

# Review of Parasites and Pathologies of the Main Bivalve Species of Commercial Interest of Argentina and Uruguay, Southwestern Atlantic Coast

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#### Abstract

A systematic review of the parasites and pathologies of the main important commercially bivalve species from Argentina and Uruguay has been done. Bacteria, algae, Perkinsozoa, Haplosporidia, Ciliophora, Apicomplexa, Turbellaria, Trematoda, Cestoda and Nemertea have been found infecting natural populations of bivalve species. Disseminated neoplasia has been recorded in mussels from culture causing gonadal castration and digestive gland atrophy. In particular, mortalities from cultured oysters have been associated predominately to *Bonamia exitiosa*. In some cases, parasites have been shown to reduce the condition index of the host. This review provides information on the individual and population effects of these conditions as well as providing suggestions for future research.

**Keywords:** Protozoans; Metazoans; Mollusc diseases; Shellfish; Southweast atlantic ocean

# Introduction

Along the Argentinean coast several bivalve species have an important economic value [1]. In Buenos Aires province, the yellow clam Amarilladesma mactroides (Mesodesmatidae) was an important economic resource during the decades of 1940 and 1950. After a maximum extraction of 1078.80 ton in 1953 [2] the production declined due to overexploitation, consequently the commercial harvest was prohibited in 1958 [3] and the fisheries targeted other species as cockles [4]. By other hand, the artisanal fisheries of the north Patagonian gulfs have been operating mainly on the Tehuelche scallop Aequipecten tehuelchus (Pectinidae). Following the marked decline due to overexploitation during the 1995s, the fisheries continued operating targeting other resources [5]. These mainly included the mussel Mytilus platensis and the ribbed mussel Aulacomya atra (Mytilidae), the Puelche oyster Ostrea puelchana (Ostreidae), the "geoduck" Panopea abbreviata (Hiatellidae), the razor clam Ensis macha (Pharidae) and the venerid Leukoma antiqua and Amiantis purpurata clams. In Uruguay, fishing companies pointed out their interest in exporting the venerid clam, Pitar rostratus as a fresh product to Europe; therefore a survey about its health status was conducted to confirm that these populations were free of pathogens included in the OIE (World Organisation for Animal Health) list of notifiable diseases [6].

The aquaculture industry is now the single fastest-growing food production process in the world [7]. Although Argentina and Uruguay have a vast platform and more than 5000 km of Atlantic coast favourable for the oceanic and sustainable culture, current aquatic production volumes are not relevant when compared to the growth of aquaculture at the regional and global levels [7]. In Argentina, bivalve molluscs occupy the third place in aquaculture production. According to their importance, culture products include Japanese Pacific oyster, *Magallana* (=*Crassostrea*) *gigas* and the mussel, *M. platensis.* Their production has reached the market since the year 2000, and by 2003, 80 tonnes were commercialized, which is equivalent to 5 percent of total aquaculture production, showing at present a sustained growth [7]. Particularly, the Puelche oyster *O. puelchana*, constitutes an important resource, since its flesh is similar to the European oyster *O. edulis.* Its fishery has been closed in San Matías gulf for the last several years [8], but its culture, including seed production, was successful until the appearance of the pathogen *Bonamia exitiosa*, which has caused an OIE notifiable aquatic disease leading to mass mortalities of oysters from culture [9,10].

Epizootic diseases have been shown to cause catastrophic mortality among marine bivalves, which can severely affect fisheries and aquaculture activities around the world [11]. Some of these diseases have become a serious constraint on the development and sustainability of shellfish farming and fishing [12]. Currently, the main cause of epizootic outbreaks is thought to be the transfer of infectious agents through the transport of live shellfish [13]. According to OIE, notifiable diseases are ones of socioeconomic and/or public health importance within countries or those that are significant in the international trade of aquatic animals and their products. The OIE has identified a series of diseases that can drastically affect the survival and growth of bivalve mollusc of economic importance. The current list [14] of notifiable bivalve's diseases and the causative pathogens comprises: marteiliosis (Marteilia refringens), bonamiosis (Bonamia ostreae and B. exitiosa) and perkinsosis (Perkinsus marinus and P. olseni).

In Argentina, the health status of several bivalve species of commercial interest regarding their parasitofauna and pathologies has been well documented: the yellow clam *A. mactroides* encompassing the entire geographic range distribution (32° 12′S, 52° 11′W Brazil-40° 55′S, 62° 25′W Argentina) [15-19]; the Tehuelche scallop *A. tehuelchus*, the mussel *M. platensis*, the ribbed mussel *A. atra*, the Puelche oyster *O. puelchana*, the false oyster *Pododesmus rudis* 

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(Anomiidae), and the geoduck *P. abbreviata*, razor *E. macha* and venerid *L. antiqua* clams from natural populations of San José gulf (42° 25'S, 64° 07'W- Chubut province); *Amiantis purpurata* from San Matías gulf (41° 30'S, 64° 15'W- Río Negro province); the stout razor clam *Tagelus plebeius* (Solenocurtidae) from Mar Chiquita (37° 46'S, 57° 27'W- Buenos Aires province); *M. platensis* from culture from Beagle Channel (54° 47'S, 68° 14'W-Tierra del Fuego province) (Tables 1 and 2). Instead, little is known about the parasites and pathologies of bivalve populations in Uruguay, where only the venerid *P. rostratus* clams have been object of study (Figure 1).



**Figure 1:** Sites of bivalve collection. References: ■ Uruguay coast; Buenos Aires province (Argentina); ● San José gulf (Chubut province, Argentina); ● Beagle Channel (Tierra del Fuego province, Argentina).

The present review comprised microbial, protozoan and metazoan parasites affecting the main important commercially bivalve species in Argentina and Uruguay.

# Parasites Included in the OIE List of Notifiable Diseases

# Perkinsus olseni

Protozoan parasites of the genus *Perkinsus* are known to infect many species of marine molluscs including oysters, abalones, clams, scallops, cockles, and mussels. The systematic affinities of the genus *Perkinsus* was controversial from its beginning. Initially, it was placed in the phylum Apicomplexa [20] subsequent phylogenetic studies support its inclusion as a unique group of Alveolates [21]. There are presently seven accepted species, *P. marinus*, *P. olseni* (=*P. atlanticus*), *P. qugwadi*, *P. chesapeaki* (=*P. andrewsi*), *P. mediterraneus*, *P. honshuensis*, and *P. beihaiensis* [22].



**Figure 2:** Trophozoites (t) of *Perkinsus olseni* evoking a severe haemocytic infiltration into the surrounding tissue and an encapsulation (arrow) by *Pitar rostratus*. Scale bar=50  $\mu$ m. Histological section stained with haematoxylin and eosin.



**Figure 3.** Microcells (arrow) inside the cytoplasm of a haemocyte of *Ostrea puelchana*. Note the heavy haemocytic infiltration. Scale bar=20  $\mu$ m. Histological section stained with haematoxylin and eosin.

*Perkinsus olseni* has been reported for the first time in South America infecting natural populations of *P. rostratus* from Uruguay with a prevalence of 22% [6]. The occurrence was observed mainly in the connective tissue surrounding the intestine, as well as the mantle and gills. A severe haemocytic infiltration and an encapsulation of the trophozoites were evidenced as host defence responses (Figure 2). *Perkinsus olseni* has been detected infecting several clam and oyster

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species from Australia, New Zealand, South Korea, Japan, China, Portugal, Spain and Italy [22].

None species of *Perkinsus* was found infecting the bivalve species studied along the Patagonian coats of Argentina (*A. tehuelchus, M. platensis, A. atra, O. puelchana, P. rudis, P. abbreviata, E. macha* and *L. antiqua*) neither by histological examination nor by culture in Ray's fluid thioglycollate medium and immunoassays [23]. The absence of this parasite may be related to the colder water (<20°C), unfavourable for its development, since proliferation of all *Perkinsus* species occurred in temperatures over 20°C [24].

physiological disorders and eventually causing the death of the animal [26,27]. Three *Bonamia* species have been identified worldwide, *B. ostreae* in Europe and North America; *B. perspora* from North America and *B. exitiosa* from North and South America, Australia, New Zealand and the Mediterranean sea [10]. In Argentina, *B. exitiosa* has been blamed for mass mortalities events among *O. puelchana* culture in San Antonio bay (San Matías gulf) during 1996 [9,10]. Most of the microscopic signs of *Bonamia* spp. infection are the haemocytic infiltration in all the connective tissue (systemic infiltration) (Figure 3), the dissociated appearance of the connective tissue containing haemocytes with picnotic nuclei and the intracellular location of the microcells (1-8 parasites in one haemocyte) [9].

# Bonamia exitiosa

Species of *Bonamia* are haplosporidian protozoan parasites [25] which infect haemocytes of several oyster species, inducing

| Parasite                   | Host   |  |
|----------------------------|--|--|
| Prokaryotic microorganisms | Aequipecten tehuelchus, Mytilus platensis, Aulacomya atra, Ostrea puelchana, Pododesmus rudis [23,33,45]; Panopea abbreviata [46];<br>Ensis macha [34]; Pitar rostratus [6]. |  |
| Coccomyxa parasitica       | M. platensis [40,41]; P. abbreviata, E. macha [42].  |  |
| Trichodina-like ciliate    | Amarilladesma mactroides [2,15,19]; A. tehuelchus [23,33].   |  |
| Other ciliates             | A. tehuelchus, M. platensis, A. atra [33,45]; P. abbreviata [45]; E. macha [34].   |  |
| Nematopsis-like            | A. tehuelchus, P. rudis [23,33]; P. rostratus [6].   |  |
| Porospora-like             | P. abbreviata [46].  |  |
| Coccidean                  | A. mactroides [15,16]; P. rudis [23]; E. macha [34]; P. rostratus [6].   |  |

Table 1: List of microbial and protozoan parasites reported in the main important commercially bivalve species from Argentina and Uruguay.

| Parasite  | Host (reference)   |
|---|--|
| Paravortex nicolli (Turbellaria, Graffillidae)    | Mytilus platensis [52].  |
| Paravortex mesodesma (Graffillidae)               | Amarilladesma mactroides [19,51].                              |
| Paravortex panopea (Graffillidae)                 | Panopea abbreviata, Ensis macha [53].                          |
| Paravortex sp. (Graffillidae)                     | Pododesmus rudis [33].   |
| Trematode sporocyst (Gymnophallidae)              | Leukoma antiqua [33].  |
| Trematode sporocyst (Fellodistomidae)             | Tagelus plebeius [61].   |
| Trematode sporocyst (Monorchiidae)                | Amiantis purpurata [54,55].                                    |
| Trematode sporocyst (Aporocotylidae)              | A. purpurata [55]; E. macha [34].                              |
| Trematode sporocyst (Bucephalidae)                | M. platensis, Aulacomya atra [59].                             |
| Trematode unidentified metacercaria               | M. platensis [45]; Pitar rostratus [6].                        |
| Trematode metacercaria (Renicolidae)              | M. platensis, A. atra [59].                                    |
| Trematode sporocyst/metacercaria (Gymnophallidae) | M. platensis, A. atra [60]; L. antiqua [33]; T. plebeius [61]. |
| Larval cestode (Tetraphyllidea)                   | Aequipecten tehuelchus, L. antiqua [33].                       |
| Larval nematode (Spirurinae)                      | T. plebeius [61].  |

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| Malacobdella arrokeana (Malacobdellidae) | P. abbreviata [68]. |
|--|---------------------|

Table 2: List of metazoan parasites reported in the main important commercially bivalve species from Argentina and Uruguay.

Infection radiated from culture area, since 1996, through the oyster populations of San Matías gulf, with highest annual values in austral autumn (from March to May) and lower levels in austral spring/early summer (from September to November) [28]. Bonamia exitiosa has also been recorded infecting several oyster species from New Zealand, Australia, northeast of Spain, Chile and North Carolina in USA [29].

# Other parasites and pathologies

Several microbial, protozoan parasites (Table 1), metazoans (Table 2) and pathologies not included in the OIE list have been detected causing both a negative effect in the condition of their hosts or evoking inflammatory/encapsulation responses as well as host castration.



**Figure 4:** Intracellular prokaryotic inclusions (arrow) infecting epithelial cells of the digestive tubule of *Pododesmus rudis*. Some colonies caused the lysis of the infected cells and were released to the lumen of the tubule (\*). Scale bar=100  $\mu$ m. Histological section stained with haematoxylin and eosin.



**Figure 5:** Prokaryote microorganisms encapsulated in the gills of *Panopea abbreviata* by an eosinophilic fibrous cover (arrow). Scale bar=50  $\mu$ m. Histological section stained with haematoxylin and eosin.

#### Prokaryotic microorganisms

Rickettsia-like (RLO's) and Chlamydia-like organisms have been reported in several marine bivalve molluscan [30]; although in most cases of mortality, the detected prokaryotes have been associated with RLO's. Rickettsiae and Chlamydiae species are commonly distinguished by using transmission electron microscopy, taking into account their morphology and division system. Few intracellular prokaryotic organisms infecting aquatic animals have been sufficiently characterized for precise taxonomic placement [31]. In the case of marine invertebrates, the principal problem in the study of intracellular obligate parasites is the absence of cell lines. Therefore, without ultrastructural data it is only possible to name these inclusions as 'prokaryotic microorganisms' [32]. Prokaryotic microorganisms were found infecting both epithelial cells of digestive tubule (Figure 4) or intestine in almost of all studied bivalve species (A. mactroides, A. tehuelchus, M. platensis, O. puelchana, P. rudis, P. abbreviata, E. macha) on the Argentinean coasts [23,33,34]. These occurred as small, rounded intracellular basophilic colonies, evoking the lysis of infected cells of the digestive tubule. The highest values of prevalence (41%) and intensity of infection (95 colonies per histological section) were recorded in *P. rudis*. Prokaryote microorganisms were detected in gills by light microscopy from histological sections in several bivalve species. They are basophilic and Gram-negative, but their intracellular location is not clearly defined by light microscopy; they are coated by an eosinophilic fibrous cover, which is interpreted as an encapsulation host response [35]. Despite their intracellularity being unclear, these "cysts" were reported as rickettsial-like organisms in the gills of M. edulis and Tridacna crocea clam [35,36]. On the Argentinean coasts, these were found as encapsulated large inclusions in the gills of M.

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*platensis, A. atra* and *P. abbreviata* (Figure 5), indicating a host defence mechanism by the hosts. The highest values of prevalence (23%) and intensity of infection (47 cysts per histological section) were recorded in *P. abbreviata*. Infections by prokaryotic microorganisms have been associated with mortalities in bivalve populations worldwide [37].

# Coccomyxa parasitica

The intracellular green alga C. parasitica was first described infecting the giant scallop *Placopecten magellanicus* (Pectinidae) from Newfoundland, Canada [38]. Then it was later recorded in M. platensis from the North Sea and Malvinas (Falkland) Islands [39] and from San José gulf (Argentina) [40,41]. Recently, C. parasitica was identified by transmission electron microscope and molecular analysis for the first time in the geoduck, P. abbreviata and in the razor clam, E. macha in Argentina [42]. The infection occurred exclusively in the distal section of the siphonal tissues evoking a severe inflammation response of the host evidenced by both haemocytic infiltration and encapsulation (Figure 6). Up to six algal cells ensheated in a common membrane were observed in a single haemocyte (Figure 7). The authors found lower condition index values in infected geoducks than in uninfected ones, associated to energetic cost required in the defence mechanisms. The prevalence in geoducks was of 51% whilst only 1 specimen was infected out 480 razor clams examined, suggesting its role as an occasional host because the siphons of this clam species remain buried, not being exposed to the sunlight [42].



**Figure 6:** Semithin section of colonies of *Coccomxya parasitica* (arrow) infecting siphonal tissues of *Panopea abbreviata* encapsulated by haemocytes. Scale bar=20  $\mu$ m. Histological section stained with haematoxylin and eosin.



**Figure 7:** Transmission electron microscope photograph of six algal cells of *Coccomxya parasitica* sheathed in a common membrane within the haemocyte cytoplasm. Scale bar=2.5 µm. References: hc: haemocyte cytoplasm; mc: membrane of haemocyte; mcm: mother cell membrane; mf: muscular fiber; n: nucleus.

# Protozoans

# Ciliophora

**Trichodina-like:** Ciliates of the genus *Trichodina* are common inhabitants of the bivalve surface gills and infrequently of the labial palps [43]. They are usually considered harmless commensals when occurred in low intensities, although mass mortalities were associated to severe infections as the case of cockles and oysters [43,44]. In Argentina, *Trichodina*-like ciliates were found infecting *A. mactroides* and *A. tehuelchus* [33]. These protozoans occurred as disc-shaped characterized by a circlet of eosinophilic denticles, ciliary fringes and a horse-shoe shaped nucleus (Figure 8). High values of prevalence (98%) and intensity of infection (564 ciliates per histological section) were recorded in *A. tehuelchus* causing a distortion of the natural architecture of the gill filaments (Figure 9) [23].

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**Figure 8:** One specimen of *Trichodina*-like (arrow) attached to the epithelial cells of the gill of *Aequipecten tehulechus*. Scale bar=50  $\mu$ m. Histological sections stained with haematoxylin and eosin.



**Figure 9:** Several specimens of *Trichodina*-like distorting the normal architecture of the *Aequipecten tehulechus* gill filaments. Scale bar=50  $\mu$ m. Histological sections stained with haematoxylin and eosin.



**Figure 10:** Seven ciliates (\*) in the gill of *Panopea abbreviata* without any pathological effect apparent observed. Scale bar=50  $\mu$ m. Histological section stained with haematoxylin and eosin.



**Figure 11:** Six *Nematopsis*-like apicomplexan oocytes (arrow) in the connective tissue of digestive gland of *Aequipecten tehuelchus*. Scale bar=50  $\mu$ m. Histological section stained with haematoxylin and eosin.

**Other ciliates:** Unidentified ciliate species were recorded on the surface of the gill filaments of scallops, mussels and ribbed mussels. These protozoans are easily identified in histological sections by having one macronucleus and several micronucleus [33,45]. Ciliates with oval-shaped, one basophilic nucleus macronucleus, several vacuoles, a cytostome and dense ciliature were recorded in gills and often in labial palps of *E. macha*, without cause any specific host response [34]. Ciliates of oval or rounded shape, with surface densely ciliated and a large polymorphic macronucleus were reported in gills of *P. abbreviata*. The relationship to the gill epithelium seemed to be superficial and no particular host response was apparent (Figure 10). The highest values of prevalence (79%) and intensities of infection (147 ciliates per

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histological section) were recorded in the austral spring season showing a negative relationship with the shell size [46].

# Apicomplexa

**Gregarines:** Nematopsis-like gregarines include oocysts bounded by a single thickened membrane with a basophilic vermiform sporozoite inside (Figure 11), were recorded in the connective tissue of almost all the organs of *A. tehuelchus* and *P. rudis*, associated usually with a focal, haemocytic infiltration, without significant health effects [33] as well as in the epithelial cells of the intestine of *P. rostratus* [6]. The prevalence was associated to the shell length of *A. tehuelchus*, indicating that bigger scallops were more frequently infected by this gregarine species [23]. These protozoans were reported infecting several bivalve species worldwide, without apparent damage to the hosts [43].

*Porospora*-like gregarines occurred as naked sporozoites (Figure 12), mainly in the connective tissue of mantle of *P. abbreviata* [46]. The presence of this protozoan evoked an inflammatory host response as haemocytic infiltration and encapsulation. The studies reporting the pathogenicity of *Porospora* spp. in bivalves are scarce; just their presence in natural populations of the venerid clam *Callista chione* was reported [47].



**Figure 12:** Naked sporozoites (arrow) resembling *Porospora* gregarine encapsulated by haemocytes in the connective tissue of the mantle of *Panopea abbreviata*. Scale bar=50  $\mu$ m. Histological section stained with haematoxylin and eosin.

#### Coccideans

Meronts and macrogamonts stages of unidentified coccidians were reported leading to hypertrophy of the epithelial cells of the nephridal tubules causing the lysis of them and the release of the parasites into the lumen of the nephridal tubule of *A. mactroides*. This apicomplexan protozoan showed a high prevalence (64%) although low mean intensity of infection (1.64) [15,16]. Different stages of a coccidian aplicomplexan (trophozoites, large and crescent-shaped macrogamonts, and both mature and immature meronts containing merozoites) were also observed in the lumen of nephridia of *E. macha* [34]. Coccideans were associated with the presence of "brown cells" in the tissues of infected clams, although no haemocyte response elicited by the host was reported. Merogonic and gamontogonic stages including immature meronts, merozoites, trophozoites, microgamonts and macrogamonts (Figure 13) were identified infecting the nephridia epithelium of P. rudis, although less frequently in epithelial cells of both digestive tubule and intestine [23]. These infections were reported to induce light to moderate lesions, evidencing a hypertrophy of the infected nephridial cells, causing the lysis of the host cells and the release of the parasites, occupying the space of the lumen of nephridia. Although the prevalence recorded in this bivalve species was low (4%), the mean intensity reached high value (37 per histological section). Nephridia of *P. rostratus* were found obstructed by different stages of an unidentified coccidian and, in heavy infections a few macrogamonts were also seen in the connective tissue that surrounds the intestine [6]. These heavy infections are considered to cause severe renal dysfunction [48]. Furthermore, the coccidian parasite of the genus Aggregata is known for play a major role in the collapse of the Iceland scallop, Chlamys islandica [49]. In addition to causing mortality, the authors pointed out that the infections significantly impacted the gonadal development, which contributes further to the collapse of the stock in the form of lower larval recruitment.



**Figure 13:** Mature macrogamonts with central nucleus (arrow) in nephridial epithelial cells of *Pododesmus rudis*. Scale bar=50  $\mu$ m. Histological section stained with haematoxylin and eosin.

# Metazoans

**Turbelleria:** "Turbellaria" of the genus *Paravortex* parasitize mainly marine bivalves, but some are associated with fish [50]. In the Northern Hemisphere (i.e., Europe and USA) five species were described [51], while in South America three species from three distinct host species were described: *P. nicolli* from *M. platensis* from Buenos Aires province, Argentina [52], *P. mesodesma* from *A. mactroides* in northern Uruguay and in Buenos Aires province, Argentina [19,51], and *P. panopea* from *P. abbreviata* and *E. macha* [53]. Since the graffilids lack endogenous digestive enzymes, they use their host enzymes to feed on partially digested host's food, and their food reserves consist mainly on glycogen; these facts represent physiological adaptations to a parasite mode of life. This high degree of metabolic dependence may be responsible for the high specificity

observed in the species of *Paravortex* [53]. Unidentified *Paravortex* species were also reported occupying the intestine lumen of *P. rudis*, without evidence of direct physical damage [33].

**Digenea:** Digeneans typically have a dramatic effect on the health of their molluscan first intermediate host. Intramolluscan stages live within the haemocoel and occupy the gonadal space causing castration [50]. Their larvae have been reported in almost all bivalves [50]. Three different sporocysts with developing cercariae, two belonging to the family Monorchiidae and the other to the Aporocotylidae, were found in the purple clam, *A. purpurata* [54,55]. In one case, of 21 infected clams, 17 individuals had no gametes in the gonads, and the remaining 4 individuals that did produce gametes harboured just a few sporocysts with poorly developed cercariae [54]. In the same way, sporocysts of the family Aporocotylidae were reported to cause complete castration in one specimen of the razor clam *E. macha* (Figure 14) [34].



**Figure 14:** Digenean trematode of the family Aporocotylidae in *Ensis macha.* Scale bar=20  $\mu$ m. References: s: oval sporocysts, ce: cercaria, gp: penetration glands, ev:excretory vesicle, gb: germ balls.

The family Gymnophallidae Odhner, 1905 is a small and homogeneous entity of marine digeneans [56,57]. Most members use molluscs as first intermediate hosts and with rare exceptions, charadriiform and anseriform birds as definitive hosts [56,57]. Gymnophallids use bivalves as first host causing castration and mainly the same or other bivalve species as second intermediate host, by hosting the metacercarial stage; eliciting diverse reactions in the mantle and valve [50]. When the bivalve is infected by the metacercarial stage and is eating raw, the may cause a zoonotic disease. For example, Gymnophalloides seoi was described from a human case with pancreatitis; the patient got infected by eating raw Japanese oyster [58]. In Argentina, the mytilids, M. platensis and A. atra from Buenos Aires and Chubut provinces (Argentina) were found infected by Gymnophallus australis [59,60]. Likewise, the stout razor clam T. plebeius was found acting as first intermediate host for two digenetic trematode species of the families Gymnophallidae and Fellodistomidae, by hosting sporocysts (mainly in gonad and digestive gland that results in the replacement of host tissues) and as second intermediate host by housing a gymnophallid metacercaria (Figure 15). The valves of the infected stout razor clams showed abnormal calcifications and strong orange colorations [61-63]. Specimens of the

*L. antiqua* living on the intertidal beds on the Patagonian coast were found infected with an unidentified gymnophallid metacercaria, eliciting pits in the inner valve surface [33].



**Figure 15:** Histological section of dorsal part where gymnophallid metacercariae are located below the dorsal shell margin of *Tagelus plebeius*. Scale bar=200 µm. References: m=metacercaria, r=rectum.

**Cestoda:** Larval cestodes have been reported from a great variety of marine invertebrates, including molluscs; their adults are parasites of elasmobranches [64]. Plerocercoids larval were found in both *A. tehuelchus* and *L. antiqua* [33]; however due to the absence of taxonomically important characteristics, their identification beyond the order level was not possible [30]. In *A. tehuelchus*, the larvae were found in the intestine lumen without apparent reaction, while in *L. antiqua*, a thick capsule formed by an inner wall composed of aggregated haemocytes and an outer thicker and formed by fibroblast-like cells concentrically ringed around the parasite [33].

**Nemertea:** The family Malacobdellidae comprises a single genus, *Malacobdella*, with 6 species inhabiting the mantle cavity of bivalves [65,66]. They are usually considered as entocommensals, although other studies supported their parasitic life style [67]. A histopathological study of mantle tissues on the commercial geoduck, *P. abbreviata* hosting the nemertean, *Malacobdella arrokeana*, was carried out to evaluate the possible damage of host mantle tissues elicited by the nemertean attachment structure [68]. This nemertean was found in high prevalence, almost in the total of the geoducks studied (P=99.4%, n=657), varying between 0 and 191 individuals (adult and young nemerteans) per clam. This study revealed that the vacuum force generated by the nemertean sucker elicited a moderate deformation of epithelial cells and an inflammatory response affecting the connective tissue between inner and outer mantle epithelium, just below the point of attachment [68].

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**Nematoda:** Although nematodes are uncommon as bivalve parasites, few species of the orders Ascaridoidea and Spirurida could be found as larval stages without specificity for their site infection. Indeed, they frequently appear in low prevalence and intensities of infection, eliciting different kind of capsules [50]. In *T. plebeius, spirurina* larval nematodes (Figure 16) were found free or individually surrounded by a capsule, mainly in the muscular wall of visceral mass (48%), but also in labial palps (28%), siphon retractor muscles (8%), adductor muscles (8%), radial muscles of the mantle border (4%), mantle (4%) [61]. These histopathology results revealed that the degree of tissue reaction elicited by the presence of this parasite varied from no reaction to the formation of a thick capsule.



**Figure 16:** Scanning electron microscope photograph of the larval spirurine nematode in *Tagelus plebeius*, showing pseudolabia, cephalic papillae (arrow) and amphids. Scale bar=10  $\mu$ m.

#### Disseminated neoplasia

This disease consists of a proliferation of abnormal circulating cells of unknown origin (likely haemocytes), with a progressive and replaced of normal cells by the neoplastic cells (involving loss of the normal architecture of tissues and organs) that often causes the death [69]. Disseminated neoplasia in bivalve mollusc species is characterized by the presence of large (2-4 times the diameter of normal haemocytes), neoplastic circulating cells that have a hyperchromatic and often pleomorphic nucleus containing 1 or more prominent nucleoli [70], and a high nuclear to cytoplasmic volume ratio in neoplastic cells [71]. The disease can be transmitted to naive animals by experimental transplantation of haemocytes [72,73]. A viral cause has been suspected, but no infectious agent has been confirmed [72,73]. The detection of reverse transcriptase activity in neoplastic cells [74] suggested the possibility of retroelement involvement, called Steamer, which its DNA copy number per genome was found at enormously high levels in neoplastic cells of the softshell clam Mya arenaria [75]. Recently, using three types of DNA markers (sequences of Steamer integration sites, mitochondrial DNA single-nucleotide polymorphisms (SNPs) and polymorphic microsatellite alleles), the genotype of neoplastic cells was found to be different from that of normal cells of the host, whereas the neoplastic cells of clams from dispersed locations in Canada and North eastern USA all had nearly identical genotype, suggesting that disseminated neoplasia is spreading among animals in the marine environment as a clonal transmissible cell derived from a single original clam [76]. Disseminated neoplasia in

cultured *M. platensis* (Figure 17) from the Beagle Channel (Tierra del Fuego province) in Southern Argentina is the only record of this disease in marine bivalve species from Argentina and Uruguay [45]. All diseased individuals were female and in advanced stages of the disease, the gonad was represented only by a few atrophied follicles invaded by neoplastic cells (Figure 18). Moreover, atrophied digestive tubules were observed; the high prevalence reported indicates an epizootic level.



**Figure 17:** Neoplastic cells (arrow head) in the gills of *Mytilus platensis.* Scale bar=50  $\mu$ m. Histological section stained with haematoxylin and eosin.



**Figure 18:** Disseminated neoplasia affecting *Mytilus platensis* where almost complete gonadal atrophy and the presence of storage cells (arrows) are observed. Scale bar=100  $\mu$ m. Histological section stained with haematoxylin and eosin.

# **Conclusions and Future Directions**

This review has covered all the parasites and pathologies of the main commercially bivalve species from Argentina and Uruguay, considering the effect of these on both individual and population health. Based on reported morphological alterations, bonamiosis, perkinsosis and disseminated neoplasia, were the most severe diseases. Nevertheless, the rest of the parasites are not considered as severe pathogens, since none caused significant damage to their hosts and none appears to be a problem to the fishery as well as to future farming, based on either low infestation levels or low pathological effects. Paid attention should be addressed to the venerid clams and mussel's consumption, since its infection by the gymnophallid digenean parasite is considered of zoonotic importance.

The research effort of the countries of Latin America and the Caribbean focuses on detecting notifiable diseases according to the OIE [77]. This fact is partly due to attempts to consolidate with international market rules for fresh exported products and that the most scientific knowledge, as well as the diagnostic methods has been developed for the diseases that have traditionally affected countries with historical shellfish culture development, mainly Spain, France and the United States. A few cases reporting the viral pathogens associated to mass mortalities and bivalve diseases would be due to lack of tools for its study, primarily, the absence of cell lines and the expensive manipulation of transmission electron microscopy [78]. Causes and mechanisms of mass mortalities events in natural populations are very difficult to ascertain, and they are probably due to a combination of factors [79]; the causes of the mass mortalities episodes affecting the yellow clam Amarilladesma mactroides, throughout its geographic range distribution remain unknown. The future studies would be addressed to investigate the co-infection of virus (OsHV-1) and different Vibrio species, since protozoans as causative agents of the mortalities have not been detected.

Furthermore, we consider that each country should be focused on investigating the parasites and diseases that are proper or affect to their bivalve species along a regional scale by developing and using specific diagnostic methods for these diseases, instead of focusing exclusively to detect diseases that are notifiable by the developed countries.

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