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Occurrence and lack of transmissibility of gonadal neoplasia in softshell clams, *Mya arenaria*, in Maine (USA) and Atlantic Canada

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Abstract

Gonadal neoplasms (germinomas) in softshell clams, *Mya arenaria*, have only been reported from locations in Maine, USA despite the fact that the geographic range of *M. arenaria* extends from Labrador to North Carolina on the east coast of North America. To more accurately determine the geographic distribution of this disease, adult clams (n = 18–60 per sample) obtained between 1989 and 1997 from sites along the entire coast of Maine and from Atlantic Canada (New Brunswick, Nova Scotia, and Prince Edward Island) were examined histologically for the presence of neoplasia. Gonadal neoplasms were present at 10 of the 28 locations sampled, including sites in Maine, New Brunswick, Nova Scotia, and Prince Edward Island, at prevalences ranging from 3.3 to 50% and at all stages of development. Prevalence and stage of development, however, were consistently greater at sites located between Penobscot Bay, Maine and Passamaquoddy Bay, New Brunswick. There was no correlation between mean clam size (shell length) and prevalence. Clams with neoplasia were predominantly female. To assess possible disease transmissibility and subsequent mortality rates, naïve clams were transplanted to a site where neoplasia is enzootic and placed in close proximity to clams having the disease. After 6 months, no evidence of neoplasia was found in the transplanted clams even though cumulative mortality (14.7%) was greater than that in local clams (3.4%). These results suggest that gonadal neoplasms in *M. arenaria* progress slowly and cause little mortality once present in an individual and may not have an infectious etiology. Loss of reproductive output is a potential long-term effect of the disease.

Résumé

Fréquence, et absence de transmissibilité de la néoplasie des gonades chez la mye des sables, *Mya arenaria*, du Maine (Etats-Unis) et de l'Atlantique canadien. Des néoplasmes (germinomes) dans les gonades de la mye des sables, *Mya arenaria*, ont été signalés dans différents sites du Maine, Etats-Unis, en dépit du fait que l'aire géographique de *M. arenaria* s'étend du Labrador à la Caroline du Nord sur les côtes est d'Amérique du Nord. Afin de déterminer plus précisément la répartition géographique de cette maladie, des coquillages adultes (n = 18–60 par échantillon), prélevés entre 1989 et 1997, et provenant de sites tout au long de la côte du Maine et de l'Atlantique canadien (Nouveau-Brunswick, Nouvelle-Ecosse, et l'île du Prince Edward) ont été examinés par histologie pour détecter la présence de néoplasie. Des néoplasmes dans les gonades étaient présents dans 10 des 28 sites échantillonnés, y compris les sites du Maine, Nouveau-Brunswick, Nouvelle-Ecosse et l'île du Prince Edward, avec une prévalence de 3,3 à 50 %, à tous les stades de développement. Cependant, prévalence et stade de développement étaient considérablement plus élevés dans les sites situés entre la baie de Penobscot, Maine, et la baie de Passamaquoddy, Nouveau-Brunswick. Il n'y avait pas de corrélation entre la taille moyenne des myes (longueur de la coquille) et la prévalence. Les myes comportant des néoplasies étaient en majorité des femelles. Pour établir une possible transmission de la maladie et des taux de mortalité en résultant, des myes saines ont été transplantées sur un site où la néoplasie est endémique et placées à proximité de myes ayant cette maladie. Après six mois, aucune évidence de néoplasie n'a été trouvée chez les myes transplantées bien que la mortalité cumulée (14,7 %) était plus élevée que chez les myes locales (3,4 %). Ces résultats suggèrent que des néoplasmes chez

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M. arenaria progressent lentement et provoquent qu'une faible mortalité quand ils sont présents chez un individu et peuvent avoir une étiologie infectieuse. Un des effets potentiels à long terme de cette maladie est la perte de la capacité reproductrice. © 2002 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS. Tous droits réservés.

Keywords: Neoplasia; Disease; Mollusks; *Mya arenaria*

1. Introduction

Gonadal neoplasms (germinomas, tumors) of marine bivalve mollusks are classified into three basic histotypes, with those of germ cell origin being the most common (Peters et al., 1994). Germinomas are characterized by the presence of monomorphic, basophilic, undifferentiated germ cells that appear initially as small foci in one or more follicles, but which may multiply to completely fill most or all follicles, and are capable of invasion and metastasis (Hesselman et al., 1988; Gardner et al., 1991b; Peters et al., 1994; Barber, 1996).

Germ cell neoplasms are rarely seen in Eastern oysters, *Crassostrea virginica*; ocean quahogs, *Arctica islandica*; bay scallops, *Argopecten irradians*; and blue mussels, *Mytilus edulis* (Farley, 1976; Harshbarger et al., 1979; Gardner et al., 1991a; Peters et al., 1994). Epizootics of germinomas, however, occur in populations of quahogs, *Mercenaria* spp. (Yevich and Berry, 1969; Barry and Yevich, 1972; Hesselman et al., 1988; Bert et al., 1993; Eversole and Heffernan, 1995) and softshell clams, *Mya arenaria* (Barry and Yevich, 1975; Gardner et al., 1991b; Barber, 1996). Prevalences of gonadal neoplasia as high as 31.5 and 42.9% have been reported for populations of *M. mercenaria* and *M. arenaria*, respectively (Hesselman et al., 1988; Barber, 1995). Although the disease appears to be progressive based on histological data, it is unclear whether mortality results. There is little question however, that affected clams exhibit abnormal gametogenesis and a reduction in fecundity (Eversole and Heffernan, 1995; Barber, 1996).

The etiology of the disease is unknown, but several potential causative factors have been identified. Initial reports of this condition were correlated with hydrocarbon or herbicide contamination (Barry and Yevich, 1975; Yevich and Barszcz, 1976, 1977; Brown et al., 1977; Gardner et al., 1991b). In addition, Landsberg (1996) suggested that the occurrence of gonadal neoplasia in *M. arenaria* parallels the distribution of harmful algal (*Alexandrium* spp.) blooms. More recently, a number of studies have documented the effects of endocrine modulating substances on reproductive processes in marine invertebrates, including bivalves (see review by Oberdörster and Cheek, 2001). Finally, high prevalence of the disease in a population could indicate susceptibility to an infectious agent (Harshbarger et al., 1979; Hesselman et al., 1988). Thus both environmental factors and infectious agents are possible causative factors.

To date, the only reported cases of gonadal neoplasia in *M. arenaria* have come from sites in Maine (USA), even though the species is distributed from Labrador to North Carolina along the east coast of North America (Abbott, 1974). The condition was initially described in clams from Long Cove, Searsport, where 1–22% of individuals examined between 1971 and 1976 had neoplasia (Barry and Yevich, 1975; Yevich and Barszcz, 1976, 1977; Brown et al., 1977). Clams collected in 1980 from Rogue Bluffs (near Machiasport) and Dennys Bay (near Dennysville) had 3 and 35% prevalences of gonadal neoplasia, respectively (Gardner et al. 1991b). More recently, Barber and Davis (1994) and Barber (1995) found neoplasia in five populations of clams from eastern Maine, at prevalences ranging from 6.7 to 42.9%. Gonadal neoplasia has not been observed in *M. arenaria* from locations to the south of Maine (Massachusetts, Rhode Island, Connecticut, and Maryland), despite extensive sampling (Brown et al., 1977; Yevich and Barszcz, 1976, 1977; Farley et al., 1986; Brousseau, 1987a).

In an attempt to further define the geographic extent of this disease, we report here on the occurrence of gonadal neoplasia in *M. arenaria* collected from Maine and Atlantic Canada (New Brunswick, Nova Scotia, and Prince Edward Island) between 1989 and 1998. We also provide results of a preliminary transplantation experiment designed to assess disease transmissibility, progression, and resultant mortality.

2. Materials and methods

2.1. Occurrence

The histological material examined for this study came from several sources. All site locations are provided in Fig. 1 and Table 1.

Adult clams were collected using a hand rake from an intertidal flat in St. Andrews (Passamaquoddy Bay) New Brunswick, Canada (site 21 in Fig. 1). Collections were made monthly (n = 19–30) from January 1989 through December 1990 as part of a study examining clam reproduction, settlement, and population dynamics (S. Robinson, unpublished data). In the laboratory, the shell lengths (calipers, ± 0.1 mm) of all clams were recorded prior to shucking and fixation in Bouin's solution (Howard and Smith, 1983). Transverse sections (3 mm) of gonadal tissue were placed in embedding capsules, dehydrated and cleared in an automated tissue processor (Tissuematon), before being embedded in paraffin. Sections (6 μm) were stained

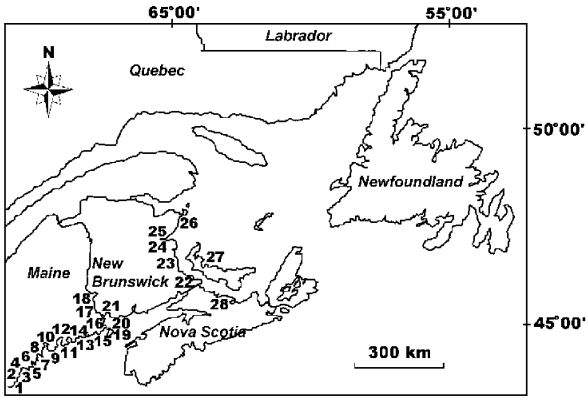

Fig. 1. Map showing relative locations of sampling sites from which clams, *M. arenaria*, were examined for the presence of gonadal neoplasms. Refer to Table 1 for exact locations.

with Harris' Hematoxylin and Eosin. In 1997, these slides were re-examined with a Leitz Dialux 20 compound microscope (25 and 250 ×) for the presence of gonadal neoplasia.

Adult clams were collected with hand rakes from four sites in New Brunswick, Canada (n = 18–30) for routine health screening between 1993 and 1996 (sites 21, 23, 24 and 26 in Fig. 1). Prior to shucking, shell length was measured with calipers (± 0.1 mm). Transverse sections of clam tissue (including gonad, gills, digestive gland, mantle, and kidney) were fixed in 1G4F seawater fixative and stained with hematoxylin and eosin (Howard and Smith, 1983), and processed and examined for the presence of gonadal neoplasia as described above for the 1989–1990 St. Andrews samples.

Table 1
Locations of sites where samples of *M. arenaria* were collected. Sites can be located by number in Fig. 1

Site number	Site name	Location	
		Latitude (N)	Longitude (W)
1	Piscataqua River, ME	43°05'56"	70°45'56"
2	Flying Point, ME (Casco Bay)	43°49'17"	70°03'25"
3	Brunswick, ME (Thomas Point Beach)	43°53'41"	69°53'23"
4	New Meadows River, ME (Mill Cove)	43°52'00"	69°51'58"
5	Kennebec River, ME	43°46'17"	69°47'38"
6	Back River, ME	43°55'20"	69°43'50"
7	Damariscotta River, ME (Lowes Cove)	43°55'08"	69°34'30"
8	Medomak River, ME	44°02'00"	69°21'00"
9	St. Georges River, ME	44°00'28"	69°14'33"
10	Long Cove, ME (Penobscot Bay)	44°27'47"	68°53'13"
11	Morse Cove, ME (Penobscot Bay)	44°26'59"	68°47'04"
12	Mt. Desert Narrows, ME	44°26'30"	68°21'27"
13	Beals Island, ME	44°31'15"	67°36'40"
14	Englishman Bay, ME (Rogue Bluffs)	44°36'38"	67°28'30"
15	Little Machias Bay, ME (Cutler)	44°39'36"	67°14'24"
16	Whiting Bay, ME (Bell Farm)	44°49'18"	67°09'46"
17	Mill Cove, ME (Dennys Bay)	44°52'30"	67°09'18"
18	Pembroke, ME (Dennys Bay)	44°52'55"	67°08'17"
19	S. Lubec, ME	44°49'04"	66°58'56"
20	Eastport, ME	44°55'16"	67°01'09"
21	St. Andrews, NB (Passamaquoddy Bay)	45°04'35"	67°03'50"
22	Shediac Bridge, NB	46°17'00"	64°33'00"
23	Cocagne, NB	46°23'30"	64°36'10"
24	Bouctouche, NB	46°30'00"	64°40'45"
25	Richibucto, NB	46°43'30"	64°52'05"
26	Shippagan, NB	47°42'37"	64°48'38'
27	Mill River, PEI	46°46'32"	64°06'15"
28	Wallace, NS	45°49'05"	63°25'15"

Adult clams were collected with a hand rake from five sites in New Brunswick, Nova Scotia, and Prince Edward Island, Canada (sites 21, 22, 25, 27 and 28 in Fig. 1 on a seasonal (spring, summer, fall) basis in both 1996 and 1997 (n = 14–60) and processed as above.

Adult clams were collected with hand rakes from 20 sites along the coast of Maine, USA between January 1994 and July 1996 (n = 28–50). Samples were returned (chilled) to the laboratory where they were measured with calipers (shell length, ± 0.1 mm), shucked, and placed in Dietrich's fixative (Gray, 1954). Transverse sections (3–4 mm) through kidney, digestive gland, gonad, gills, and foot were removed, placed in cassettes, and dehydrated and cleared in a Fisher Histomatic Tissue Processor. Tissues were embedded in Paraplast, sectioned (6 μm), and stained with Shandon Instant Hematoxylin and Eosin Y before being coverslipped. Finished slides were examined with a Nikon Labophot microscope (100 ×) for the presence of gonadal neoplasia (Barber, 1995, 1996).

Individual cases of neoplasia were categorized as to their extent of development using the criteria of Barber (1996) as follows: stage 1 neoplasms consisted of undifferentiated germ cells partially or totally filling one or more (but less than half of the total) follicles, with no loss of gonadal architecture (Fig. 2a); stage 2 neoplasms were characterized by the presence of undifferentiated germ cells in over half of the follicles, along with some loss of tissue architecture, but no evidence of invasion or metastasis (Fig. 2b); stage 3 neoplasms involved most to all follicles, a loss of tissue architecture was common, and invasion or metastasis was evident (Fig. 2c).

The sex ratio of clams with neoplasia was compared to a 1:1 ratio using the χ^2-test, and for samples with clams having neoplasia, mean shell height was correlated with prevalence (Zar, 1974).

2.2. Transmissibility, progression and mortality

Clams were transplanted from the Brunswick site in southern Maine (where gonadal neoplasia has never been found) to the Whiting Bay site, where neoplasms occur at consistently high prevalences (Barber, 1996). To avoid potential disease introduction to southern Maine waters, the reciprocal transplant was not conducted.

On 5 June 1995, 500 clams were collected from Brunswick (site 3 in Fig. 1; Table 1) and on 6 June 1995, 500 clams were collected from Whiting Bay (site 16 in Fig. 1; Table 1). All clams were brought to the University of Maine, Orono, ME, where they were individually measured (shell length, mm) and numbered with a felt tip pen (permanent ink). An additional 50 clams from each source population

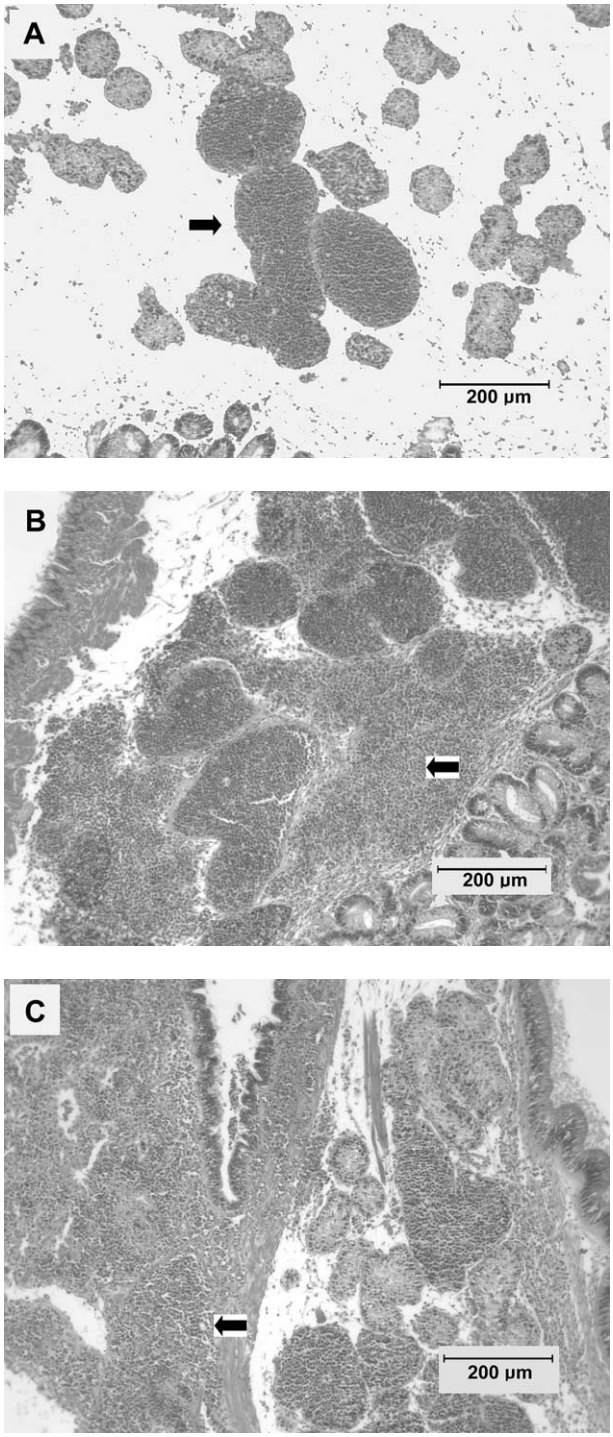

Fig. 2. Micrographs of clam, *M. arenaria*, tissue exhibiting various stages in the development of gonadal neoplasia. A, stage 1 case with few follicles containing neoplastic germ cells in otherwise normal (undeveloped) gonad; B, stage 2 case with all follicles filled with neoplastic germ cells and some loss of architecture; C, stage 3 case showing invasion of adjacent red gland.

were processed histologically to determine the initial prevalence of neoplasia in both populations.

On 8 June 1995, all the clams were taken to Whiting Bay. Fifty clams from each source population were placed into each of 10 replicate 1 m^2 predator exclusion cages at mid-tidal height (density = 100 clams per m^2). Cages were constructed of pressure treated wood (2.5 × 15.2 cm) frames, covered with 1.3 cm^2 mesh polypropylene screen. Each cage was buried so its surface was flush with the surrounding sediment.

At monthly intervals, from July through December 1995, clams from all cages were re-moved from the sediment; all dead clams were counted and removed. In August, October, and December, five transplanted clams from each source population were removed from each cage for histological processing ($n = 50$ per source) as described above. In September and December, the shell lengths of all living clams (calipers, ± 0.1 mm) were re-measured. After counting and measuring was completed, living clams were put back into their respective cages.

The null hypotheses of no differences between source location and among sampling dates for both growth (shell length) and mortality (number of dead clams) were tested using repeated measures analysis of variance (DataMost, 1995). Cumulative mortality was calculated for clams from each source population as the sum of instantaneous mortality rates, using a life table (Krebs, 1994).

3. Results

3.1. Occurrence

A total of 3480 clams collected from 28 sites in Maine, New Brunswick, Nova Scotia, and Prince Edward Island between January 1989 and October 1997 were examined histologically for this study (Table 2). Mean shell length of clams ranged from 33.6 to 73.0 mm among samples. Gonadal neoplasms were found in 116 clams, for an overall prevalence of 3.3%. Of the 116 clams with neoplasms, 61 were classified as stage 1, 47 as stage 2, and eight as stage 3 (Fig. 2). Stage 3 neoplasms included invasion of kidney and metastasis in the gills. Diseased clams were predominantly female: 72 were female, 40 were male, a sex ratio that differed significantly from 1:1 ($P \leq 0.05$). Sex could not be determined in four clams. There was no correlation ($P = 0.47$) between prevalence of gonadal neoplasia and clam size (mean shell length) in those samples in which neoplasia was found.

Thirty-one of the 91 discreet samples had at least one clam with a gonadal neoplasm. Among those samples, prevalence ranged from 1.7 to 50.0% (Table 2). Of the 28 sites sampled, 10 were positive for neoplasia on at least one occasion. All of these sites were located to the north of Penobscot Bay, ME (USA). Prevalences exceeding 10% were found at only three locations (St. Andrews, New Brunswick; Mill Cove, ME; and Whiting Bay, ME). A prevalence of 50% was found at Whiting Bay, ME in June of 1996. Advanced (stage 3) cases were only found at locations in Maine.

Table 2
Dates (listed chronologically), locations, number, and mean shell lengths (± S.D.) of clams (*M. arenaria*) examined, and prevalence and stage of development of gonadal neoplasms

Date month/year	Location	*n*	Shell length(mm)	Prevalence (%)	Stage		
					1	2	3
1/89	St. Andrews, NB	19	39.2 ± 13.0	0	0	0	0
2/89	St. Andrews, NB	24	45.8 ± 11.3	8.3	1	1	0
3/89	St. Andrews, NB	24	43.7 ± 9.2	4.2	1	0	0
4/89	St. Andrews, NB	28	33.6 ± 10.3	0	0	0	0
5/89	St. Andrews, NB	28	44.6 ± 12.5	0	0	0	0
6/89	St. Andrews, NB	25	40.5 ± 9.5	0	0	0	0
7/89	St. Andrews, NB	25	34.6 ± 11.6	0	0	0	0
8/89	St. Andrews, NB	27	42.3 ± 13.1	7.4	1	1	0
9/89	St. Andrews, NB	28	45.9 ± 14.7	3.6	0	1	0
10/89	St. Andrews, NB	27	41.9 ± 12.2	0	0	0	0
11/89	St. Andrews, NB	22	46.4 ± 14.0	0	0	0	0
12/89	St. Andrews, NB	29	44.2 ± 10.6	17.2	3	2	0
1/90	St. Andrews, NB	26	41.1 ± 7.6	30.8	6	2	0
2/90	St. Andrews, NB	26	41.7 ± 9.9	7.7	1	1	0
3/90	St. Andrews, NB	28	45.1 ± 9.5	3.6	1	0	0
4/90	St. Andrews, NB	29	41.9 ± 9.0	3.4	1	0	0
5/90	St. Andrews, NB	26	46.5 ± 11.3	3.8	1	0	0
6/90	St. Andrews, NB	27	42.5 ± 9.8	0	0	0	0
7/90	St. Andrews, NB	29	46.1 ± 15.0	0	0	0	0
8/90	St. Andrews, NB	30	51.5 ± 12.3	6.7	2	0	0
9/90	St. Andrews, NB	30	42.8 ± 9.7	10.0	3	0	0
10/90	St. Andrews, NB	30	41.4 ± 11.5	0	0	0	0
11/90	St. Andrews, NB	26	42.3 ± 12.2	7.7	2	0	0
12/90	St. Andrews, NB	27	41.6 ± 10.6	0	0	0	0
8/93	Cocagne, NB	30	62.2 ± 7.3	0	0	0	0
1/94	Mill Cove, ME	30	52.3 ± 7.8	16.7	2	2	1
6/94	Cocagne, NB	30	56.8 ± 6.5	0	0	0	0
10/94	Cocagne, NB	30	52.0 ± 11.7	0	0	0	0
10/94	Mill Cove, ME	30	48.8 ± 4.7	10.0	2	1	0
10/94	Whiting Bay, ME	30	46.9 ± 3.1	10.0	2	1	0
3/95	Shippagan, NB	25	56.6 ± 4.1	4.0	1	0	0
6/95	Brunswick, ME	48	49.9 ± 1.1	0	0	0	0
6/95	Whiting Bay, ME	50	51.9 ± 0.9	18.0	3	5	1
8/95	Brunswick, ME	50	No data	0	0	0	0
8/95	Whiting Bay, ME	50	52.6 ± 6.0	24.0	5	6	1
10/95	Brunswick, ME	50	No data	0	0	0	0
10/95	Whiting Bay, ME	50	No data	30.0	5	9	1
12/95	Whiting Bay, ME	50	No data	12.0	4	2	0
5/96	Shippagan, NB	34	53.4 ± 7.5	5.8	2	0	0
5/96	St. Andrews, NB	60	59.7 ± 5.5	0	0	0	0
5/96	Richibucto, NB	60	55.2 ± 5.5	0	0	0	0
5/96	Wallace, NS	23	54.3 ± 7.7	0	0	0	0
6/96	Shediac Bridge, NB	34	59.5 ± 7.5	0	0	0	0
6/96	Mill River, PEI	60	62.8 ± 4.3	0	0	0	0
6/96	New Meadows River, ME	30	61.5 ± 4.8	0	0	0	0
6/96	Kennebec River, ME	28	71.8 ± 7.4	0	0	0	0
6/96	Back River, ME	30	65.8 ± 6.6	0	0	0	0
6/96	Flying Point, ME	30	58.5 ± 6.1	0	0	0	0
6/96	Piscataqua River, ME	28	73.0 ± 6.0	0	0	0	0
6/96	Long Cove, ME	30	59.5 ± 6.0	6.7	0	1	1
6/96	Morse Cove, ME	30	62.7 ± 5.1	0	0	0	0
6/96	Mt. Desert Narrows, ME	30	57.0 ± 3.3	3.3	0	1	0
6/96	Damariscotta River, ME	30	58.3 ± 4.0	0	0	0	0
6/96	Medomak River, ME	30	63.4 ± 4.9	0	0	0	0
6/96	Whiting Bay, ME	30	59.4 ± 7.3	50.0	9	3	3
6/96	Englishman Bay, ME	30	56.9 ± 3.4	3.3	1	0	0
6/96	St. Georges River, ME	30	63.4 ± 4.3	0	0	0	0
7/96	St. Andrews, NB	18	55.8 ± 10.3	0	0	0	0
7/96	Bouctouche, NB	30	52.6 ± 9.8	0	0	0	0
7/96	Bouctouche, NB	30	41.3 ± 6.5	0	0	0	0
7/96	St. Andrews, NB	23	39.8 ± 8.4	0	0	0	0
7/96	Little Machias Bay, ME	30	58.9 ± 4.5	0	0	0	0
7/96	Pembroke, ME	30	53.0 ± 3.8	0	0	0	0

Table 2 (continued)

Date month/year	Location	*n*	Shell length(mm)	Prevalence (%)	Stage		
					1	2	3
7/96	S. Lubec, ME	30	54.2 ± 2.9	0	0	0	0
7/96	Eastport, ME	30	50.2 ± 4.7	0	0	0	0
7/96	Beals Island, ME	30	58.3 ± 6.8	0	0	0	0
7/96	Richibucto, NB	30	50.7 ± 4.1	0	0	0	0
8/96	St. Andrews, NB	56	47.6 ± 6.8	0	0	0	0
8/96	Shediac Bridge, NB	51	50.0 ± 7.9	0	0	0	0
8/96	Wallace, NS	55	46.6 ± 5.8	0	0	0	0
8/96	Mill River, PEI	57	55.3 ± 5.7	0	0	0	0
10/96	St. Andrews, NB	45	53.3 ± 10.7	0	0	0	0
10/96	Shediac Bridge, NB	60	65.3 ± 5.1	0	0	0	0
10/96	Richibucto, NB	60	55.3 ± 4.2	0	0	0	0
10/96	Wallace, NS	60	40.2 ± 5.6	0	0	0	0
10/96	Mill River, PEI	60	54.0 ± 5.1	0	0	0	0
5/97	St. Andrews, NB	14	54.2 ± 4.6	0	0	0	0
6/97	Shediac Bridge, NB	57	52.6 ± 4.6	0	0	0	0
6/97	Richibucto, NB	60	58.6 ± 6.9	0	0	0	0
6/97	Wallace, NS	60	45.1 ± 7.2	3.3	2	0	0
6/97	Mill River, PEI	54	56.5 ± 3.4	0	0	0	0
8/97	St. Andrews, NB	60	52.6 ± 7.5	0	0	0	0
8/97	Shediac Bridge, NB	60	54.6 ± 7.5	0	0	0	0
8/97	Richibucto, NB	60	60.2 ± 6.8	0	0	0	0
8/97	Wallace, NS	60	46.6 ± 6.6	5.0	0	2	0
8/97	Mill River, PEI	60	58.2 ± 3.5	1.7	0	2	0
10/97	St. Andrews, NB	60	48.1 ± 9.7	0	0	0	0
10/97	Shediac Bridge, NB	60	55.4 ± 5.0	0	0	0	0
11/97	Richibucto, NB	60	56.0 ± 6.2	3.3	0	2	0
10/97	Wallace, NS	60	45.2 ± 5.8	3.3	0	2	0
10/97	Mill River, PEI	60	58.2 ± 4.6	0.0	0	0	0

3.2. Transmissibility, progression and mortality

Over the 6-month period, gonadal neoplasms were only seen in clams originating from Whiting Bay, at prevalences ranging from 8 to 18.8% (Table 3). All stages of disease development were present.

Clams did not grow appreciably over the study period, as mean shell length of both groups remained the same ($P = 0.56$) between June and December (Table 3). Clams originating from Whiting Bay had a mean shell length of 51.9 mm in June and 51.8 mm in December. For clams originating from Brunswick, initial mean shell length was 49.9 mm and final mean shell length was 49.4 mm. Overall, Whiting Bay clams had a greater ($P \leq 0.0001$) mean shell length than Brunswick clams.

Mortality was greater ($P \leq 0.04$) for clams transplanted from Brunswick than for clams from Whiting Bay (Table 3). Over the 6-month study period, a total of 68 clams from Brunswick died compared to 13 clams from Whiting Bay. The greatest mortality in Brunswick clams occurred in the July sample ($n = 22$) but decreased in each succeeding month. No more than four Whiting Bay clams died in any month. Cumulative mortality at the end of the study was 14.7% for Brunswick clams and 3.4% for Whiting Bay clams.

Table 3
Mean shell length (± S.D.) of transplanted clams, number of dead clams, and prevalence of gonadal neoplasia in clams, *M. arenaria*, from Whiting Bay and Brunswick, ME source populations. Clams killed as a result of handling were not counted

Date	Whiting Bay			Brunswick		
	Shell length (mm)	# Dead	Prevalence (%)	Shell length (mm)	# Dead	Prevalence (%)
8 June 1995	51.9 ± 0.9	0	18.0	49.9 ± 1.1	0	0
10 July 1995		1			22	
15 August 1995		4	8.0		18	0
20 September 1995	52.5 ± 0.8	3		50.3 ± 1.1	15	
18 October 1995		1	18.8		7	0
16 November 1995		3			4	
13 December 1995	51.8 ± 1.1	1	18.0	49.4 ± 1.1	2	0

4. Discussion

The results presented here more clearly define the geographic distribution of gonadal neoplasia in *M. arenaria* and its occurrence in populations in Maine and Atlantic Canada. The southern distributional limit of germinoma is Penobscot Bay, ME (Long Cove site). Yevich and Barszcz (1976, 1977) examined over 14 000 clams from Maine, Massachusetts, Rhode Island, Maryland and California, but only found gonadal neoplasms in Maine clams (also Long Cove). Brown et al. (1977) examined 1325 clams at 10 sites between Maine and Rhode Island and found gonadal neoplasms only at Long Cove, ME. Brousseau (1987a) collected samples from three sites in Long Island Sound, Connecticut between 1983 and 1985 and observed no cases of gonadal neoplasia in the 3963 clams examined. Similarly, Farley et al. (1986) examined over 3500 clams from 51 sites in Chesapeake Bay, and reported no cases of gonadal neoplasia. The finding of positive cases of gonadal neoplasia in clams from New Brunswick, Nova Scotia, and Prince Edward Island, as reported here, has therefore, extended the known range of this disease to the north. The limited distributional range of this disease near the northern geographic limit of the species suggests that low temperature may be a prerequisite for development of neoplasia. Additional sampling from eastern Nova Scotia and the Bay of Fundy and north to Labrador is clearly warranted to further delineate its geographic distribution.

Within the range of occurrence of the disease reported here, neoplasia prevalence and stage of development were both greatest within the Passamaquoddy Bay–Cobscook Bay region (St. Andrews, New Brunswick and Mill Cove and Whiting Bay, ME sites). These were the only sites where neoplasia consistently occurred and for which stage 3 neoplasms were found. The 50% prevalence found in the June 1996 sample from Whiting Bay, ME is the highest yet recorded for this disease. It is interesting to note that at the St. Andrews site, neoplasms were found in 13 of 24 samples collected in 1989 and 1990, but were absent from six samples taken in 1996 and 1997.

This study reinforces the finding that a significant majority of clams having gonadal neoplasia (both *Mercenaria* spp. and *M. arenaria*) are females (Barry and Yevich, 1972; Hesselman et al., 1988; Barber, 1996), while clam populations in general have a 1:1 sex ratio (Coe and Turner, 1938; Brousseau, 1987b; Barber, 1996). One of two possibilities could account for these observations. The first is that females are somehow more susceptible to the development of gonadal neoplasia. The second is that the disease affects sexual differentiation, resulting in an unusually high proportion of females in affected clam populations. Since this disease represents an impairment of normal gametogenic processes, it is not unreasonable to suggest that the disease might affect sexual differentiation as a result of a disruption of normal genetic or hormonal control mechanisms. This is supported by the observation of Barber (1996), who noted that the effects of neoplasia were general in nature, affecting the entire gonad (not just the follicles filled with undifferentiated cells). A secondary factor might be the timing of the disease relative to sexual maturity. Since protandry does not occur in *M. arenaria* (Coe and Turner, 1938), expression of the disease prior to sexual differentiation might lead to a higher proportion of females than males. Disease expression after sexual differentiation has occurred is unlikely to alter the sex of the individual, but would nonetheless reduce fecundity (Barber, 1996).

It might be expected that larger clams would have greater disease prevalence due to a presumably longer exposure to potential causative factors, as is often the case with infectious diseases (Sindermann, 1990). The lack of a correlation between clam size (mean shell length) and prevalence as seen in this study is thus somewhat difficult to interpret. One factor attributing to the lack of a correlation between clam size and disease prevalence may be the limited size range of clams examined (33.6–73.0 mm), since only adult (sexually mature) clams can have the disease. Second, due to differential growth rates among the various sites, shell height is probably a poor predictor of age. For example, growth rate has been found to vary between sites by as much as 30% (S. Robinson, unpublished data). Finally, a correlation between clam size (age) and disease prevalence would not be evident if the disease affects clams soon after reproductive maturity is reached and progresses slowly or affects clams at any age and progresses quickly, resulting in mortality.

Cases of gonadal neoplasms reported here ranged in severity from stage 1 to stage 3, suggesting that the disease is progressive and may result in clam mortality. The rate of disease progression, however, is difficult to determine since individual clams cannot be repeatedly examined, as is the case with hemic neoplasia (Barber, 1990). Over the course of the 6-month transplant study reported here, mean cumulative mortality of Whiting Bay clams (having an initial prevalence of 18%) was only 3.4%, suggesting that disease progression is slow and mortality is low but continual. Mortality of transplanted Brunswick clams was much higher, but not as a result of disease. Most of these mortalities (which occurred immediately after the transplant) were likely the result of stresses associated with transport and acclimation to the Whiting Bay environment. Overall, the fact that very few advanced (stage 3) cases were found suggests that once the disease progresses beyond a certain point, mortality occurs quickly.

Harshbarger et al. (1979) found two types of intranuclear inclusion bodies in clams from the Long Cove, ME site. One type of inclusion, found in atypical hemocytes of healthy clams, contained non-enveloped particles 55 nm in diameter that resembled Papovavirus. The other type of inclusion was found only in the nucleus of germinoma cells, but a viral nature for these inclusions was not confirmed (Harshbarger et al., 1979; Peters et al., 1994). In the present study, the fact that germinomas did not develop within 6 months in naive clams transplanted from Brunswick to

Whiting Bay and placed in close proximity to diseased clams, suggests that an infectious etiology is either not involved or takes longer than 6 months to manifest itself.

In spite of the limited geographic distribution of gonadal neoplasia in *M. arenaria* (northern Maine, USA and Atlantic Canada), the etiology of this disease remains elusive. An infectious agent has yet to be identified, and some combination of environmental and genetic factors may be involved. Regardless of etiology, once initiated, the disease progresses slowly, causing minimal mortality. In populations having a high prevalence of the disease, however, reproductive potential could be diminished (Barber, 1996).

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