

Available online at www.sciencedirect.com



Aquaculture 249 (2005) 23-33

Aquaculture

www.elsevier.com/locate/aqua-online

# A histopathological survey of some commercially exploited bivalve molluscs in northern Patagonia, Argentina

Florencia Cremonte<sup>a,\*</sup>, Antonio Figueras<sup>b</sup>, Eugene M. Burreson<sup>c</sup>

<sup>a</sup>Centro Nacional Patagónico (CONICET), Boulevard Brown s/no., 9120 Puerto Madryn, Chubut, Argentina <sup>b</sup>Instituto de Investigaciones Marinas (CSCIC), Eduardo Cabello 6, E-36208 Vigo, Spain <sup>c</sup>Virginia Institute of Marine Science, College of William and Mary, PO Box 1346, Gloucester Point, Virginia 23062, USA

Received 4 August 2004; received in revised form 4 January 2005; accepted 23 January 2005

#### Abstract

A survey of pathological conditions affecting natural beds of the most economically important bivalve species was performed for first time in the Argentinean Sea. The bivalves studied were *Aequipecten tehuelchus* (Pectinidae), *Mytilus edulis*, *Aulacomya atra* (Mytilidae), *Protothaca* (=*Venus*) *antiqua* (Veneridae), *Ostrea puelchana* (Ostreidae) and *Pododesmus rudis* (Anomiidae), all from San José gulf (42°20'S, 64°20'W), in northern Patagonia, Southwest Atlantic Ocean. Samples of about 30 adult individuals from each molluscan species were collected and processed by standard histological techniques. *Rickettsia*-like organisms were the commonest parasites found, followed by ciliates, *Nematopsis*-like spores, sporocysts and metacercariae of Trematoda, larval Cestoda, Turbellaria and two crustaceans (an isopod and a pea crab). A protozoan of uncertain taxonomic affinities, similar to that formerly reported as *Perkinsus karlssoni* in *Argopecten irradians*, was found in *A. tehuelchus*. DNA analysis demonstrated that it does not belong to a *Perkinsus* species. The commercially exploited bivalves here studied seem to be devoid of serious pathogens. None of the parasites appear to be a problem to future farming due either to low infestation levels or low pathological effect. Moreover, none of these parasites is OIE notifiable, although to confirm these initial results it is necessary to perform a more exhaustive survey in several beds from different seasons. In order to manage Argentinean stocks, a continuous, organized survey, satisfying international standards, is strongly recommended. © 2005 Elsevier B.V. All rights reserved.

Keywords: Exploited bivalve molluscs; Pathology; Parasites; Argentina; Southwestern Atlantic Ocean

#### 1. Introduction

<sup>\*</sup> Corresponding author. Fax: +54 2965 451543.

*E-mail addresses:* fcremont@cenpat.edu.ar (F. Cremonte), pato1@iim.csic.es (A. Figueras), gene@vims.edu (E.M. Burreson).

To date, knowledge about molluscan bivalves diseases from South America is very scarce. By contrast, in other parts of the world, the parasitofauna of commercially exploited bivalves is well known and is increasing as aquaculture increases (Lauckner,

<sup>0044-8486/\$ -</sup> see front matter  ${\rm \odot}$  2005 Elsevier B.V. All rights reserved. doi:10.1016/j.aquaculture.2005.01.024

1983; Bower et al., 1994). In Argentina, aquaculture is poorly developed and limited, in part, because of the lack of information about the health status of the marine bivalves (Pascual and Zampatti, 1998). Most knowledge available about bivalve pathology in Argentina concerns metazoans (Ciocco, 1990; Cremonte, 1999; Cremonte et al., 2001; Ituarte et al., 2001; Cremonte and Ituarte, 2003). The only parasites reported are an unidentified ciliate, Rickettsia-like colonies, and an intracellular organism similar to Bonamia from the puelche oyster, Ostrea puelchana (Ostreidae) (Kroeck and Montes, 2005). The presence of Bonamia-which causes an OIE (Office International des Epizooties) notifiable aquatic diseasecaused the failure of O. puelchana culture because of high mortalities and difficulties exporting fresh product (Kroeck and Montes, 2005).

With the exception of the native oyster *O. puelchana*, mass mortalities have not been observed in commercially exploited bivalves on the Patagonian coast. However, base-line information about which parasites are already present, where they are distributed, and their pathological developments. Knowledge of parasite status prior to possible mass mortalities can facilitate the interpretation of the cause of the disease outbreak and may allow subsequent control, and limit spread of the disease (Fisher and Figueras, 1987).

This study was performed to determine the presence of parasites and symbionts and the histopathological condition of the following commercially important bivalve species in San José Gulf, Argentina: *Aequipecten tehuelchus* (Pectinidae), *Mytilus edulis*, *Aulacomya atra* (Mytilidae), *Protothaca* (=Venus) *antiqua* (Veneridae), *O. puelchana* (Ostreidae) and *Pododesmus rudis* (Anomiidae).

## 2. Materials and methods

#### 2.1. Study area fishery activities

San José Gulf ( $42^{\circ}20'$ S,  $64^{\circ}20'$ W) is located in the north of Península Valdés, Chubut Province, in northern Patagonia, Argentina. The gulf has an area of 814 km<sup>2</sup> and a mean depth of about 30 m. The water temperature is 9–10 °C in winter and 16–17 °C in summer; the mean salinity is 33.9‰. Peninsula Valdés is a Protected Provincial Area and Natural Human Patrimony (UNESCO) where some human activities (e.g., tourism and artisanal fisheries) are carried out. The main bivalve exploited in San José gulf is the tehuelche scallop, *A. tehuelchus*. Approximately 20 small boats catch about 500–600 ton/year. Other molluscs commercially exploited are the common mussel *M. edulis* and the ribbed mussel *A. atra*, the venerid clam *P.* (=*Venus*) *antiqua* and the oyster *O. puelchana*. *P. rudis*, called false oyster, is a by-catch of *O. puelchana* and domestically consumed (Lasta et al., 1998). The bivalves are sold by the fishermen to the fisheries companies or directly to the internal market of Puerto Madryn city. However, high international prices suggest that the exportation of fresh bivalves is a potential option.

#### 2.2. Bivalve characteristics

The tehuelche scallop, A. tehuelchus (d'Orbigny, 1846) is distributed from Rio de Janeiro (23°S, Brazil) to the south of Chubut Province (45°S, Argentina) (Scarabino, 1977). After 25 years of regular activity, the commercial diving fishery of San José Gulf was closed during 1996-1998 due to the collapse of the stock (Ciocco and Orensanz, 1997). The fishery was reopened partially in 1999 and normally since 2000 (Ciocco et al., 2004). The scallop is sold within Argentina, with or without valves, during the regular fishery season (autumn-winter). During the red tide closed season (summer), only the adductor muscle is processed in local markets and occasionally exported, mainly to the USA). Some culture attempts were carried out with good results (Pascual and Zampatti, 1998). The mussel, M. edulis (d'Orbingy, 1842), is found from southern Brazil to the Magellan Strait (Scarabino, 1977). It constitutes the main volume of shellfishes harvested in the Argentinean Sea (Ciocco et al., 1998) and its culture proved to be an commercially viable activity in northern Patagonia (Pascual and Zampatti, 1998). The ribbed mussel, A. atra (Molina, 1782) is distributed from Buenos Aires Province in Argentina to Peru in the Pacific Ocean (Scarabino, 1977). The flesh is considered of lower quality than that of *M. edulis*, and it is sold on the internal market, fresh, frozen, and for the canning industry. This mytilid could represent the second one in importance in San José Gulf (Ciocco, 1995) and is cultured in Chile (Zaixso, 1980). The venerid clam, P. (=Venus) antiqua (King and Broderip, 1832) is found from southern Brazil to Peru in the Pacific Ocean (Scarabino, 1977) and is the third most important species fished in the San José Gulf (Ciocco, 1995). The puelche oyster, O. puelchana d'Orbigny, 1842 is distributed from Rio de Janeiro to San José Gulf; in San Matías Gulf where this species forms economically important beds (Scarabino, 1977). The main interest in the puelche oyster is its culture, since its flesh is similar to the European oyster, Ostrea edulis. The fishery has been closed in San Matías Gulf for the last several years (Lasta et al., 1998), but its culture, including seed production, was successful until the appearance of Bonamia (Kroeck and Montes, 2005). P. rudis (Broderip, 1834) is distributed from Antillas Sea to San José Gulf (Carcelles, 1944). It is exploited only domestically, but constitutes a potential resource for canned industry (Zaixso, 1980).

### 2.3. Samples

Samples of the following market-sized bivalves were collected: *A. tehuelchus* (*N*=31, June 2002), *M. edulis* (*N*=30, January 2002), *A. atra* (*N*=28, January

2002), *P.* (=*Venus*) antiqua (N=30, January 2002), *O.* puelchana (N=22, July 2002) and *P. rudis* (N=6, July 2002). They were transported to the laboratory and maintained in aquaria with aerated seawater at 10–13 °C for about 48 h, until processing.

#### 2.4. Gross morphology and condition index

The maximum length of each individual was measured with callipers. Shells and soft parts of all specimens were observed individually for the presence of epibionts, symbionts, and calcareous abnormalities. The soft parts were carefully removed from their shells, and separately weighted to calculate the condition index (soft part weight/shell weight). Before weighting, epibionts were removed and the shells dried. Soft parts were fixed in Davidson's solution (Shaw and Battle, 1957) for 24 h and stored in 70% alcohol.

#### 2.5. Histological assay

Oblique transverse sections, approximately 5 mm thick, were taken from each specimen to including

Table 1

Characteristics of the bivalve samples (shell size in mm; condition index and sex ratio given as mean value with range in parentheses) and percentage of epibionts and shell abnormalities of some exploited bivalves in San José Gulf, northern Patagonia, Argentina

Bivalve species	Ν	Shell size	Condition index	Sex ratio (M:F)	Epibionts	Shell abnormalities	
Aequipecten tehuelchus	31	69 (56-89)	0.84 (0.59–1.13)	monoicus	Spirorbis sp. (Polychaeta) (42%), oyster seed (3.22%)	Mud blisters on the inner shell surface (6.5%) (Fig. 1	
Mytilus edulis	30	64 (55-79)	0.92 (0.62-1.29)	54:46	none	none	
Aulacomya atra	28	122 (110–138)	0.45 (0.23–0.64)	48:52	Corallina sp. (Alga) (100%), Crepidula sp. (Gastropoda) (3.57%), O. puelchana (3.57%), Litophaga patagonica (Bivalvia: Mytilidae) (3.57%), Spirorbis sp. (Polychaeta) (3.57%)	Pearls in the mantle (25%) (Fig. 2) and calcareous blisters on the inner shell surface (7.14%)	
Protothaca antiqua	30	43 (34–97)	0.32 (0.20-0.69)	41:59	none	Calcarean ridges below the umbo, and/or pits, and/or brown spots in the center part of the inner shell surface (100%) (Fig. 3)	
Ostrea puelchana	22	93 (57–111)	0.15 (0.08–0.35)	monoicus	<i>Spirorbis</i> sp. (0.36%), Alga (0.18%), <i>O. puelchana</i> (0.18%), mussels (0.14%), Briozoa (0.09%), <i>Cliona</i> sp. (Porifera) (0.09%), <i>L. patagonica</i> (0.05%)	none	
Pododesmus rudis	6	53 (35–66)	0.28 (0.20-0.32)	43:57	Briozoa (0.43%), <i>Spirorbis</i> sp. (0.29%), <i>Balanus</i> sp. (Crustacea: Cirripedea) (0.29%)	none	

mantle, gills, gonad, digestive gland, nephridia and foot. Tissue samples were embedded in Paraplast and 5  $\mu$ m sections were stained with haematoxylin and eosin. Histological sections were examined under a light microscopy for presence of parasites and pathological alterations.

Histological sections of parasitized clams were deposited at Museo de La Plata, La Plata, Argentina in the Protozoological Collection (MLP Nos. 12, 18–21) and Helminthological Collection (No. 5351), and some valves of the examined clams in the Malacological Collection (MLP Nos. 7519–7523) of the same institution.

#### 2.6. In situ hybridization assay

A representative tissue section of the scallop A. *tehuelchus* infected with a protist resembling what

was originally described as *Perkinsus karlssoni* by McGladdery et al. (1991) was evaluated at the Virginia Institute of Marine Science by in situ hybridization with a *Perkinsus* genus-specific DNA probe (Elston et al., 2004). Assays were performed as previously described (Elston et al., 2004).

# 3. Results

A summary of the main characteristics of the bivalve samples, the percentage of epibionts and shell abnormalities, and the results of the histological examinations are presented in Tables 1 and 2.

The shell abnormalities found (Table 1) include the mud blisters observed in *A. tehuelchus* (Fig. 1), which are probably caused by the shell-boring *Polydora* 

Table 2

Prevalences (P) and intensities (I) of the different parasites and symbionts in some exploited bivalves from San José Gulf northern Patagonia, Argentina

Bivalve species	Parasite or symbiont	Organ or tissue parasitized	P (%)	Range I	Mean I
Aequipecten	Rickettsia-like	Digestive tubules epithelium	16	1-9	4
tehuelchus	Trichodina sp. (Protozoa: Ciliata)	Mainly in gills, also in nephridia, labial palps and mantle cavity (Fig. 6)	100	1–163	48
	Ciliate sp. 1	Gills	3	-	3
	Ciliate sp. 2	Gills	3	-	1
	Nematopsis-like	Connective tissue, mainly in gonad but also in	100	1-98	21
	(Protozoa: Apicomplexa)	digestive gland, gills, and labial palps (Fig. 7)			
	Unidentified protozoa <sup>a</sup>	Mainly in connective tissue of intestine, also in intestine epithelia (Figs. 4 and 5)	16.13	_	_
	Larval Cestoda (Tetraphyilidea)	Intestine lumen (Fig. 8)	3	-	1
Mytilus edulis	Basophilic colonies of bacteriae	Gills connective tissue (Fig. 9)	10	3-80	44.33
	Rickettsia-like	Intestine epithelium	16.67	1-4	2.40
	Ciliate sp. 3	Gills, mantle cavity and intestine lumen (Fig. 10)	76.67	1 - 84	13.48
Aulacomya atra	Rickettsia-like	Gills connective tissue of the base of gills (Fig. 11)	4	-	1
	Ciliate sp. 4	Gills (Fig. 12)	96	1-13	5.4
	<i>Edotea magellanica</i> (Crustacea: Idoteidae)	Mantle cavity	3.45	_	2
	Tumidotheres maculatus (Crustacea: Pinnotheridae)	Mantle cavity	3.45	_	1
Protothaca antiqua	Trematode sporocysts	Mainly in gonad, also in mantle, gills and nephridia (Fig. 13)	6.67	_	-
	Larval Cestoda (Tetraphyilidea)	Gonad connective tissue (Figs. 14 and 15)	3.33	_	1
	Gymnophallidae metacercariae	Between mantle and shell	_	_	_
	(Trematoda) <sup>b</sup>	(general extrapallial space)			
Ostrea puelchana	Rickettsia-like	Digestive epithelium and gonad (Fig. 16)	31.82	2-16	5.71
Pododesmus rudis	Nematopsis-like	Connective tissue, mainly in labial palps,	33.33	11-12	11.5
	(Protozoa: Apicomplexa)	also in gonad and digestive gland (Fig. 17)			
	Turbellaria	Intestine lumen (Fig. 18)	16.67	_	1

<sup>a</sup> Very similar in morphology to that formerly reported as *P. karlssoni* by McGladdery et al. (1991) in the bay scallop.

<sup>b</sup> Most of these parasites were lost during the fixing process.

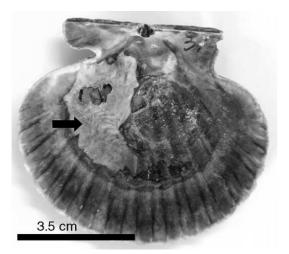


Fig. 1. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Mud blister (arrow) on the inner shell surface of *Aequipecten tehuelchus*.

*websteri*, the pearls in the mantle of *A. atra* (Fig. 2) from unknown etiology, and the pits and brown spots found on the inner shell surface of *P. antiqua* (Fig. 3). The calcarean abnormalities present in 100% of the specimens of *P. antiqua* were most probably caused by the presence of gymnophallid metacercariae (Digenea) (Table 1 and 2). These metacercariae were

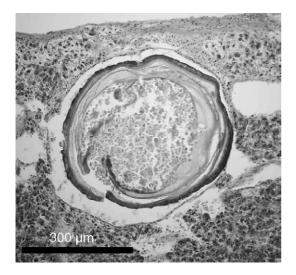


Fig. 2. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Decalcified pearl embedded in the connective tissue of the mantle of *Aulacomya atra*.

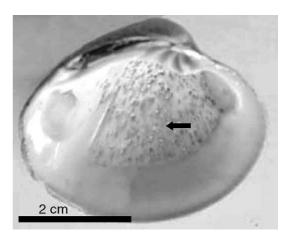


Fig. 3. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Pits (arrow) on the inner shell surface of *Protothaca antiqua*.

observed in the extrapallial space (between the mantle and shell) during sampling, but most of them were lost when the valves were taken off for fixation.

Each of the six bivalves species studied were parasitized by at least one parasite species. The commonest parasites or symbionts found in the histopathological samples were *Rickettsia*-like organisms, which were present in four of the six bivalve species studied, and ciliates which were the second more abundant parasite (Table 2).

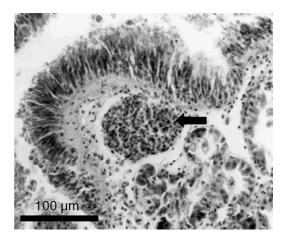


Fig. 4. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). General view (arrow) of the hemocyte encapsulation process caused by an unidentified protozoan in the connective tissue subjacent to intestine epithelial of *A. tehuelchus*.

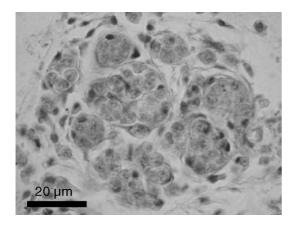


Fig. 5. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Detail of the unidentified protozoan in *A. tehuelchus*, note hemocyte encapsulation.

The pathogens of *A. tehuelchus* were a *Rickettsia*-like organism, three taxa of ciliates, a protozoan similar in morphology to *Nematopsis* sp. (Apicomplexa), and a larval tetraphyillidian cestode (Fig. 6). The parasites more frequently found in the scallop were the ciliate *Trichodina* sp. and *Nematopsis*-like, which occurred in the 100% of the individuals with high intensities, followed by colonies of *Rickettsia*-like organisms (Table 2). In addition, a protozoan of uncertain taxonomic affinities, similar to that formerly reported as *P. karlssoni* in *Argopecten irradians* in Canada, was found in 5 of 31 scallops examined (Figs. 4 and 5). The *Perkinsus* genus-specific DNA probe did not

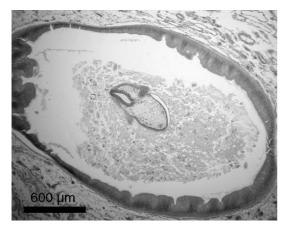


Fig. 6. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Plerocercoid larva of a tetraphyillidian cestode in the intestine lumen of *A. tehuelchus*.

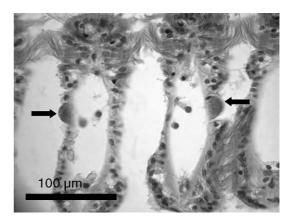


Fig. 7. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Basophilic colonies of bacteriae (arrows) in the gill connective tissue of *Mytilus edulis*.

bind to this parasite in in situ hybridization assays. *M. edulis* harboured bacteriae (Fig. 7), *Rickettsia*-like organisms, one ciliate (Fig. 8), and the ribbed mussel, *A. atra*, had *Rickettsia*-like organisms, one ciliate species (Fig. 9), and two metazoans inhabiting the mantle cavity: the isopod *Edotea magellanica* and the pea crab *Tumidotheres maculatus* (Table 2). In the venerid clam *P. antiqua*, only metazoan parasites were found. These included trematode sporocysts (Fig. 10), metacercariae of a gymnophallid trematode, and larval tetraphyillidian cestodes (Figs. 11 and 12) (Table 2). In *O. puelchana* only *Rickettsia*-like organisms were recorded. The false oyster *P. rudis* also had, in addition to *Rickettsia*-like organisms, gregarines

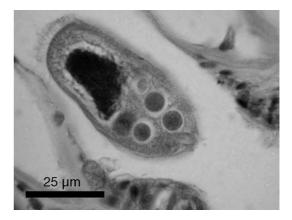


Fig. 8. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Ciliate on the gills of *Mytilus edulis*.

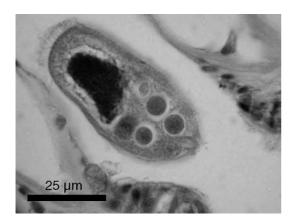


Fig. 9. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Ciliates on the gills of *A. atra*.

similar in morphology to *Nematopsis*, and turbellarian parasites (Table 2).

There was no obvious defense response against *Rickettsia*-like organisms in any of the samples examined. It was the same case for ciliates (Figs. 8 and 9), *Nematopsis*-like protists, and for metazoans inhabiting the mantle cavity and the digestive lumen (i.e., isopod and pea crab and cestode and turbellaria, respectively). On the contrary, a conspicuous host reaction consisting of hemocyte infiltration and encapsulation was observed in scallops infected by the unidentified protozoan (Figs. 4 and 5). Moreover, strong responses were observed in the specimens of *P. antiqua* parasitized by two trematode stages (spor-



Fig. 10. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Trematode sporocysts (s) in the gonad of *Protothaca antiqua*.

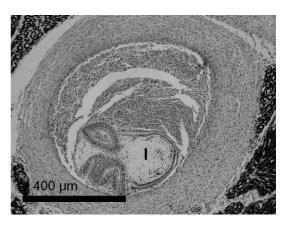


Fig. 11. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Plerocercoid larva of a tetraphyllidian cestode (l) encapsulated in the gonad connective tissue of *P. antiqua*.

ocyst and metacercaria) (Figs. 3 and 10) and by the larval Cestoda (Figs. 11 and 12). In the case of the individuals parasitized by sporocysts, the gonad was not developed even though non-parasitized individuals sampled were mature. The sporocysts contained germinal balls inside, but not developed cercariae, and were surrounded by a strong hemocyte infiltration in all the parasitized organs (i.e., mantle, gills and nephridia) (Fig. 10). In the case of the larval Cestoda, a thick capsule formed by two walls was present. The inner wall was composed of aggregated hemocytes and the outer was thicker and formed by fibroblast-

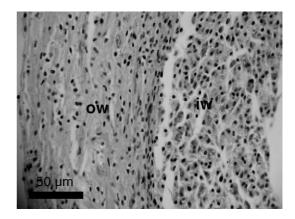


Fig. 12. Parasites and pathologies of commercial bivalves from San José Gulf, northern Patagonia, Argentina (histological sections are H&E stained). Detail of the thick capsule formed by *P. antiqua* around the larval cestode; note the outer wall (ow) composed of a compact network of fibers and the inner wall (iw) formed by hemocytes.

like cells concentrically ringed around the parasite (Figs. 11 and 12).

# 4. Discussion

This paper presents the first histopathological survey of the main commercially exploited bivalves molluscs in Argentina. From the six bivalve species studied, four were parasitized by *Rickettsia*-like organisms with prevalences varying from 4% to 67%, but in all cases with low intensities of infection. Ciliates were the second commonest parasite, found in three of the six molluscs species studied.

Except the *Rickettsia*-like organisms, which were reported in *O. puelchana* from San Matías Gulf (Kroeck and Montes, 2005), all parasites reported in the present study constitute the first record for their hosts and for South America. Of the three previously reported protozoan parasites from *O. puelchana* from San Matías Gulf (Kroeck and Montes, 2005), the unidentified ciliate, and *Bonamia*-like protist were not found in the present work. This could be attributed to the collection site and sample size; however, a more exhaustive sampling is necessary to determine if the parasites are present in San José Gulf.

*Rickettsia*-like organisms have been detected in different tissues of a variety of bivalve molluscan species. Nevertheless, heavy infections of intracellular colonies of these organisms could be very pathogenic since infections by *Rickettsia*-like organisms have been associated with mortality in some bivalves such as scallops (Gulka et al., 1983; Le Gall et al., 1988), clams (Norton et al., 1993; Villalba et al., 1999) and abalones (Friedman et al., 1997). However, in the present study the light infection intensities observed in all cases did not cause any significant damage.

Some controversy surrounds the pathogenic role of *Trichodina* and other ciliate genera. Mortalities attributed to *Trichodina* sp. have been reported in cockles and oysters (Bower et al., 1994; Boussaïd et al., 1999). Nevertheless, numerous individuals identified as *Trichodina* sp. were seen in 100% of the tehuelche scallops throughout the present study without apparent damage. The other ciliate species observed were in low intensities.

Gregarines of the genus *Nematopsis* utilize bivalves as intermediate hosts and marine arthropods

as final hosts (Lauckner, 1983). In the bivalves, they occur within the connective tissue of most organs, but are more frequently observed in the gills. Infection is usually associated with a focal, benign inflammatory response, without significant health effects (Bower et al., 1994). In the present study, the oocysts containing the sporozoites were found mainly in the gonad of *A. tehuelchus* and in the labial palps of *P. rudis*, without host reaction in either of the bivalves.

The protozoan found in the tehuelche scallop is very similar in morphology to that formerly reported as *P. karlssoni* in *A. irradians* from the Atlantic coast of Canada and United States by McGladdery et al. (1991). In addition, both parasites are from scallops and evoked very similar host reactions. The taxonomic affinities of the parasite are uncertain, but results of the in situ hybridization assay support previous conclusions (Goggin et al., 1996) that it is not a species of *Perkinsus*. It is possible that it is a thraustochytrid (Susan Bower, personal communication).

Larval cestodes have been reported from a great variety of marine invertebrates, including molluscs; their adults are parasites of elasmobranches (Cheng, 1967). The plerocercoids, which were the larval stages found in both *A. tehuelchus* and *P. antiqua* in the present study, lack taxonomically important characteristics; thus identification beyond the order level is not possible (Lauckner, 1983). The thick capsule of host origin which surrounds the cestode larva in the connective tissue of P. *antiqua* (Figs. 11 and 12) was similar to those described by Sparks and Chew (1966) and Rifkin and Cheng (1968). In *A. tehuelchus*, the larvae were found in the intestine lumen without apparent reaction (Fig. 6).

Molluscs are the main hosts for trematode larvae and they are well adapted to each other (Lauckner, 1983). As a rule, the asexually reproducing larval stages of digenetic trematodes do not become encapsulated when found in their natural intramolluscan habitats (Cheng and Rifkin, 1970). *P. antiqua* was found acting as first intermediate host for an unidentified trematode by hosting the sporocyst stage; thus, the observed hemocyte reaction is uncommon. The gonad was not developed in infected clams (Fig. 10) even though in the rest of the examined clams the gonad is clearly visible. Parasitic castration without

31

affecting host survival is widely documented (Lauckner, 1983). P. antiqua also acts as second intermediate host for the same or another trematode species, since gymnophallid metacercariae were observed inhabiting the extrapallial space. In general, the pathology caused by metacercariae of the family Gymnophallidae is variable and depends upon the parasite and the host species involved (e.g., deposition of additional shell material in the form of calcareous concretions, blisters and crests, ridges, or igloo-like structures on the inner surface of the valves, pearls, shell erosions, depletion of host body reserves, alterations of host behaviour, and general debilitation and morbidity) (Lauckner, 1983; Ituarte et al., 2001; Cremonte and Ituarte, 2003). In the present case, the shell lesions in the form of pits (Fig. 3) are probably due to parasiteinduced alterations in the chemical composition of the extrapallial fluid. Unfortunately, the mechanism involved in their formation is not well understood (Cheng and Rifkin, 1970).

Turbellaria belonging to the orders Rhabdocoela and Allocoela associate intimately with marine molluscs. Although sometimes considered as commensals and other times as parasites, they have to be regarded as harmless (Lauckner, 1983). As members of Rhabdocoela inhabit the alimentary tract of their hosts, the worms found in *P. rudis* could belong to this order.

Shell-boring polychaetes belong to the genus *Polydora* and are globally distributed (Bower et al., 1994). In Patagonian waters, the only species reported is *P. websteri* and it occurs in *A. tehuelchus* (Ciocco, 1990). The intensity of infestation of *P. websteri* in *A. tehuelchus* increases with the age of the bivalves and is inversely related with scallop size. Moreover, the intensity of infection varies significantly among scallop banks (Ciocco, 1990). It was reported as causing mud blisters on the inner shell surface, identical to those observed in the 6.5% of the scallops sampled in present study (Fig. 1).

Bivalves are not normal hosts for Isopoda (Lauckner, 1983). The isopod *E. magellanica* was previously reported as a non-obligatory commensal in natural intertidal banks of *Mytilus chilensis* from Magellan Strait; the isopod has no influence on the flesh content of the mussels (Jaramillo et al., 1981).

The pea crab *T. maculatus* was reported in the mantle cavity of *M. edulis*, *O. puelchana* and *A. te-*

*huelchus* from banks deeper than 40 m (Fenucci, 1975; Gómez-Simes, 1993) In the present study, this crab was only found in *A. atra*. Disease conditions associated with the presence of pinnotherid crabs in the mantle cavity include emaciation, reduced filtering capacity and damage to gill, palps and mantle (Bierbaum and Ferson, 1986; Tablado and López Gappa, 1995).

The commercially exploited bivalves here studied seem to be devoid of serious pathogens. None of the parasites appear to be a problem to future farming due either to low infestation levels or low pathological effects. Moreover, none of these parasites is OIE notifiable, although to confirm these initial results it is necessary to perform a more exhaustive survey in several beds from in different seasons. Due to disease problems in the production of bivalve molluscs in Europe and USA, the availability of disease-free specimens indeed represents a valuable resource. However, the risks of introducing pathogenic agents must not be ignored when considering any introduction of live bivalve mollusc in Argentina. In order to manage Argentinean stocks, a continuous, organized survey, satisfying international standards, is strongly recommended.

# Acknowledgments

We wish to thank the fishermen, Marta Piñeiro and José Ascorti, and the diving technician, Hormiga Diaz, for the bivalve samples, and Emilio Topa, Begoña Villaverde and Susana Otero for their technical assistance. We are also grateful to Dr. Susan Bower for her help with the unidentified scallop protozoan parasite, to Dr. Kimberly S. Reece, Virginia Institute of Marine Science, for conducting the in situ hybridization assay and to Dr. Nestor Ciocco for the critical review of the manuscript. The valuable suggestions of both anonymous reviewers are gratefully acknowledged. The present study was funded by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP 2714), and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (PICT 01-11902). Florencia Cremonte belongs to CONICET and is grateful to Harengus S.A. for its partial financial support.

#### References

- Bierbaum, R.M., Ferson, S., 1986. Do symbiotic pea crabs decrease growth rate in mussels? Biol. Bull. 170, 51–61.
- Boussaïd, B., Grippari, J.L., Renault, T., Tige§, G., Dorange, G., 1999. *Trichodina* sp. infestation of *Crassostrea gigas* oyster gills in Brittany, France. J. Invertebr. Pathol. 73, 339–342.
- Bower, S.M., McGladdery, S.E., Price, I.M., 1994. Synopsis of infectious diseases and parasites of commercially exploited shellfish. Annu. Rev. Fish Dis. (199 pp.).
- Carcelles, A., 1944. Catálogo de los moluscos marinos de Puerto Quequén (República Argentina). Rev. Mus. La Plata, NS, Secc. Zool. 3, 233–309.
- Cheng, T.C., 1967. Marine Molluscs as Hosts for Symbiosis with a Review of Known Parasites of Commercially Important Species. Academic Press Inc., London. 424 pp.
- Cheng, T.C., Rifkin, E., 1970. Cellular reactions in marine molluscs in response to helminth parasitism. In: Snieszko, S.F. (Ed.), A Symposium on Diseases of Fishes and Shellfishes. American Fisheries Society, pp. 443–496.
- Ciocco, N.F., 1990. Infestación de la vieyra tehuelche (*Chlamys tehuelcha* (d'Orbigny)) por *Polydora websteri* Hartman (Polychaeta: Spionidae) en el golfo San José (Chubut, Argentina): un enfoque cuantitativo. Biol. Pesq. 19, 9–18.
- Ciocco, N.F., 1995. 1—Marisquería mediante buceo en el Golfo San José: 2. Primeras experiencias privadas de cultivo de bivalvos en los golfos San José y Nuevo GEF-PNUD-WCS-FPN. Informe técnico del Plan de Manejo Integrado de la Zona Costera Patagónica, pp. 1–39.
- Ciocco, N.F., Orensanz, J.M., 1997. Collapse of the tehuelche scallop (*Aequipecten tehuelchus*) fishery from San José gulf (Argentina). 11th Pectinid Workshop, La Paz (México), Extended Abstracts Book, pp. 42–43.
- Ciocco, N.F., Lasta, M.L., Bremec, C.S., 1998. Pesquerías de bivalvos: mejillón, vieiras (tehuelche y patagónica) y otras especies. In: Bischi, E.E. (Ed.), El Mar Argentino y Sus Recursos Pesqueros. Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, pp. 143–166.
- Ciocco, N.F., De Garin, N., Diaz, M.A., Ortiz, N., Ascorti, J., Cabrera, J., Rodríguez, J., Diaz, O., Real, L., Loto, L., Bazterrica, M.C., 2004. Relevamiento de bancos de moluscos bivalvos de interés marisquero en el golfo San José. Resultados de la campaña Sanjo/04. Informe Técnico No. 16 del LAPE-MAR, laboratorio de peces y mariscos de interés comercial (CENPAT). 41 pp.
- Cremonte, F., 1999. Estudio parasitológico de bivalvos que habitan ambientes marinos y mixohalinos en Argentina. Tesis Doctoral, La Plata. 196 pp.
- Cremonte, F., Ituarte, C.F., 2003. Pathologies elicited by the gymnophallid metacercariae of *Bartolius pierrei* in the clam *Darina solenoides*. J. Mar. Biol. Assoc. U. K. 83, 311–318.
- Cremonte, F., Kroeck, M.A., Martorelli, S.R., 2001. A new monorchiid (Digenea) cercaria parasitising the purple clam *Amiantis purpurata* (Veneridae) from the Southwest Atlantic Ocean, with notes on its gonadal effect. Folia Parasitol. 48, 217–223.

- Elston, R.A., Dungan, C.F., Meyers, T.R., Reece, K.S., 2004. *Perkinsus* sp. infection risk for Manila clams, *Venerupis philippinarum* (Adams and Reeve, 1850) on the Pacific coast of North and Central America. J. Shellfish Res. 23, 101–105.
- Fenucci, J.L., 1975. Los cangrejos de la familia Pinnotheridae del litoral argentino (Crustacea, Decapoda, Brachyura). Physis, Secc. A. 34, 165–184.
- Fisher, W.S., Figueras, A.J., 1987. La Patología de los Bivalvos Marinos. Maryland Sea Grant Publication, Maryland. 85 pp.
- Friedman, C.S., Thomson, M., Chun, C., Haaker, P.L., Hedrick, R.P., 1997. Withering syndrome of the black abalone, *Haliotis cracherodii* (Leach): water temperature, food availability, and parasites as possible causes. J. Shellfish Res. 16, 403–411.
- Goggin, C.L., McGladdery, S.E., Whyte, S.K., Cawthorn, R.J., 1996. An assessment of lesions in bay scallops *Argopecten irradians* attributed to *Perkinsus karlssoni* (Protozoa, Apicomplexa). Dis. Aquat. Org. 24, 77–80.
- Gómez-Simes, E., 1993. Pinnixa brevipollex y Pinnotheres maculatus (Crustacea: Brachyura: Pinnotheridae) en el Golfo San José, Chubut, Argentina. Physis, Secc. A. 48, 25–28.
- Gulka, G., Chang, P.W., Marti, K.A., 1983. Procaryotic infection associated with mass mortality of the sea scallop *Placopecten magellanicus*. J. Fish Dis. 6, 355–364.
- Ituarte, C.F., Cremonte, F., Deferrari, G., 2001. Mantle–shell complex reactions elicited by digenean metacercariae in *Gaimardia trapesina* (Lamarck, 1819) (Bivalvia: Gaimardiidae) from the Magellan Strait. Dis. Aquat. Org. 48, 47–56.
- Jaramillo, E., Navarro, J., Winter, J., 1981. The association between Mytilus chilensis Hupe (Bivalvia, Mytilidae) and Edotea magellanica Cunningham (Isopoda, Valvifera) in southern Chile. Biol. Bull. 160, 107–113.
- Kroeck, M.A., Montes, J., 2005. Occurrence of the haemocyte parasite *Bonamia* sp. in flat oysters *Ostrea puelchana* d'Orbigny farmed in San Antonio Bay (Argentina). Dis. Aquat. Org. 63, 215–230.
- Lasta, M.L., Ciocco, N.F., Bremec, C.S., Roux, A.M., 1998. Moluscos bivalvos y gasterópodos. In: Boschi, E.E. (Ed.), El Mar Argentino y Sus Recursos Pesqueros: Tomo 2. Los Moluscos de Interés Pesquero. Cultivos y Estrategias Reproductivas de Bivalvos y Equinoideos. Instituto Nacional de Investigación y Desarrollo Pesquero, pp. 115–142.
- Lauckner, G., 1983. Diseases of Mollusca: bivalvia diseases of marine animals. In: Kinne, O. (Ed.), Introduction Bivalvia to Scaphopoda, vol. 2. Biologische Anstalt Helgoland, Hamburg, pp. 477–977.
- Le Gall, G., Chagot, D., Mialhe, E., Grizel, H., 1988. Branchial *Rickettsiales*-like infection associated with a mass mortality of sea scallop *Pecten maximus*. Dis. Aquat. Org. 4, 229–232.
- McGladdery, S.E., Cawthorn, R.J., Bradford, B.C., 1991. Perkinsus karlssoni n. sp. (Apicomplexa) in bay scallops Argopecten irradians. Dis. Aquat. Org. 10, 127–137.
- Norton, J.H., Shepherd, M.A., Abdon-Naguit, M.R., Lindsay, S., 1993. Mortalities in the giant clam *Hippopus hippopus* associated with rickettsiales-like organisms. J. Invertebr. Pathol. 62, 207–209.

- Pascual, M., Zampatti, E., 1998. Cultivo de moluscos bivalvos. In: Boschi, E.E. (Ed.), El Mar Argentino y Sus Recursos Pesqueros. Instituto Nacional de Investigación y Desarrollo Pesquero, pp. 167–193.
- Rifkin, E., Cheng, T.C., 1968. The origen, structure, and histochemical characterization of encapsulating cysts in the oyster *Crassostrea virginica* parasitised by the cestode *Tylocephalum* sp. J. Invertebr. Pathol. 10, 54–64.
- Scarabino, V., 1977. Moluscos del Golfo San Matías (Provincia de Río Negro, República Argentina). Inventario y claves para su identificación. Com. Soc. Malacol. Urug. 4, 177–285.
- Shaw, B.L., Battle, H.I., 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). Can. J. Zool. 35, 325–347.

- Sparks, A.K., Chew, K.K., 1966. Gross infestation of litleneck clam, *Venerupis staminea*, with a larval cestode (*Echeneibothrium* sp.). J. Invertebr. Pathol. 8, 413–416.
- Tablado, A., López Gappa, J., 1995. Host-parasite relationships between the mussel, *Mytilus edulis* L., and the pea crab, *Tumidotheres maculatus* (Say), in the southwestern Atlantic. J. Shellfish Res. 14, 417–423.
- Villalba, A., Carballal, M.J., López, C., Cabada, A., Corral, L., Azevedo, C., 1999. Branchial *Rickettsia*-like infection associated with clam *Venerupis rhomboides* mortality. Dis. Aquat. Org. 36, 53–60.
- Zaixso, H.E., 1980. Moluscos argentinos de interés comercial y sus posibilidades de cultivo. Contrib.-Cent. Nac. Patagón. 22, 1–15.