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GONAD ATROPHY CAUSED BY DISSEMINATED NEOPLASIA IN *MYTILUS CHILENSIS* CULTURED IN THE BEAGLE CHANNEL, TIERRA DEL FUEGO PROVINCE, ARGENTINA

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ABSTRACT Disseminated neoplasia in cultured *Mytilus chilensis* (Mytilidae) from the Beagle Channel (Tierra del Fuego Province) in southern Argentina has been detected for the first time. The disease is characterized by the infiltration of neoplastic cells with enlarged nuclei and high nuclear-to-cytoplasmic volume ratios. All specimens with disseminated neoplasia were female and exhibited gonadal atrophy in advanced stages of the disease. The high prevalence reported (13.3%) indicates an epizootic level.

KEY WORDS: neoplasia, gonadal atrophy, mussel, *Mytilus chilensis*, Argentina, southwestern Atlantic Ocean

INTRODUCTION

Cases of disseminated neoplasia in bivalve mollusc species are characterized by the presence of large (2–4 times the diameter of normal hemocytes), neoplastic circulating cells that have a hyperchromatic and often pleomorphic nucleus containing 1 or more prominent nucleoli (Barber 2004), and a high nuclear-to-cytoplasmic volume ratio in neoplastic cells (Mix 1975). Although prevalence is usually low, levels as high as 90% have been reported. Disseminated neoplasia is progressive and can produce substantial mortality in affected populations. In some areas, epizootic prevalence in affected bivalve species has caused serious regional economic damage to aquaculture industries (Peters 1988, Elston et al. 1992, Barber, 2004).

In South America, there have been only three reports of disseminated neoplasia: in *Crassostrea rhizophorae* from Brazil in the Atlantic Ocean, and in *Ostrea chilensis* (Ostreidae) and in *Mytilus chilensis* (Mytilidae) from Chile in the Pacific Ocean (Peters 1988, Campalans et al. 1998, Rojas et al. 1999, Campalans et al. 2000). In both cases from Chile, the authors reported disseminated neoplasias primarily affecting connective tissue associated with the digestive system, causing its destruction.

M. chilensis has been grown in hanging culture at the Beagle Channel (Tierra del Fuego Province, Argentina) since 2001. Seed are collected from natural beds, and market size is reached in about 8 mo. Reproduction occurs over an extended annual period of about 10–11 mo, with individuals spawning gradually during this time (Calvo et al. 1998). No epizootic mortality events have been reported to date in mussel culture from this area.

The aim of this study is to report the first case of disseminated neoplasia and its pathology in *M. chilensis* from the Atlantic coast of South America. Other parasites found concurrently are reported as well.

MATERIALS AND METHODS

A sample of 30 market-size mussels (mean, 78 mm; range, 64–92 mm in the longest axis) was collected in September 2004 from a culture at Bahía Brown (54°52′ S, 67°31′ W), Beagle Channel (Fig. 1). The soft parts of the specimens were carefully removed from their shells and fixed in Davidson's solution (Shaw & Battle 1957) for 24 h. Oblique transverse sections, approximately 5 mm thick and including mantle, gills, gonad, digestive gland, nephridia, and foot were taken from each specimen. Tissue samples were embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin stain. Histological sections were examined using a Leica DM 2500 light microscope for the presence of pathological alterations, and measurements were taken with a Leica DFC 280 digital camera and its software. Parasites and symbionts present in these sections were noted.

RESULTS

A disseminated neoplasia prevalence of 13.3% was found in the mussels from the Beagle Channel. Diseased mussels showed heavy levels of hemocyte or neoplastic cell infiltration in connective tissues, particularly in subepithelial regions. Thus, digestive gland tubules, intestine, nephridia, gills, gonad, and connective tissue of the mantle were the main sites invaded.

In healthy mussels, the hemocytes were typical granulocytes displaying pseudopodia that average 8.79 ± 1.97 μm in length (SD; $n = 50$), with nuclei 3.25 ± 0.46 μm in length ($n = 50$; Fig. 2). In mussels with disseminated neoplasia, neoplastic cells were larger (12.62 ± 1.73 μm in length, $n = 50$), and varied in appearance from round to ovoid or spindle shaped. Their nuclei (8.77 ± 1.09 μm in length, $n = 50$) were hyperchromatic and pleomorphic, and sometimes bilobed or multilobed (Fig. 3). Nuclear-to-cytoplasmic volume ratios were high (mean, 0.70; SD, 0.10; $n = 50$) relative to those of normal hemocytes (mean, 0.37; SD, 0.08, $n = 50$). The cytoplasm of neoplastic cells contained granules and a smooth margin. No mitotic figures were observed. The sex ratio was 0.43 male versus 0.53 female overall, and gonads of all healthy specimens were mature. All specimens with disseminated neoplasia were female. Three different stages of the disease were observed. During an initial stage, gills, nephridia, connective tissue of mantle, gonad, and digestive system were infiltrated by hemocytes, and a few neoplastic cells were apparent (Fig. 3). During an intermediate stage, infiltration was heavier and the proportion of neoplastic cells increased, with a disruption of normal tissue architecture and the beginning of gonadal atrophy (Fig. 4). During an advanced stage, normal hemocytes were nearly absent and were largely replaced by

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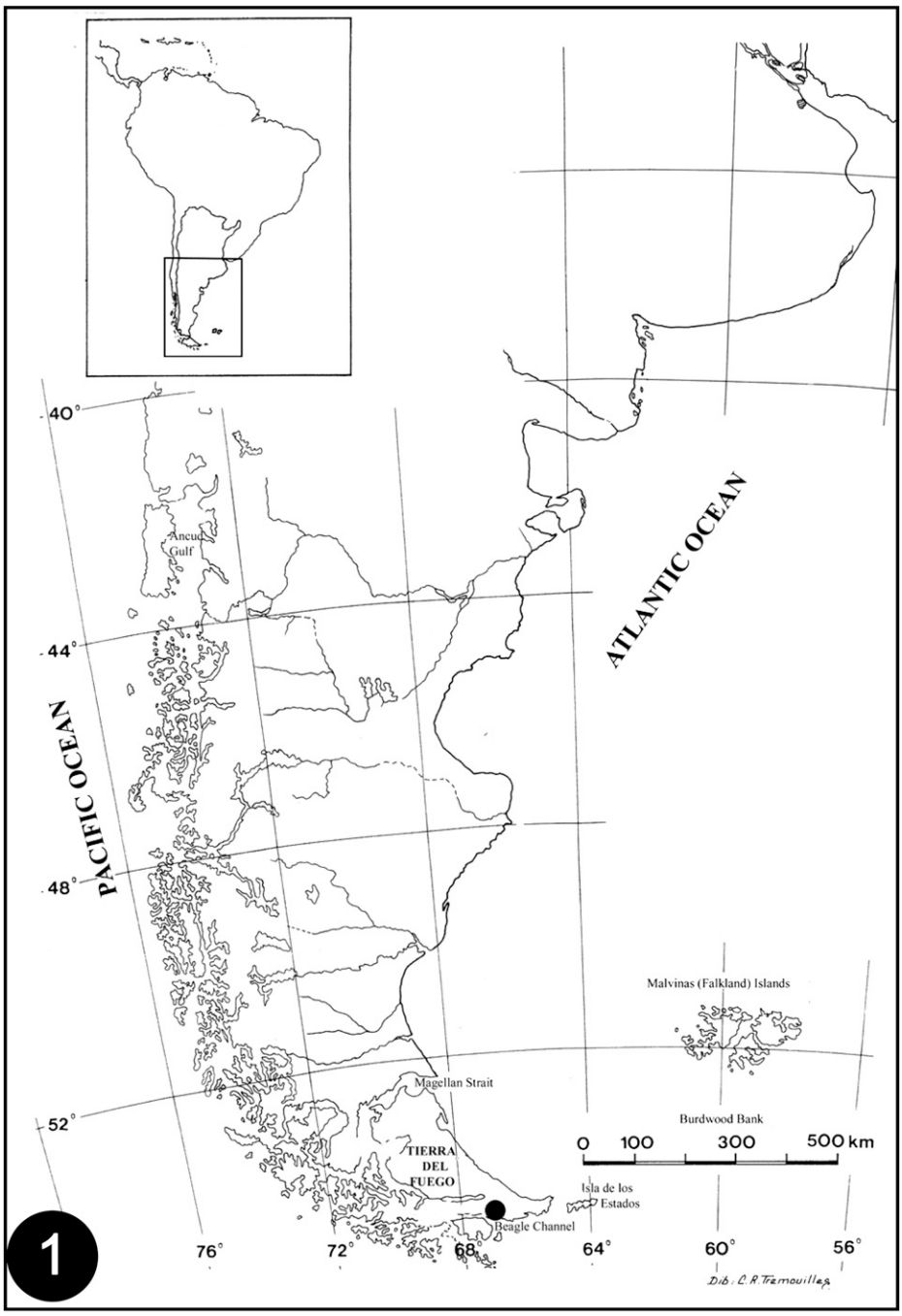

Figure 1. Sampling site: Bahía Brown, Beagle Channel (Tierra del Fuego Province, Argentina).

neoplastic cells; the gonad was represented only by a few atrophied follicles invaded by neoplastic cells (Fig. 5). Connective tissue of diseased specimens showed abundant storage cells (Fig. 5), in contrast to nondiseased specimens. An abundance of neoplastic cells occurred in the spaces between digestive tubules that also showed signs of atrophy in some cases (Fig. 6).

A summary of the parasites recorded—their prevalence, mean intensity, as well as their host location—is presented in Table 1. *M. chilensis* harbored bacteria in the gills (Fig. 7); ciliates, observed mainly in gills (Fig. 7) and also in the mantle cavity; and trematode metacercariae inhabiting the foot or byssus gland (Fig. 8).

DISCUSSION

A review of disseminated neoplasias in *Mytilus* spp. by Ciocan and Sunila (2005) stated that they occur in *Mytilus galloprovincialis* and *Mytilus edulis* at a very low prevalence. In our study, a higher prevalence was found compared with *M. chilensis* cultured in Chile (13.3% versus 2.4%) (Campalans

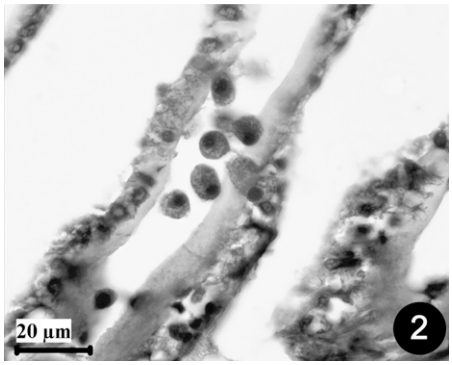

Figure 2. Hemocytes of healthy mussels *M. chilensis*.

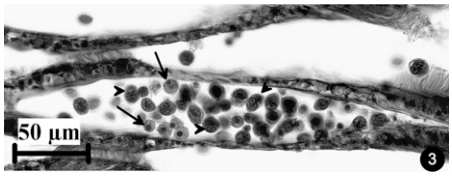

Figure 3. Initial stage of disseminated neoplasia showing heavy infiltration by normal hemocytes (arrow) with some neoplastic cells (arrowhead) in gill branches.

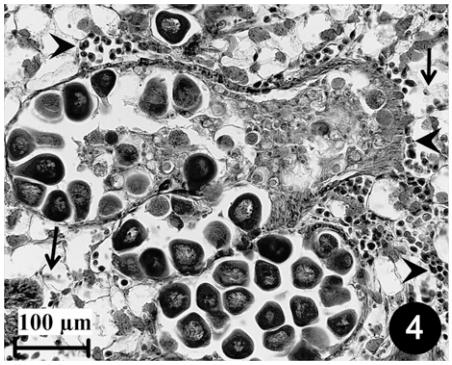

Figure 4. Intermediate stage of disseminated neoplasia showing initial gonad atrophy and heavy infiltration by normal hemocytes (arrow) and neoplastic cells (arrowhead).

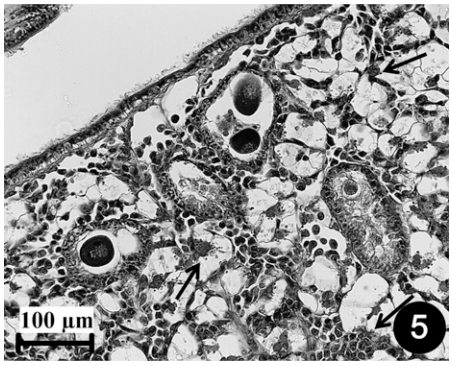

Figure 5. Advanced stage of disseminated neoplasia showing almost complete gonadal atrophy and the presence of storage cells (arrow).

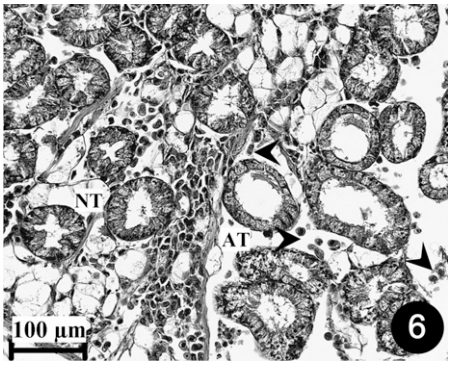

Figure 6. Advanced stage of disseminated neoplasia showing atrophied digestive tubules (AT) and neoplastic cells (arrowhead), with normal tubules (NT).

TABLE 1.

Prevalence and mean intensity of parasites and symbionts recorded in *Mytilus chilensis* cultured in the Beagle Channel, Tierra del Fuego Province, Argentina.

Parasite or Symbiont	Location	Prevalence (%)	Mean Intensity
Basophilic colonies of bacteria	Gills (Fig. 7)	16.67	10.8
Ciliates	Gills (Fig. 7)	53.33	3.94
Ciliates	Mantle cavity	3.33	4
Metacercariae (Trematoda)	Foot or byssus gland (Fig. 8)	10	1

et al. 1998). In a previous report for *M. chilensis*, Campalans et al. (1998) found neoplastic cells mainly infiltrating the digestive gland, accompanied by the destruction of the digestive tubules. The authors did not mention signs of gonadal atrophy, as found in the current study.

Although there is no evidence regarding the origin of proliferating cells, different stages of disease progression were assigned. We considered an early stage to be the presence of a heavy infiltration of normal hemocytes with a few neoplastic cells, in contrast to the presence of small-foci neoplastic cells reported by Mix (1983). Ford et al. (1997) remarked that, in the literature, the effects of neoplasia on gametogenesis were equivocal, ranging from arrested development (Farley 1969, Cosson-Mannevy et al. 1984, Brousseau 1987) to no apparent consequences (e.g., Alderman et al. 1977, Peters 1988, Elston et al. 1992, Barber 2004, Ciocan & Sunila 2005). Ford et al. (1997) found a relationship between disseminated neoplasm and the gonadal cycle of *Crassostrea virginica*, where diseased oysters had measurable gonads but their gonadal indices were generally below the mean for nonneoplastic individuals. Although clearly distinct from the gonadal neoplasm described in other bivalves, disseminated neoplasms have two hypothesized associations with reproduction (Elston et al. 1992): (1) a gonadal origin and (2) an inhibitory effect on gametogenesis. Elston et al. (1992) found neoplasia associated with retarded gametogenesis in *M. edulis* during periods of low ambient food supply. This suggested that energy allocation favored neoplastic cells over gametes at times when food was limiting.

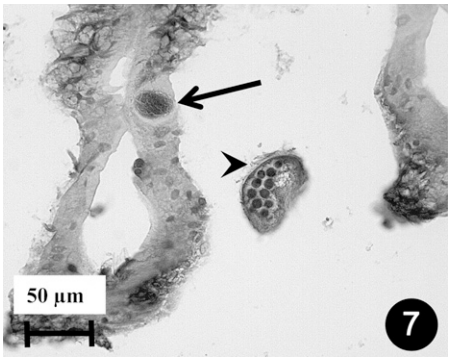

Figure 7. Basophilic colony of bacteria (arrow) and a ciliate (arrowhead) in gills of *M. chilensis*.

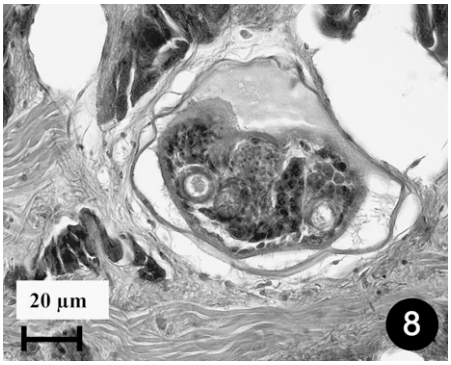

Figure 8. Example of metacercaria inhabiting the byssal gland of *M. chilensis*.

In the current case, mussels with neoplasia exhibited gonadal atrophy, whereas nonneoplastic individuals from the same sample had mature gonads. Diseased specimens showed abundant connective storage cells, which argues against a food depletion hypothesis, in agreement with findings by Ford et al. (1997) for neoplastic oysters.

For disseminated neoplasias, there is only one case reporting differences in sex affected (Brousseau & Baglivo 1994), whereas there are numerous reports for gonadal neoplasia (e.g., Alonso et al. 2001, Bert et al. 1993, Barber 1996). In our study, all diseased individuals were female, in contrast with the reports of Brousseau and Baglivo (1994), who found a higher prevalence of disseminated neoplasia in males of *Mya arenaria*. Alonso et al. (2001) also found that males of *M. galloprovincialis* were the most affected by gonadal neoplasia. However, most report gonadal neoplasia as occurring mainly in females (e.g., Barry & Yevich 1972, Bert et al. 1993, Barber 1996), suggesting that this alteration could be related to some hormonal disorder.

This is the first report of neoplasia disease in *M. chilensis* from the South Atlantic coast and the southernmost region of Argentina. The high prevalence reported here suggests a potential epizootic origin. Subsequent studies, including larger samples and expanded geographic coverage, are needed to determine whether males may also be affected, and whether disseminated neoplasia are present in other culture sites.

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