., 2011) identified *Polydora rickettsi* Woodwick, 1961, as the causative agent. Infestation was documented for Tehuelche scallops (*Aequipecten tehuelchus* (d'Orbigny, 1846), Pectinidae), ribbed mussels (*Aulacomya atra* (Molina, 1782), Mytilidae), false oysters (*Pododesmus rudis* (Broderip, 1834), Anomiidae), flat oysters (*Ostrea puelchana* d'Orbigny, 1842, Ostreidae) and stripped clams (*Ameghinomya antiqua* (King, 1831), Veneridae). *P. rickettsi* is a common shell borer in other regions as well, having been reported for the northeast and southeast Pacific Ocean and for some localities in Brazil (Radashevsky et al., 2006).

The assessment of infestation patterns in relation to season, location or host age requires the development of standards based on a damage typology (Cañete and Cárdenas, 2004; Silina, 2006), but there are few detailed studies of the structural alterations caused by shell-boring polydorids (e.g., Zottoli and Carriker, 1974; Sato-Okoshi and Okoshi, 2000) that can provide objective support for such a typology. The purpose of this study was to characterize infestation patterns of Tehuelche scallops by polydorids in San José Gulf (Argentine Patagonia), and to develop an objective typology of shell damage. To that end we utilized X-rays, computerized tomography, shell sections, scanning electron microscope, Energy Dispersive X-rays, mineralogical analysis and geometric morphometrics (GM, Márquez et al., 2010).

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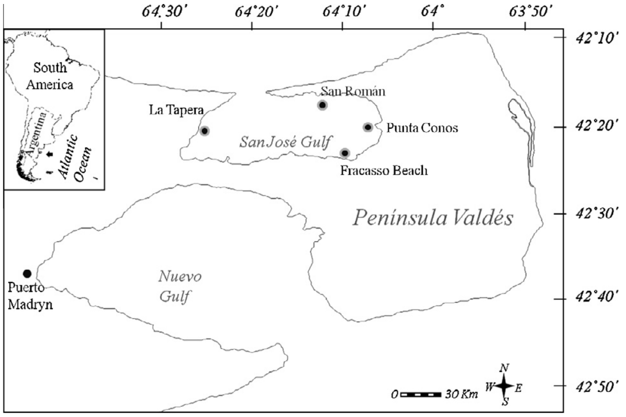

Fig. 1. Exploited beds of the Tehuelche scallop (*Aequipecten tehuelchus*) in San José Gulf, northern Patagonia, Argentina.

2. Materials and methods

2.1. Study site and sample collection

The study was conducted in San José Gulf (northern Argentine Patagonia), where Tehuelche scallops have been harvested by commercial divers since 1973 (Orensanz et al., 1991; Ciocco et al., 2005) (Fig. 1). During the summer of 2010, a total of 253 scallops of commercial size were collected from four exploited beds by divers: La Tapera, Fracasso Beach, Punta Conos and San Román (Fig. 1; Table 1). Scallops were transported to the laboratory and kept in aquaria up to 48 h before processing.

2.2. Shell damage

Scallop shells were observed macroscopically and under a stereoscopic microscope in order to typify damage. Pattern of infestation was described in relation to growth rings; formation of the latter has been described in detail by Orensanz (1986) and Orensanz et al. (1991). Some shells were then broken with pliers and hammer, and polydorids were removed and identified under light microscopy. Voucher specimens were deposited in the Invertebrate Collection of Centro Nacional Patagónico, Puerto Madryn, Argentina (CNP-inv-05-076 to 078, *n* = 6). Burrow shape and location in the shell were established using X-rays (*n* = 20). Computed Axial Tomography (CAT) serial scan slices (1 mm thick) made with a General Electric CT/e tomographer were used to determine the number of worms present in the mud blisters, and the structure of the latter. Tissue density was expressed in Hounsfield units (HU), which express radiodensity (radiation attenuation in different tissues) in a quantitative scale.

2.3. Shell microstructure

Shells were sectioned at different orientations using a low-speed saw and diamond blades. Sections 0.4-mm thick were obtained and mounted with cyanoacrylate adhesive. The exposed face of each section was polished and observed under a Philips XL 30 SEM, and EDAX was used to describe elementary composition in the most harmful types of damage. Additional infested shells were used for mineralogical analysis with X-ray powder diffraction (XRD) in a Philips 3020 goniometer.

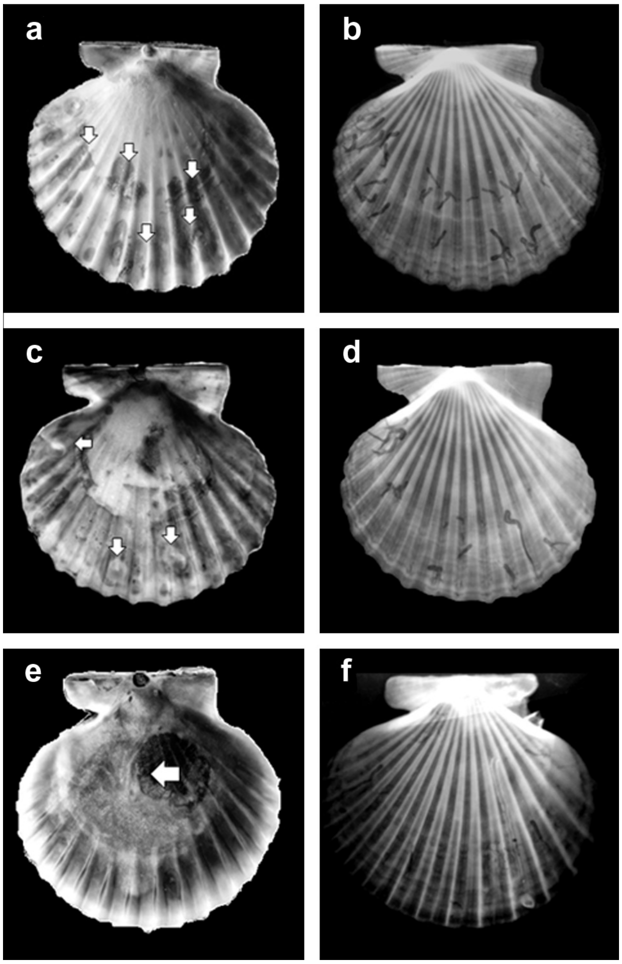

Fig. 2. Types of damage in scallop shells caused by *Polydora rickettsi*. (a–b) spots, (c–d) calcareous alterations, (e–f) mud blister. (a, c and e) macroscopic view, internal shell surface (arrows indicate damaged areas); (b, d and f) X-ray images of the same specimens, captured from the external shell surface.

2.4. Geometric morphometrics

After removing the soft parts, right shell outlines were digitized (concave side upwards) with a digital camera. To capture the individual form we used the landmarks configuration defined by Márquez et al. (2010). All specimens were digitized with the software TPS dig2. Landmark configurations were superimposed by generalized Procrustes analysis (Slice et al., 1996). To assess and control putative allometric effects, we computed the multivariate regression of shape (Procrustes coordinates used as dependent variables)

Table 1
Prevalence and intensity of *Polydora rickettsi* infestation of *Aequipecten tehuelchus*, by bed, San José Gulf. S: Spots, CA: Calcareous alterations, MB: Mud blister.

Beds	Latitude S	Longitude W	N (scallops)	Prevalence (%)	Intensity (range)	Type of damage (%)
La Tapera	42°20′	64°35′	63	27	1–8	S (25.4), CA (0), MB (1.6)
Fracasso Beach	42°25′	64°07′	64	90	1–8	S (84), CA (1.5), MB (4.7)
Punta Conos	42°20′	64°02′	83	100	1–30	S (65), CA (23), MB (12)
San Román	42°15′	64°12′	43	76.7	1–7	S (63), CA (4.7), MB (9)

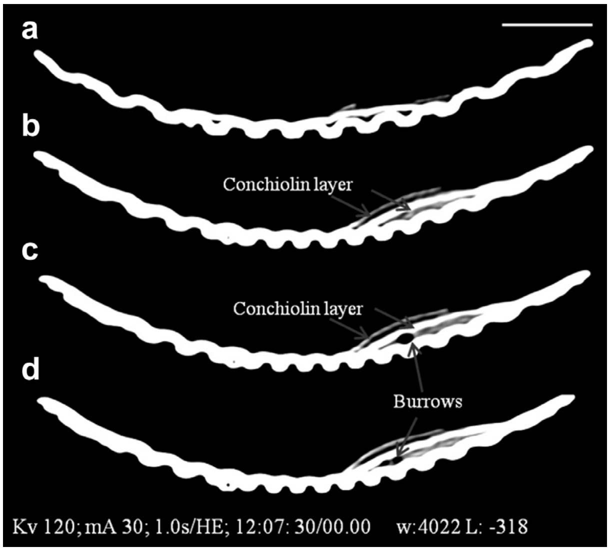

Fig. 3. Computed tomography of serial scan slices (a–d) of a scallop shell with a mud blister caused by *Polydora* infestation. Arrows indicate conchiolin layers and burrows. Scale bar 15 mm.

on size (independent variable). Furthermore, we used the presence or absence of polydorids as classifier in the regression graph to investigate the effect of infestation on scallop's allometric growth. The principal components of shape were calculated from the variance–covariance matrix of the Procrustes coordinates to display the major features of shape variation along the axes.

2.5. Variation of infestation in relation to geographic location (exploited beds), valve orientation (left vs. right) and size

Burrows were counted under a stereomicroscope to estimate intensity of infestation. Infestation intensity was calculated as the number of burrows per shell examined. Mean infestation intensity was compared among beds with a Kruskal Wallis one-way analysis of variance by ranks, and between valves (left vs. right) with a Chi-square test (0.05 significance level).

3. Results

3.1. Shell damage

Three types of damage were identified: (1) spots, (2) calcareous alterations, and (3) mud blisters (Fig. 2), in all cases caused by *Polydora rickettsi* infestation. X-ray observations revealed that most burrow openings associated with spots and calcareous alterations were aligned along the concentric growth rings (Fig. 2b, d, and f), while mud blisters were located close to the insertion of the adductor muscle (Fig. 2e); only in a few instances were blisters observed in the periphery of the shell.

Most spots were brown coloured, darker in the middle; a few were clearer in the middle and had a light protuberance (Fig. 2a).

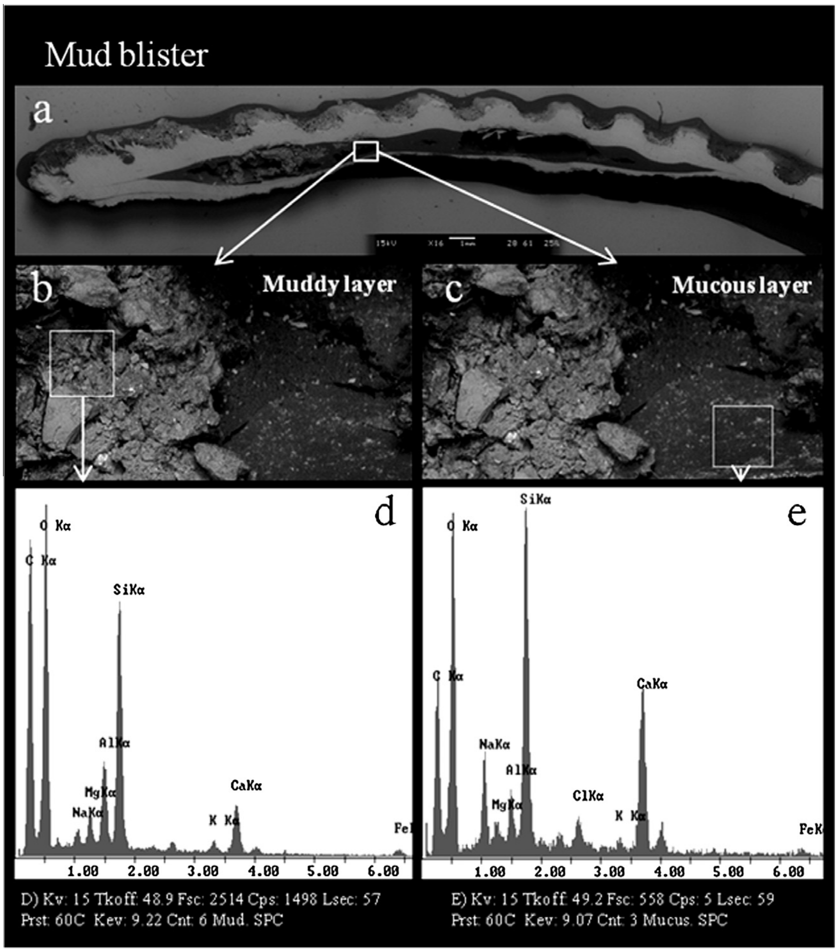

Fig. 4. Mud blisters. a: Transversal section through a mud blister. (b–c) Detail showing two layers and spots selected for elementary analysis (rectangular boxes) in the muddy (b) and mucous (c) layers. (d–e) EDAX analysis of the two layers, muddy (d) and mucous (e). In (d) and (e) the *x*-axis corresponds to energy in keV (kilo-elektronnvolts) and the *y*-axis to counts per second (cps).

Each spot was associated with a single worm. A U-shaped burrow was observed when the shell was broken. The burrow has a characteristic elevation in the middle, filled with mucus and mud and covered by an organic layer presumably formed of conchiolin. Calcareous alterations were gray-coloured, with a calcified protuberance surrounded by a brown area (Fig. 2c). Each alteration was occupied by a single worm. Mud blisters consisted of greyish or brownish-coloured camera. Most were occupied by four to five live worms. Tomography revealed that there is a layer of conchiolin covering the burrow of each individual worm (Fig. 3). These layers pile up to form a large blister (Fig. 3a–d). Burrow age is correlated with the density of the conchiolin layer. In the case illustrated the oldest layer (3 mm thick) had a density of 744 HU, and the most recent (1 mm thick) 700 HU.

3.2. Shell microstructure

Microstructural observation of shell sections under SEM (Figs. 4a and 5a) revealed that the most harmful damages were mud blister and calcareous alteration. Spots presented a simple organic membranous layer. Mud blisters consisted of several membranous layers of conchiolin piled up, covering sediment and organic material (Fig. 4b and c). Calcareous alterations presented 2–3 layers of calcium with different degree of calcification, separated by interleaved organic layers (Fig. 5b and c).

Elementary composition determined with EDAX revealed that mud blisters and calcareous alterations differed in microstructure and composition (Figs. 4 and 5). Two different surface morphologies were visualized in sections of shells with mud blisters (Fig. 4): a “muddy layer” of granulate appearance (Fig. 4b), and a “mucous layer” (Fig. 4c), formed by mucus and fine sediment. Elemental analysis (EDAX) of the mud layer (Fig. 4d) indicated that it is composed mainly of carbon (45%) and oxygen (29%), with small amounts of silicon (9%), and calcium (7%). In comparison, the mucous layer (Fig. 4e) was richer in calcium (16%), oxygen (35%) and silicon (12%), and lower in carbon (28%). Calcareous alteration, as seen in shell sections (Fig. 5a), consisted of a series of interleaved layers of different degree of calcification. Two different surface morphologies were identified based on the degree of apparent calcification and named “calcium layers 1 and 2” (Fig. 5b and c), layer 1 being the most calcified. Elemental analysis EDAX indicated that layer 1 (Fig. 5d) was composed mainly of carbon (44%), oxygen (26%) and calcium (24%). The amount of calcium (9%) was lower in layer 2 (Fig. 5e), while carbon (66%) was higher.

Mineralogical analysis showed that, in all cases (including non-infested shells), calcite was the calcium carbonate polymorph present.

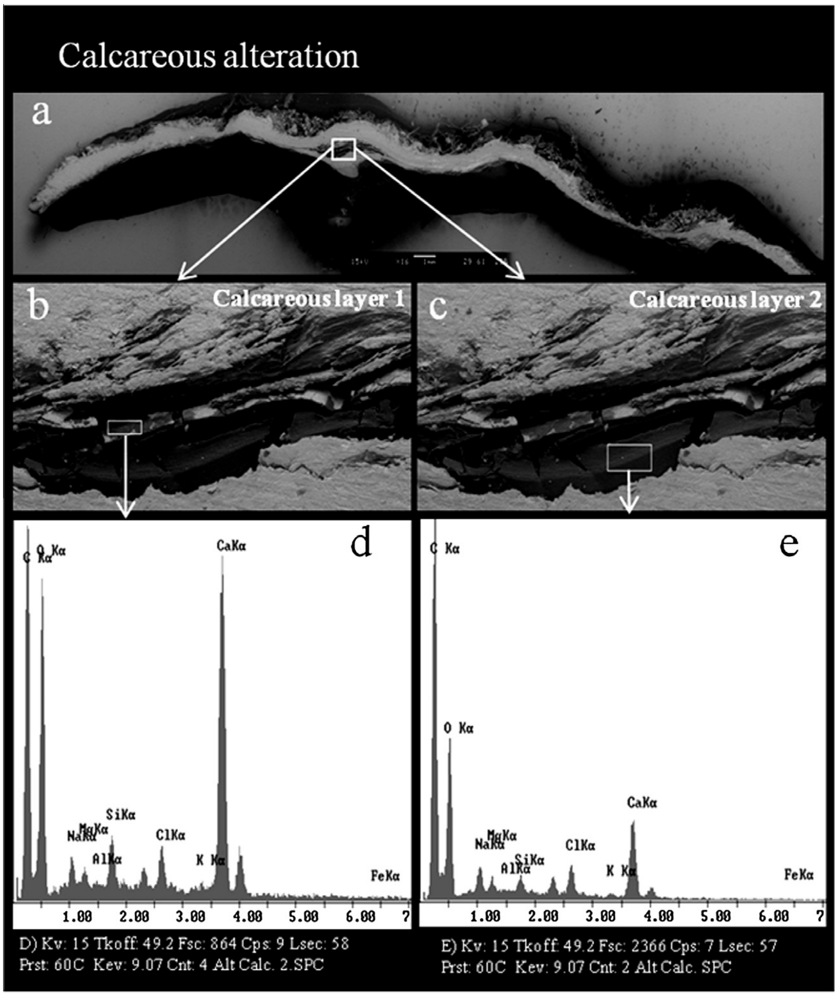

Fig. 5. Calcareous alterations. a: Transversal section through a calcareous alteration. (b–c) Detail showing calcium layers and spots selected for elementary analysis (rectangular boxes) in calcium layers 1 (b) and 2 (c). (d–e) elementary analysis (EDAX) of calcium layers 1 (d) and 2 (e). In (d) and (e) the *x*-axis corresponds to energy in keV (kilo-elektronnvolts) and the *y*-axis to counts per second (cps).

3.3. Geometric morphometrics

Growth was allometric. Multivariate regression of shape on centroid size was statistically highly significant (permutation test with 10,000 random permutations, $P < 0.0001$), and accounted for 5.6% of the total amount of shape variation. In order to describe the size-unrelated shell shape features we performed a principal component analysis (PCA) of the residuals from the regression of shape on centroid size (allometric correction). The first 8 PCs explained 88% of the total variation. Specimen's overlapped widely in the shell shape space, irrespective of the type of damage (Fig. 6).

3.4. Variation of infestation in relation to geographic location (exploited beds), valve orientation (left vs. right) and size

Significant differences were found between four exploited beds (Table 1) in the intensity ($H = 180.98$, $P < 0.05$) and prevalence of infestation. Left shells were found significantly more infested than right shells (X^2: 7.81, $P < 0.05$). Larger Tehuelche scallops were more frequently infested than smaller ones (Fig. 7).

4. Discussion

Polydorid infestation of Tehuelche scallops from San José Gulf was reported by Orensanz (1986), Ciocco (1990) and Orensanz et al. (1991), who apparently misidentified *P. rickettsi* as *P. websteri* Hartman. A recent study (Diez et al., 2011) could not confirm the presence of the latter in the study area. According to our results, infestation by *P. rickettsi* is the primary cause of shell damage affecting product quality in this scallop fishery.

We identified three types of damage: spots, calcareous alterations and mud blisters. These were characterized in terms of appearance and structure, elementary composition and shell morphology. Compared to calcareous alterations, mud blisters were richer in organic matter (as revealed by the high Carbon content) and silicon, indicative of trapped fine sediment. Modest change

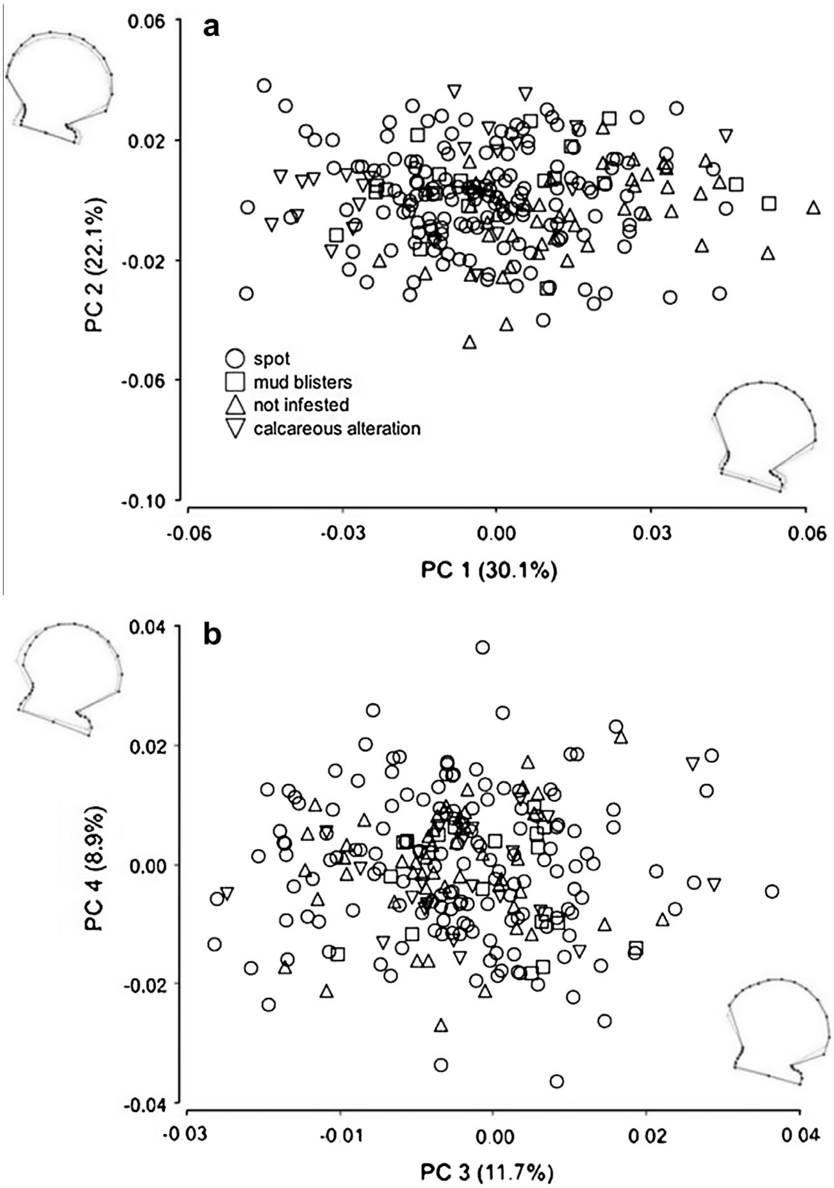

Fig. 6. Plot of the principal components (PC) of shell shape based on Procrustes distances. Wireframe diagrams show the shape change associated with variation along the first PC axis, from the overall mean shape (light grey wireframe) to the positive extreme shape (black wireframe). Shape changes have been exaggerated (scale factor = 0.1) for better visualization. (A) PC1 vs. PC2. (B) PC3 vs. PC4. Percentages of explained variance for each axis are in parentheses.

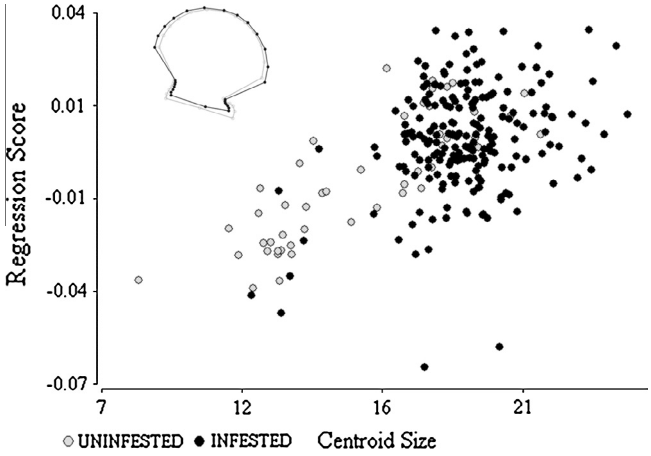

Fig. 7. Shape scores as a function of size (centroid size was used as a proxy of size, calculated as the square root of the sum of the squared deviations of landmarks from the centroid) illustrating the relation between scallop allometric growth and worm infestation. (●) Infested scallops, (○) uninfested scallops. The wireframe diagram in the left corner shows average shell shape; change from grey to black outline indicates the predicted landmark shift corresponding to an increase of centroid size by a 15 scale factor.

in shell morphology in relation to infestation has been documented also in other scallop-polydorid interactions (e.g. Silina, 2006; Silina and Zhukova, 2009). Silina and Zhukova (2008) showed that in *Mizuhopecten yessoensis* infected with *Polydora brevipalpa* the degree of infection (as captured by the bioerosion of the scallop's shell) is inversely correlated with shell height (standardized by age), tissue mass, and internal volume and growth rate, which seems to result from reduced food consumption. In our case morphometric geometrics showed that infestation-related change in shape is small, too subtle to be incorporated in monitoring routines. Studies conducted during the 1970s (at the onset of the fishery), when scallops abundance and longevity were higher (Orensanz, 1986), sugest that the effects of *Polydora* infection were more significant (Orensanz et al., 1991). It is likely that the modest effect of infection on shell shape detected in our study is a result from released density-dependence.

Infestation intensity was higher on the left valve, upwards oriented in the living animal. This phenomenon, reported before for the Tehuelche scallop (Ciocco, 1990) as well as for other species (e.g. Silina and Zhukova, 2009, for *Patinopecten yessoensis*), could be due to the fact that the left valve is more exposed to larvae advected by near-bottom flows. The types of shell damage reported here were not registered in other molluscs exploited in the same area, despite being infested by this polydorid species as well (Cremonte et al. 2005; Diez et al., 2011, and unpublished data). The type of damage caused by polydorids seems to depend more on the host than on the worm species involved.

Damage types and infestation intensity define a sequence of stages in polydorid infestation. Spots correspond to the initial stage, while calcareous alterations and mud blisters are different forms of more advanced infestation. A relation between polydorid infestation and the age of scallop hosts has been well documented in other studies (e.g. Silina, 2006; Silina and Zhukova, 2009), including the Tehuelche scallop from San José Gulf (Ciocco, 1990). Most of the shells observed by us presented spots, which are often found along the growing rings. Orensanz (1986) suggested that polydorid infestation intensifies following periods of slow scallop growth, when growth rings are marked. A likely reason is that *Polydora* larvae settle preferentially in the micro-crevices which form the growth rings. Density-dependent depression of growth rate in individual scallops belonging to an exceptionally strong scallop year-class resulted in unusually well-marked growth rings, which in turn led to high infestation intensity along the shell edge, and marginal shell erosion. It has been claimed (Tinoco-Orta and Cáceres-Martínez, 2003; Silina, 2006) that settling polydorid larvae show preference for pre-infested host molluscs, which would enhance the type of feedback postulated for the Tehuelche scallop stock. As scallops grow, marginal infestation results in a characteristic pattern of damage intensity along growth rings (Orensanz et al., 1991). Consistently, in our study calcareous alterations were more frequently observed between the adductor muscle and the growing edge of the shell. Zottoli and Carriker (1974) also found that shell damage caused by polydorid infestation is most frequent along growth rings, which they attributed to the accumulation of organic matter in shell interstices. We found that mud blisters were located close to the adductor muscle, where the oldest infestation episodes are to be expected, and inhabited by about 4–5 worms.

Intensity of scallop infestation by polydorids varied significantly among harvested beds, Punta Conos being the most affected in prevalence and intensity. These differences could result from ecological characteristics of the sites or from the age structure and density of the host populations (Ciocco, 1990).

In recent years there were growing concern among fishers, processors, managers and consumers of scallops from San José Gulf regarding product quality problems caused by polydorid infestation, mostly of the half-shell product. Similar problems have been reported for commercial bivalve harvests, worldwide. In the case of aquaculture production research has focused on methods to treat infected bivalves (e.g. Dunphy et al., 2005), but this is not an alternative to improve the quality of the product from exploited natural stocks. The dynamics of the scallop stocks from San José Gulf are reasonably well understood and regularly monitored (Orensanz, 1986; Ciocco et al., 2005; Amoroso, 2012). Given that infestation intensity varies with scallop age and bed location, the timing and allocation of the harvests could be possibly fine-tuned to improve product quality, e.g. by increasing harvest rate (and consequently reducing the average age in the catch) in areas of high scallop density and/or intensity of infestation. The results of this study provide a basis for objectively diagnose and monitor infestation severity of harvested scallop stocks.

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