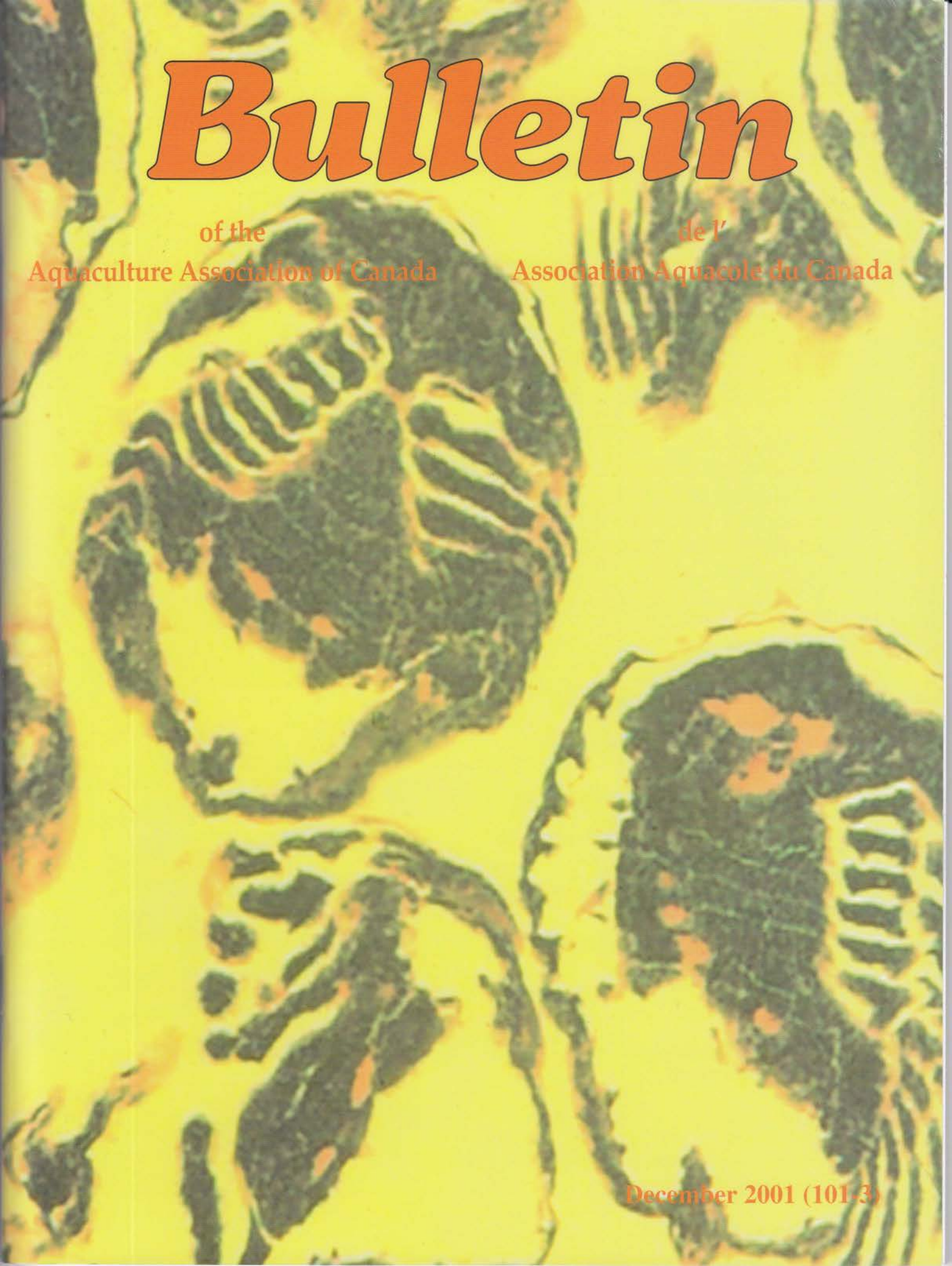
Bulletin

of the
Aquaculture Association of Canada

de l'
Association Aquacole du Canada



December 2001 (101-3)

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Cover: Unidentified copepod-like organisms in cross-section through a grey "egg mass" attached to the abdomen of a northern shrimp [Gregory MacCallum photo]

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Shellfish Health — Risks and Management

Proceedings of a special session held at Aquaculture Canada 2001

Linda D. Hiemstra

The molluscan shellfish culture and enhancement industries in Canada continue to grow, prosper and diversify. As they do, avoiding, minimizing and controlling disease outbreaks becomes more challenging. Increasing awareness of the potential for disease to significantly impact aquaculture development is bringing industry, science and management expertise together in a concerted effort to avoid the avoidable and minimize the impact of the unavoidable. The Aquaculture Association of Canada has long provided the opportunity for shellfish researchers, culturists and resource managers to meet and discuss the latest research findings as well as the imperative health problems facing the industry. In a continuation of the Aquaculture Canada 2000 special session *Shellfish Health and Wealth,* a session was organized at Aquaculture Canada 2001 by Dr. Sharon McGladdery entitled *Shellfish Health Management.* The 2001 session covered a broad range of issues currently being faced by the shellfish culture industry and resouce managers. Invited speakers, including experts from both the east and west coasts of Canada and the United States, provided a broad perspective on national shellfish health issues.

The 2001 session opened with a keynote presentation by Dr. Ralph Elston of AquaTechnics and the Pacific Shellfish Institute in Sequim, Washington. With over 20 years of experience in shellfish health management, Dr. Elston provided the backdrop for the session by presenting an overview of the parallel evolution of shellfish health expertise and shellfish aquaculture. His examples spanned the history of the industry from the inception of shellfish hatcheries, through the development of regional and national health management policies, to the health protocols laid down by the World Organization for Animal Health (OIE – Office International des Épizooties) and their implications for international trade. The strict and well-defined disease lists of the OIE provided an interesting segue, and contrast, to the scenarios presented in the other presentations in the session. The most common problems faced by shellfish health diagnosticians are the management and the control of stock losses where the cause cannot be identified or where the significance of an infection cannot be determined. The papers included in this issue of the *Bulletin* provide some insight into how these challenges are being addressed.

Dr. Carol Reinisch, of the Marine Biological Laboratory in Woods Hole, Massachusetts provided the second invited presentation. Dr. Reinisch focused on the difficult issue of understanding and managing shellfish diseases that have strong and complex environmental influences. Dr. Reinisch's model is a leukemic condition in soft-shell clams, on which she has been working since the early 1980s. As with vertebrate leukemia and related neoplastic conditions, the triggers are complex and span genetic, environmental and infectious possibilities. These studies are now revealing biomolecular parallels between invertebrate models and the vertebrate models that pioneered medical investigation into leukemia. Her work includes studies ranging from the eastern United States to Sydney, Nova Scotia and focus on the strong links between PCB contamination and alterations in clam blood cells. Dr. Reinisch's presentation set the stage for the presentation by Dr. Sharon McGladdery who has been investigating a similar problem.

Dr. McGladdery and her co-workers have been studying mass mortalities in soft-shell clams in Prince Edward Island and New Brunswick that appear to be attributable to the same, or similar, haemic neoplasia (leukemia) described by Dr. Reinisch. In the PEI-NB study, however, there is no obvious relationship between PCB contamination and neoplasia. The results indicate that multi-aetiological factors can be associated with the proliferation of this disease and reinforce the complexities of studying and managing the problem. Dr. McGladdery emphasized the need to ensure that all possible triggers are considered when attempting to identify the cause of the disease in a particular area. Without such a broad perspective, tools being used (such those developed by Dr. Reinisch) to identify the actual trigger from among the many possible candidates will be ineffective. Since much of Dr. McGladdery's and Dr. Reinisch's presentations cov-

ered similar diagnostic work and provided interesting comparative results, the two talks were combined into a single paper for this publication.

Mark Ball shifted the theme of the session from understanding mortalities in clams to assessing the health risks to scallops being domesticated for aquaculture. He presented preliminary results from his work on the parasites, pests and diseases of sea scallops and bay scallops. Although he did not find any severe infections, several interesting infections, including rickettsial-like organisms (intracellular bacteria) and trichodinid ciliates, were identified as having the potential to become opportunistic pathogens when scallops are held under confined or high density conditions. Investigations involving experimental challenges were identified as being required before a risk assessment could be conducted for these infections.

An excellent example of why such knowledge and risk assessments are required was provided by Sonia Belvin and her colleagues from the Université du Québec à Rimouski and MAPAQ, Grande-Rivière. Sonia described the challenges facing scallop culture development along the lower north shore of Québec. Recurring mortalities are having a severe impact on some stocks, with no obvious causative agent. Sonia presented the results of research that is being conducted in partnership with industry stakeholders. The question of whether the responsible agent could be transmitted between stocks or whether the cause was more probably environmental/physiological or genetic was investigated. Since seed transfers between locations are a crucial component in the development of scallop aquaculture in Québec, the initial focus of the study was on the question of whether transmission was possible. Initial results presented at Aquaculture Canada 2000 in Moncton, NB suggested a transmissible agent. This was reinforced by the research presented in this session, which answered previous questions of inoculant-induced, as opposed to pathogen-induced pathology.

The next speaker, Greg MacCallum, continued the theme of health management and risk assessment. He presented the results of preliminary work conducted on both coasts of Canada on new candidate species for aquaculture. These included sea urchins, sea cucumbers, abalone, shrimp, several clams and flat oysters. His results, like those of Mark Ball, showed some interesting findings, including many new species requiring identification and experimental investigation to assess pathogenic potential under culture conditions.

Dr. John Morgan concluded the session with research conducted at Malaspina University-College, on the west coast of Canada. Dr. Morgan continued the crustacean theme by introducing everyone to a new direction for both the study and handling of live crustaceans. The issues involved in humane approaches to handling live animals were brought to everyone's attention as well as the increasing pressure to meet these obligations. Anaesthesia using clove oil was investigated for efficacy on three crab species. Results suggested efficacy for teaching and research purposes, although the role of clove oil as a sedative to reduce handling stress requires more study. The need for increased attention to reducing stress in species being cultured was highlighted, making this presentation a useful conclusion to the session. An interesting discussion of the implications for future shellfish research and culture development followed.

Linda Hiemstra is the coordinator of aquaculture, extension and safety courses in the Fisheries and Aquaculture Extension Program at Malaspina University-College in Nanaimo, BC. (e-mail hiemsta@mala.bc.ca).

Dr. Sharon McGladdery organized the Special Session on Shellfish Health at Aquaculture Canada 2001.

Disease Issues Relevant to the Culture of Shellfish in Atlantic and Pacific Canada

Gregory S. MacCallum, Janice Blackbourn, Sharon E. McGladdery, Susan M. Bower, and Jeffery T. Davidson

A project initiated in October, 2000 at the Atlantic Veterinary College and Pacific Biological Station (PBS) assessed the health/disease issues relevant to the culture of indigenous shellfish species. This project included a thorough geographic survey of infections affecting the Stimpson's bar clam *(Mactromeris polynyma)*, European oyster *(Ostrea edulis)*, green sea urchin *(Strongylocentrotus droebachiensis)*, orange-footed sea cucumber *(Cucumaria frondosa)*, and northern shrimp *(Pandalus borealis)* on the east coast, and the green and red sea urchin *(S. droebachiensis, S. franciscanus)*, California sea cucumber *(Parastichopus californicus)*, cockle *(Clinocardium nuttali)*, varnish clam *(Nutallia obscurata)* and pinto abalone *(Haliotis kamtschatkana)* on the west coast. All species are currently under culture development, or of culture interest, on their respective coasts. Gross observations found the presence of the boring sponge *(Cliona vastifica)* in the shells of *O. edulis* and *Cliona* sp., and *Polydora* sp. in the shells of abalone. Histological examination revealed organisms such as *Trichodina* sp., unidentified intestinal ciliates, *Rickettsia*-like organisms, unidentified copepods, *Nematopsis*-like gregarine spores, and digenean metacercarian cysts in or near tissues in the bivalves, echinoderms and crustaceans. It is essential to establish baseline information on what is "normal" for species going into culture production to: i) accurately assess disease risks, and ii) differentiate true pathogens from opportunists taking advantage of sub-optimal culture conditions. This proactive research approach sets a precedent for the development of shellfish culture species, since health research rarely occurs before a disease crisis occurs.

Introduction

Shellfish culture in Canada has expanded rapidly over the last 10 years in both the number of species and the production volume and value. For example, blue mussels *(Mytilus edulis)* cultured on Prince Edward Island had a gross value of CDN$30 million in 1999.[1,2] Currently, the Pacific Coast shellfish aquaculture industry concentrates on growing the Pacific oyster *(Crassostrea gigas)*, manila clam *(Ruditapes philippinarum)* and Japanese scallop *(Patinopecten yessoensis)* which had a combined wholesale value of CDN$12 million in 1998.[6]

This increased interest in shellfish culture inevitably includes increased pressure to transfer live stock (seed, larvae, and broodstock, as well as animals for processing) from one coastal site to another. In addition, culture techniques designed to enhance productivity (e.g., the use of hatcheries and grow-out techniques such as the suspension culture of oysters and scallops) provide conditions that differ significantly from those encountered by wild populations. These factors all pose demands on, and risks to, the health of cultured shellfish. Primary pathogens and opportunistic infections can proliferate either by "accident" via live transfers and exposure to naïve stocks, or because of physiologically-stressful culture conditions.

Another crucial fact important to shellfish culture is that aquaculture most frequently occurs in habitats shared with wild stocks of the same (or closely related) species. Consequently, any "new" infectious agent that is introduced and becomes established in a cultured stock has the potential to impact both wild and cultured stock production and sustainability. It is essential, therefore, to establish baseline information on what is "normal" for species coming into culture production to accurately assess disease risks and differentiate between true pathogens and opportunists taking advantage of suboptimal culture conditions.

Table 1. Summary of shellfish collections on the Pacific and Atlantic coasts.

Species and Common Name	Location	Coast	Number Examined
Echinoderms			
Strongylocentrotus droebachiensis green sea urchin	Malaspina Inlet	Pacific	60
	Pacific Biological Station	Pacific	11
	Passamaquoddy Bay	Atlantic	60
	Canso	Atlantic	60
Strongylocentrotus franciscanus red sea urchin	Pacific Biological Station	Pacific	60
Cucumaria frondosa sea cucumber	Passamaquoddy Bay	Atlantic	60
Parastichopus californicus california sea cucumber	Pacific Biological Station	Pacific	2
Bivalves			
Clinocardium nuttali cockle	Icarus Point	Pacific	60
Nutallia obscurata varnish clam	Piper Lagoon	Pacific	60
Mactromeris polynyma Stimpson's bar clam	Grand Banks	Atlantic	60
Ostrea edulis European oyster	Port Medway	Atlantic	150
Gastropods			
Haliotis kamtschatkana pinto abalone	Island Scallops Ltd. (hatchery)	Pacific	5
Crustaceans			
Pandalus borealis boreal red shrimp	Digby	Atlantic	60
	Passamaquoddy Bay	Atlantic	34
	Grand Banks	Atlantic	34
Total			776

Established culture species such as Pacific oysters *(Crassostrea gigas)*, American/Eastern oysters *(C. virginica)*, Japanese scallops *(Patinopecten yessoensis)* and the blue mussel *(M. edulis)* all have relatively well-established health profiles on both coasts of Canada. With such knowledge, Canada has managed to remain free from all but one pathogen listed as "significant" by the Office International des Epizooties (OIE–World Animal Health Organization). In contrast, baseline knowledge of what is normal for species coming into culture production is scarce or completely lacking. This is especially true for sea scallops *(Placopecten magellanicus)* on the Atlantic coast and pinto abalone *(Haliotis*

kamtschatkana) on the Pacific coast. It is unusual to have the opportunity to conduct health research before a disease crisis occurs, and such information will undoubtedly help increase the chance for success for the entrepreneurs and investors involved in shellfish aquaculture. It will also set a precedent for similar culture initiatives elsewhere, where health research has rarely been funded before a disease crisis and the resultant challenges posed in controlling losses occur. This paper summarizes the results of a simultaneous histological survey of infections in indigenous shellfish under culture development, or of culture interest, on both coasts of Canada conducted between October 14, 2000 and March 31, 2001.

Materials and Methods

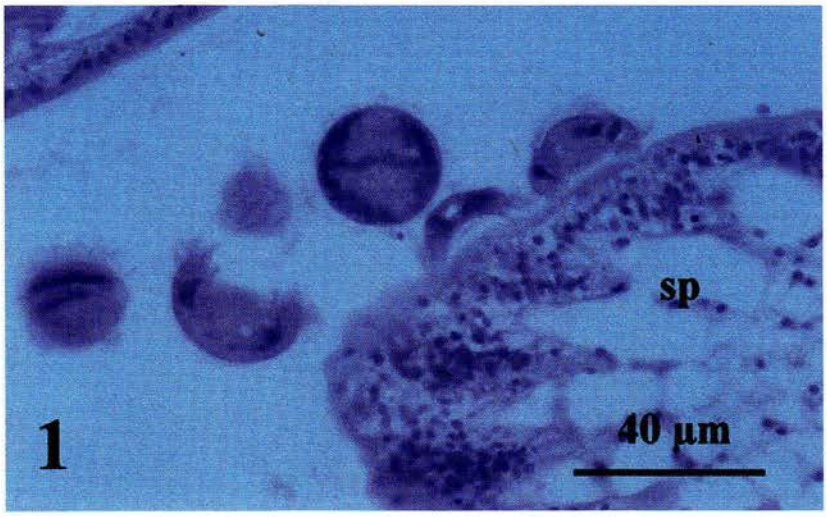
Sample collection and holding

The survey included examination of wild and cultured shellfish, as well as samples from active or potential culture areas experiencing abnormal growth or mortalities. All organisms found on and in the tissues of the shellfish were identified as far as possible before March 31, 2001 and cataloged for future cross-reference and teaching use. Data collected included sample size, date of collection, geographic location and habitat (i.e., wild, hatchery, grow-out facility), along with details on the individual specimens (sex, state of maturity, size, surface observations).

A summary of species collected between October 15 and December 21, 2000 are shown in Table 1. On the Atlantic coast, sea urchins were collected by SCUBA or provided by growers, or provincial, federal and private institutions (Table 1). Shellfish samples from the Atlantic coast were processed within 24 hours of being removed from the water. On the Pacific coast, all the invertebrates, except the abalone, were maintained in tanks with flow-through seawater (9° to 11°C) at the Pacific Biological Station in Nanaimo, B.C. until examined.

Examination procedures and histology

All external shells and soft-tissues were examined for grossly visible abnormalities. Representative tissues and organs from all sampled individuals were selected for histological examination and were preserved in Davidson's fixative[7] for at least 24 hours



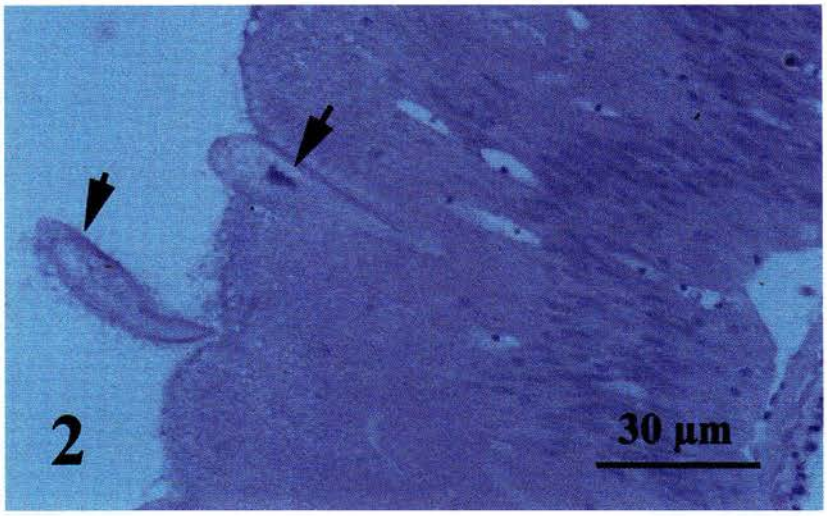


Figure 1. *Trichodina* sp. ciliates near the spine (sp) of a green sea urchin. Haemotoxylin and eosin stain.

Figure 2. Type A ciliates (arrows) in the intestine of a green sea urchin. Ciliates are imbedded in the lumen of the intestine. Haemotoxylin and eosin stain.

prior to processing. All sea urchin test samples were decalcified after fixation prior to being processed for routine paraffin histology. De-paraffinized 5-6 μm sections were stained in Harris's hematoxylin and eosin and examined under a compound microscope (up to 1000x magnification).

Results

Pacific coast

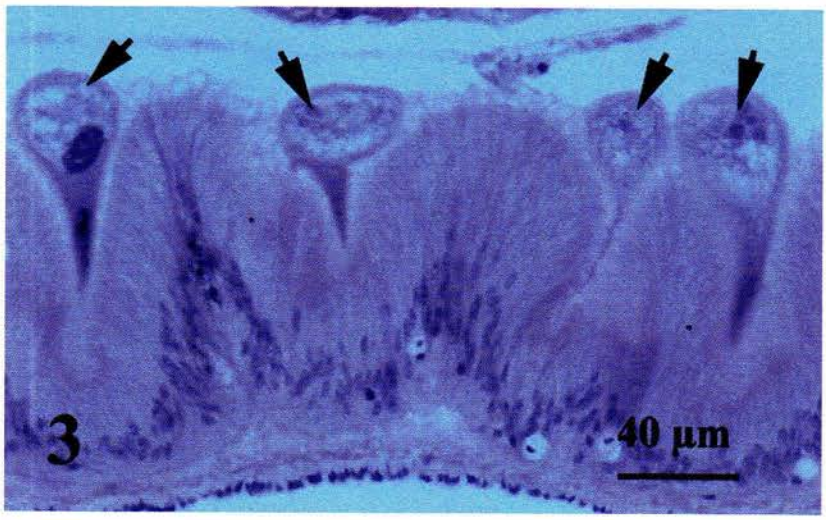
Echinoderms

No gross indications of disease were observed except in one sea urchin (1.7%) which had thin and watery soft tissues. Trichodinids were observed histologically on or adjacent to the epithelial cells covering the outer surface of the test of 45 (75%) sea urchins sampled from Malaspina Inlet (Fig. 1).

Unidentified ciliates (A and B) were found in the lumen of the digestive tract of 22 (36.7%) green sea urchins sampled from Malaspina Inlet (Fig. 2, 3). Both types of ciliate were observed partially embedded between the epithelial cells of the intestine and also free in the lumen. Type B ciliates (approximately 39.3 µm x 68.4 µm) found in 5 (8.3%) sea urchins were larger than the type A ciliates and had a more rounded shape. Four sea urchins (6.7%) were observed with both type A and B ciliate infections, and a single sea urchin was found infected with only type B ciliates.

The 11 green sea urchins in the sample from the Pacific Biological Station (PBS) all had dark red/brown patches on the test that lacked spines or normal structure. The patches ranged from 4 to 15 mm in diameter; 10 patches were located on the oral surfaces, one on the aboral surface and four on lateral surfaces. Histologically, the patches were necrotic and denuded of spines, pedicellariae and tube feet. The underlying calcaraeous test was deteriorating and high concentrations of hemocytes and ceroid bodies were observed in the areas surrounding the lesion. Bacterial infections were not evident in the histological preparations of sea urchins.

Trichodina sp. were found in association with the external epithelium of the test in 6 of the 11 (54.5%) green sea urchins examined from PBS. Parasitic ciliates A and B occurred together in 10 of 11 (90.9%) PBS green sea urchins. No pathology was observed in association with these organisms.



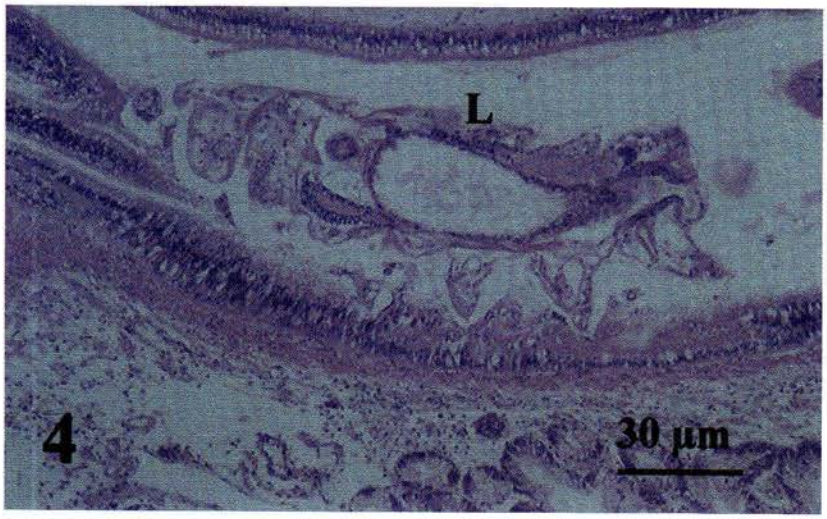


Figure 3. Type B ciliates (arrows) in the intestine of a green sea urchin. Haemotoxylin and eosin stain.

Figure 4. Near longitudinal section through the parasitic copepod *Myticola* sp. in the lumen (L) of the intesine of a cockle. Haemotoxylin and eosin stain.

Histologically, 19 red sea urchins (31.7%) were found to be harbouring the type B parasitic ciliate in the lumen and between the epithelial cells of the intestine. Pathology was not observed in connection with the presence of this ciliate.

Two sea cucumbers were preserved for histological examination in October 2000. Histology revealed the presence of mature oocysts of unidentified coccidia in the epithelium of the respiratory tree and cloaca. In addition, numerous ciliates (25 per section) were observed free within the lumen and also in close association with the epithelium lining the lumen of the respiratory tree. There was no host reaction to the presence of the cysts, or to the ciliates.

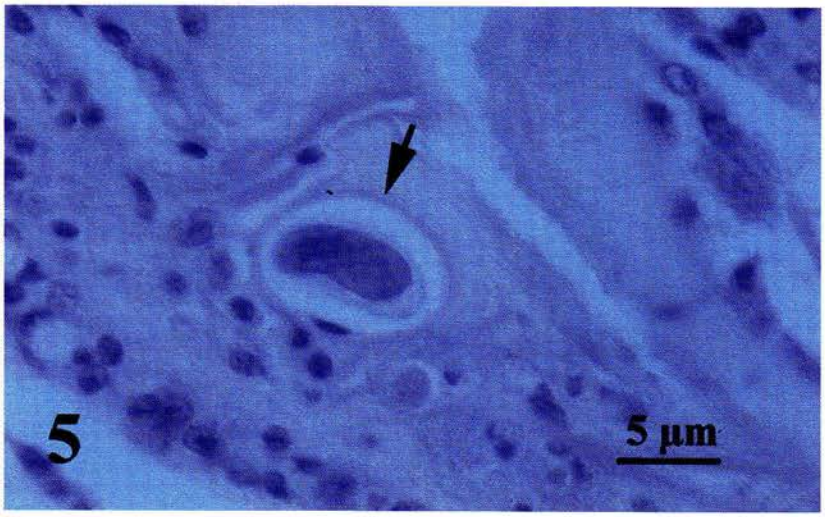
Molluscs

Gastropods (abalone). All the abalone (5/5) submitted for examination were found to harbour *Polydora* (possibly *P. limicola, P. ligni,* and/or *P. websteri*) and the shell-boring sponge *Cliona* sp. at low to moderate intensity (the internal surface of the shell was free of deformities or discoloration).

Large amoeboid cells containing orange/brown granules or droplets were observed within the histological preparations of abalone muscle and connective tissues.

Bivalves. Gross abnormalities of cockles included a pink tinge in the mantle fluid of 8 (13.3%) cockles. Two of 60 cockles were found with lemon-yellow interior valve surfaces instead of the usual off-white color, and a pearl of 3.3-mm diameter was discovered in the mantle of a single cockle. Histologically, intracellular packets of *Rickettsia*-like or *Chlamydia*-like organisms (RLO) occurred in 37 (61.7%) of this sample of cockles. They were observed within hypertrophied epithelial cells of the siphons, gills, and/or digestive gland tubules. A colony of these organisms was found in an epithelial cell of the intestine of two cockles.

The spores of a *Nematopsis*-like gregarine were observed within the connective tissues and muscle tissues of 38 (63.3%) cockles (Fig. 4). Two cockles were observed with low numbers (< 10) of gregarine spores in the muscle of the siphon or heart or in the connective tissues of the digestive gland, intestine, palps, and kidney. Twenty-seven cockles had low numbers of spores in the gill connective tissue and one cockle was



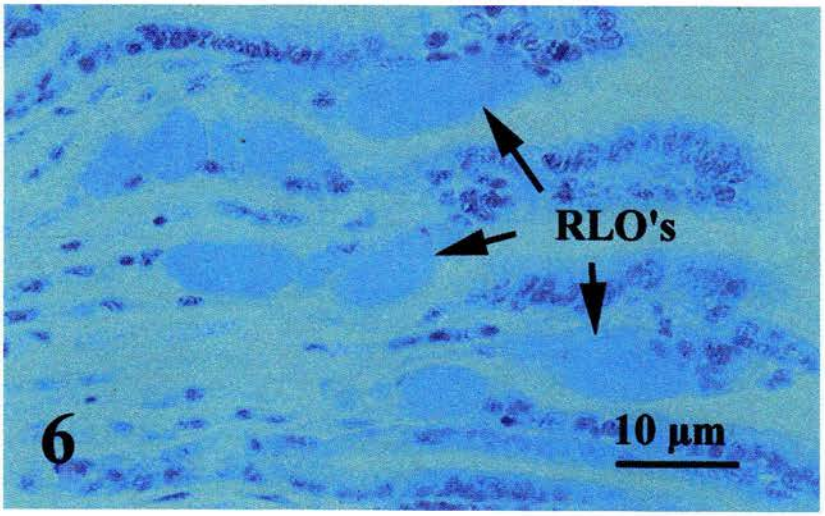


Figure 5. Oocyst (arrow) containing a sporozite of an unidentified *Nematopsis* sp. in the connective tissue of a cockle gill. Haemotoxylin and eosin stain.

Figure 6. *Rickettsia*-like organisms (RLO's) in the gill filaments of a Stimpson's bar clam. Haemotoxylin and eosin stain.

found with moderate numbers (17) in the gills. The parasitic copepod, *Mytilicola* sp. (probably *Mytilicola orientalis*) was observed in the histological preparations of 12 cockles (20%); one copepod was found in each of nine cockles and three copepods were observed in each of three cockles (Fig. 5).

No gross abnormalities were observed in the sample of varnish clams. Histologically, the spores of a *Nematopsis*-like gregarine were observed within the connective tissues of the gills in 46 (76.7%) varnish clams. They appeared to be identical to those observed in the cockles. Thirty-three clams were observed with low numbers (< 10) and 13 clams were found with moderate numbers (10 to 20) of gregerine spores per histological preparation. A single clam was found with two unidentified ciliates in the lumen of the stomach, but they did not appear to be pathogenic and may have been food items.

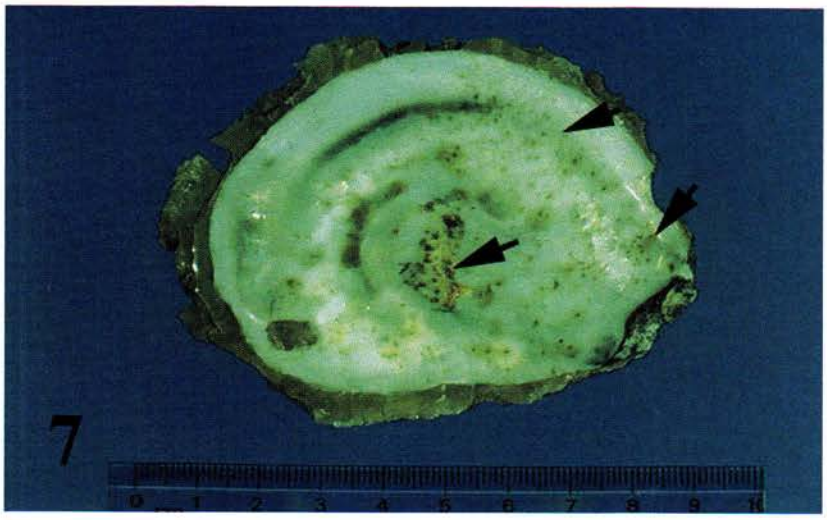
Atlantic coast

Echinoderms. No gross indications of health problems were found in the green sea urchins. Histologically, trichodinids were observed on, or adjacent to, the epithelial cells of the test in 80% (48/60) of the Canso sample and 1.7% (1/60) of the Passamaquoddy Bay sample. Ciliates (A and B) were found in the lumen of the digestive tract of 23/60 (38.3%) and 1/60 (1.7%), respectively, in sea urchin samples from Canso and Passamaquoddy. Type A ciliates (70-80 µm diameter) were embedded between the intestinal epithelial cells and were also found free in the lumen.

Type B ciliates (approximately 70 µm long) were also found in the intestinal tract of both sea urchin samples (1.7%). Macronu- clei appeared divided with up to 10 spheres. The impact of these ciliates on sea urchins is unknown. No pathological effects or any host reaction were observed in response to either of the ciliates.

Unidentified metazoans, possibly copepods (110-130 µm length), were observed adjacent to the test epithelium in 15/60 (25%) of sea urchins in the Passamaquoddy sample. None were attached to the spines or test epithelium. A single metazoan cyst, possibly a digenean metacercariae (43 µm diameter), was found in the intestinal epithelium of one Canso sea urchin (1.7%). No host response or pathology was noted.

Gross observation of sea cucumber bodies revealed "shrimp-like" amphipods (5



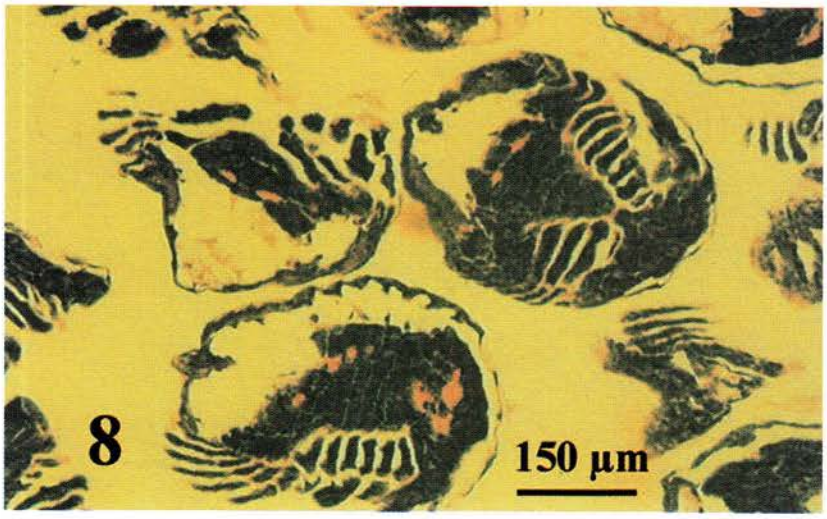


Figure 7. Holes etched by the boring sponge *Cliona* sp. (arrows) on the inside surface of the shell of a European oyster.

Figure 8. Unidentified copepod-like organisms in cross-section through a grey "egg-mass" attached to the abdomen of a northern shrimp.

mm in length) called the skeleton shrimp (*Caprella* sp.) attached to, and crawling over, the surface of 3/60 (5%) sea cucumbers in the sample. The amphipods did not appear to negatively impact the sea cucumbers. Histologically the only organisms found were ciliates in the intestinal lumen of 1/60 (1.7%) of the sample. They appeared similar to the type A ciliates (60-70 µm length) found in the intestinal tract of the sea urchins. No host response or pathology was associated with their presence.

Bivalves. No gross abnormalities were observed in or on the bar clams examined. Histologically, *Rickettsia*-like organisms (RLOs) were observed in the gills of 5% of the sample (Fig. 6). An unidentified cyst, resembling a digenean metacercariae, was found in the intestinal epithelial of one clam. No pathology or host reaction was noted in response to the cyst, indicating low pathogenicity. Eosinophilic "swirls" (30-60 µm diameter) were observed in the gills and digestive gland tubules in 8/60 (13.3%) of the bar clam sample. No pathology or host reaction was associated with their presence and their aetiology/pathology was not determined from the preliminary histological examination.

Gross observation of the shell and soft tissues of European oysters revealed that the boring sponge *Cliona vastifica* was responsible for extensive damage in one oyster (1/50 = 0.7%; Fig. 7).

Histologically, the sample from Port Medway, Nova Scotia, showed no evidence of any significant pathogens, including *Bonamia ostrae*. Eosinophilic globules (30-60 µm length), present in the gill epithelia 138/150 (92%) were tentatively attributed to secretory activity.

Crustaceans (Decapoda). Shrimp collected from Passamquoddy Bay on October 11, 2000 contained no gross abnormalities or infections detectable histologically. Shrimp collected from the same location on December 5, 2000 contained an unidentified grey "egg mass" on the dorsal abdomen (10 mm length) of two shrimp (prevalence 8.3%). A histological section through the abdomen and unidentified mass revealed numerous (65) copepods of undetermined identity (180-200 µm length; Fig. 8). The copepods did not penetrate the carapace of either shrimp. No host response or histopathology was observed.

Histologically, RLOs were found in the gill epithelial cells of shrimp from Sable Island (3.3%). Ciliates similar to those observed in sea urchins were found in the gill epithelia of shrimp from Passamaquoddy Bay, NB (8.3%) and from Digby, NS (68.3%). They appeared to penetrate the gill epithelia but no host response or pathology was observed. A second ciliate ("type C") was found in the gill epithelium of one shrimp from Passamquoddy Bay (4.1%; intensity = 2 organisms). The two ciliates (180 and 250 µm length) contained 6-8 nucleic spheres around the periphery of the organism. Infected gill epithelia appeared hypertrophied, but no surrounding histopathology was observed.

Discussion

This is the first time that a geographic survey of infections affecting shellfish species of this variety (echinoderms, molluscs and crustaceans) has been undertaken simultaneously on both coasts of Canada. Gross observations included assessment of damage by the boring sponge *(Cliona vastifica)* in the shells of European oysters, and *Cliona* sp. and *Polydora* sp. in the shells of pinto abalone. Damage was minimal in all cases, but it should be noted that *Polydora* sp. is described as a significant infectious disease (organisms that are detrimental under certain conditions), but not considered to be lethal.[5] Histological examination provided the first observations of infection by ciliates belonging to the genus *Trichodina*, along with at least three other as yet unidentified ciliates in sea cucumbers, sea urchins and shrimp. *Trichodina* spp. are disc-shaped ciliates, characterized by a circlet of eosinophilic denticles, ciliary fringes and a horse-shoe shaped macronucleus.[6,7] They are found in association with oysters, scallops and clam gill epithelia worldwide and are thought to be ubiquitous.[7-8] Despite heavy infections in the Canso sample, no pathology or host response was observed. Due to their well-documented opportunistic pathogenicity under culture conditions for salmonids (there are many freshwater species of *Trichodina*), their pathogenic impact in cultured sea urchins merits further investigation.

Rickettsia-like organisms were also found associated with the bivalve and crustacean tissues examined. RLOs are Gram-negative, intracytoplasmic, membrane-bound, oval to rod shaped bacilli, measuring 0.3 to 0.6 µm.[9] Generally, they cause negligible tissue pathology.[7,10-15] RLOs are well documented in many bivalves, including clams from the Maritime Provinces, in prevalences ranging from 1.6 to 43.3% with no associated pathology or adverse host response to infection.[15] RLOs have been reported in the soft-shell clam *(Mya arenaria)*, quahaug *(Mercenaria mercenaria)*, Pacific razor clam *(Siliqua patula)*, manila clam *(Ruditapes philippinarum)*, Pacific oyster *(Crassostrea gigas)*, American oyster *(Crassostrea virginica)*, European oyster *(Ostrea edulis)* and sea scallop *(Placopecten magellanicus)*.[10-14,16-18]

In Atlantic bivalves, digenean metacercarian cysts and copepods were associated with sea urchins and

bivalves, while *Nematopsis*-like gregarine cysts and *Myticola* sp. were found associated with bivalves and sea cucumbers on the Pacific coast.

Although none of these organisms showed evidence of overt pathology, it should be noted that most samples originated from open water or wild populations. Thus, their impact under culture conditions could not be accurately assessed from this preliminary study. It is essential to establish the significance of these observations in order to accurately assess disease risks and differentiate true pathogens from opportunists taking advantage of suboptimal culture conditions. This proactive research approach sets a precedent for development of new shellfish culture species, since health research rarely occurs before a disease crisis and the resultant challenges posed in controlling losses.

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Scallop Parasites, Pests and Diseases: Implications for Aquaculture Development in Canada

Mark C. Ball and Sharon E. McGladdery

Scallops harvested from wild stocks off the Atlantic coast of Canada have considerable value to the Canadian shellfish industry. However, in recent years, wild stocks have been depleted while the market value of scallop meat has dramatically increased. To maintain the viability of the scallop industry, considerable research has been conducted on the culture of native scallop species, principally the giant sea scallop *(Placopecten magellanicus)*. One of the major concerns in the development of any bivalve culture industry is the impact of disease on growth rates, spawning and product quality. The effects of disease on clam, oyster and mussel populations are well documented, and a solid database has been developed in Atlantic Canada for infectious agents. Such information has yet to be obtained for scallop species. The goal of this project is to develop a database that can be used to protect the health of cultured scallops in Atlantic Canada. In addition to giant sea scallops, two other commercially important species, the bay scallop *(Argopecten irradians)* and the Iceland scallop *(Chlamys islandica)* are included in this comprehensive examination of infectious agents.

Introduction

Atlantic Canada is a major component of the shellfish industry of North America, with its value being driven by such economically-important species as the Malpeque oyster *(Crassostrea virginica)* and blue mussels *(Mytilus edulis* and *M. trossulus)*. Scallops are also an important part of the industry; in 1999, nearly 58,000 tonnes of scallop meat were harvested from wild stocks on the East Coast of Canada.[1] However, with the recent decline in inshore stocks and the high market value of scallops, there has been significant investment and interest in the development of a sustainable scallop industry through aquaculture. Initially, failures in the hatchery production of scallop seed impeded development, but the successful collection of wild spat has helped reinforce both fishery enhancement and aquaculture activities. Techniques for spat collection and grow-out, and criteria for site selection, are still being refined to optimize production. Major challenges include unexplained mass mortalities and continuing losses in the hatchery phase of production.[2] There is a critical lack of baseline knowledge of infectious agents and other health-related characteristics of scallops, especially in Atlantic Canada. Data are available for species traditionally cultured on the West Coast (Japanese or yesso scallop, *Patinopecten yessoensis*),[3] as well as European scallop species (queen scallop, *Aequipecten opercularis*, and coquille St. Jacques, *Pecten maximus*),[4] but are lacking for the giant sea scallop *(Placopecten magellanicus)*, bay scallop *(Argopecten irradians)* and Iceland scallop *(Chlamys islandicus)*.

One of the major concerns facing the development of any new bivalve culture industry is the potential impact of disease. Not only can disease affect survival, but it can also have more subtle, yet equally significant, economic effects resulting from reduced growth rates, inhibition of spawning, and a reduction in the quality of the meat or shell. Shellfish health problems in Atlantic Canada are negligible compared with other shellfish-producing areas (notably Europe and the eastern United States), and this region has taken a proactive approach to assessing disease risks associated with shellfish culture. An extensive specimen collection and database of normal and abnormal conditions has been developed for oysters, clams and mussels. Such material is essential to accurately differentiate between opportunistic health challenges and primary pathogens. These health hazards require different control or management intervention, so an inaccurate diagnosis can lead to the use of control measures that are ineffective and costly. A pivotal component of developing health/disease profiles of culture species is to identify the effects of handling and culture strategies on the physiology of the animal being grown, as such effects may influence the development of disease. For example, slight changes in en-

vironmental conditions have the potential to induce pathogenic characteristics in otherwise latent infections.

Presently, there is little information available on the diseases and parasites infecting scallops in Atlantic Canada,[6] so research was undertaken to:

- complement existing disease/health knowledge of Atlantic Canadian scallop populations,
- enhance existing data on the distribution of infectious agents, to provide accurate health zonation and expedite risk assessments associated with live introductions and transfers of scallops, and
- help identify the research required to enhance scallop culture development.

Materials and Methods

Target Scallop Species

Placopecten magellanicus (giant sea scallop) (Gmelin 1791). Giant sea scallops are endemic to the Atlantic coast of North America, ranging from Cape Hatteras (North Carolina) to Labrador. They are limited to water temperatures less than 20°C and this results in scallops aggregating at varying water depths (ranging from 10 to 100 m). This species forms the basis for a rapidly developing aquaculture industry in Nova Scotia, Newfoundland, and Québec (Magdalen Islands and Basse Côte Nord).[2]

Interest in stock selection to promote optimal production characteristics has resulted in the movement of live scallops between provinces and oceanographic water bodies (Bay of Fundy, Gulf of St. Lawrence and the Atlantic coasts of Nova Scotia and Newfoundland). Since these stocks have never been mixed historically, the potential of exposing naive populations to "new" infectious agents is high and baseline information on the occurrence of infectious agents is required to accurately assess risk. The timing of our project is therefore critical, as scallop culture and related enhancement activities are beginning to accelerate.

Argopecten irradians (bay scallop) (Lamarck 1819). Taxonomically, there are three subspecies of *Argopecten irradians*, each having a different distribution pattern. In Atlantic Canada, *Argopecten irradians irradians* is of interest for culture. This subspecies typically ranges from Maine to New Jersey in the United States, occupying shallow sub-tidal depths of less than 10 m. The bay scallop is coveted for aquaculture purposes due to its rapid growth and production turnover. This species, which is not endemic to Atlantic Canada, entered the region in quarantine-controlled introductions in 1979, 1989 and 1991.[2] However, development of a bay scallop culture industry has been impeded by problems with disease, parasites and marketing. Originally, broodstock were reserved from the late summer/fall harvest of market-size scallops and overwintered in hatcheries. Recently, however, some stocks appear to have successfully adapted to the coastal waters of Prince Edward Island.[6] Since a major economic investment in this species involves the overwintering of broodstock, the existence of a self-sustaining population may rekindle interest in culturing this species. The health of these Canadian-adapted stocks should be compared to bay scallop populations in other areas to see if they have overcome the disease challenges faced by earlier generations.

Chlamys islandicus (Iceland scallop) (Müller 1776). The Iceland scallop has a range that extends from the Arctic Ocean to Casco Bay, Maine, and locally to Cape Cod, Massachusetts. There is little known about the health profile of this species and its cold, deep-water, habitat makes it currently unsuitable for culture. However, there is a small but significant fishery for this species on the north shore of Québec (Basse Côte Nord), and on the west and south shores of Newfoundland.[3] Since these locations are potential culture areas for giant sea scallops, it is important to have health information on the Iceland scallop. The results will also provide useful comparative data on host-specificity and physiological stress challenges.

Examination methods

One hundred and twenty giant sea scallops *(P. magellanicus)* were collected from wild stocks (60 from the stock off Digby, NS (November 5, 2000) and 60 from the stock off St. John's, NF (December 18, 2000). Sixty bay scallops (*A. irradians irradians*) were collected from naturally overwintering stocks off Ellerslie, PEI, on November 21, 2000. Due to the fall closure of the Iceland scallop fishery, no samples could be collected before the preparation of this article. All specimens were shipped live, on ice, to the Atlantic Veterinary College, University of Prince Edward Island (AVC-UPEI) for immediate processing.

Each animal was weighed (wet weight, whole specimen), measured (shell height) and any abnormalities of the external shell were recorded. Scallops were opened using a shucking knife. The appearance of the inner surface of the shell and soft tissues was recorded. Macroparasites were immediately fixed in 10% buffered formalin and stored in catalogued specimen vials until specific identification was done.

The soft-tissues of each scallop were detached from the shell:

- In smaller specimens (shell height < 2 cm), a complete dorsiventral section was made through the digestive gland, kidney, gonad, gills and mantle.

- In larger specimens (shell height > 2 cm), separate sections were taken from the gill, gonad, digestive gland, kidney and mantle.

Duplicate sections were taken for histological processing and for evaluation by transmission electron microscopy, if required. Sections were placed in tissue cassettes labeled according to species, location, date and diagnostic test. All tissue for light microscopy was fixed and stored in 1G4F solution until processing. The remaining soft tissues were pressed between 2 glass plates (4" x 4" x 0.2" (10 cm x 10 cm x 0.5 cm) and examined for the presence of macroparasites using a dissecting scope at 10x magnification.

Results and Discussion

Placopecten magellanicus

Gross observations of the scallops collected from Digby showed 4/60 (7%) had damage to the shell caused by *Cliona* sp.[7] (Porifera) (Table 1). Three specimens (5%) had damage caused by *Polydora* sp. (Polychaeta).[8] There were no visible anomalies to the soft tissues underlying the sponge or polychaete tunnels. Both of these shell-penetrating organisms can cause extensive damage by penetrating the inner nacre surface of the shell and irritating the underlying soft tissues. Scallop responses to such damage are energetically costly and chronic perforation can cause significant weakening and death. Since *Polydora* worms line their tunnels with mud, chronic incursion of mud into the extrapallial cavity can result in grossly visible mud-blisters as the scallop envelops the mud to separate it from their soft tissues. However, shell damage caused by *Polydora* sp. is usually less severe than that created by clionid sponges, which can penetrate the inner surface of the shell and cause significant shell loss at the hinge. Such penetration can also increase access to the soft tissues by secondary microbial infectious agents, such as those that cause bacterial abscess disease, which causes visible pustules on the adductor muscles of scallops rendering them unmarketable.

Histologically, *Rickettsia*-like organisms (RLOs) were present in 40% (24/60) of the Digby scallops with 46% of those demonstrating heavy infections (> 50 colonies per tissue section) (Table 1). Typically, these basophilic microcolonies are located in the gill epithelial tissue. However in this sample, most infections were found in the epithelial cells of the digestive tubules. RLO infections of scallops have not been directly associated with mortalities of scallops in Atlantic Canada. However, they have been associated with mass mortalities in *P. magellanicus* from Maine,[9,10] *Pecten maximus* from France,[11-13] and larval *A. irradians* from hatcheries in Maine.

Trichodinid ciliates were found in the gills of 8% of the Digby scallops (Table 1). These organisms are readily recognized by their large horseshoe macronucleus, circlet of hooklets and disc shape [5,14] (Fig. 1). Since whole specimens of these ciliates were not located, we were unable to identify the species. *Trichodina* sp. are known to have opportunistic,

Table 1. Prevalence (%) of parasites and fouling agents infecting scallops collected in Atlantic Canada during the winter of 2000 (values given as mean ± standard error)

Common name	Site	Mean Shell Height (cm)	RLO[a]	Perkinsidae	*Trichodina* sp.	Digenea	Turbellaria	*Polydora* sp.[b]	*Cliona* sp.[c]
Giant sea scallop	Digby, NS	67±5[d]	40	–	8	2 (larval)	2	5	7
Giant sea scallop	Avalon, NF	119±5	23	–	2	–	–	–	8
Bay scallop	Ellerslie, PEI	61±6	–	12	–	–	–	–	–

[a] *Rickettsia*-like organisms
[b] Likely *Polydora concharum* based on tunnel shape
[c] Likely *Cliona vastifica* based on pore size and shape

pathogenic potential in intensive culture systems. Thus, their presence on scallop soft tissue surfaces warrants close investigation. Similar trichodinid ciliates have been described from scallops in China,[14] as well as the Sea of Japan and Chile.[15-17]

An unidentified turbellarian flatworm parasite was found on the gills of one scallop from Digby, and a digenean metacercarian was found encysted in the digestive gland of another specimen from the same area. Most turbellarians found in bivalves usually occur in low numbers and do not provoke an obvious soft-tissue response. However, the turbellarian *Urastoma cyprinae*, which is commonly found in wild and cultured American oysters *(Crassostrea virginica)* in Atlantic Canada, and has been associated with mortalities in cultured blue mussels *(Mytilus galloprovincialis)* in Spain, has been associated with soft-tissue responses and changes in gill mucous biochemical properties.[18,19] Thus, turbellarians found on the gills of scallops warrant further investigation to clarify their identity, as well as their pathogenic potential under culture conditions.

Mollusc infections by digenean larvae usually stimulate focal haemocytic infiltration and encapsulation, but are rarely associated with extensive tissue damage (unlike the sporocyst larval stage of digeneans, which can cause significant pathology and castration of other bivalve species, such as mussels). There was little evidence that the metacercariae we discovered were pathogenic; however, the species and source of infection (other hosts involved in the life cycle) warrant further investigation. This is especially important with respect to meat quality, since encapsulated metacercariae can evoke calcareous encystment by host tissues, resulting in pearls.

Giant sea scallops from Newfoundland revealed clionid sponge invasion of the shells, comparable to that in the Digby sample, with a prevalence of 8%. No polydoriid polychaetes were found in any specimens from this area.

Rickettsia-like organisms infected 23% of the sample. Some of these infections were associated with high concentrations of ceroid cells in the digestive tubules (Fig. 2). The correlation between the RLOs and ceroid cells could not be determined. Ceroid cell accumulation has been associated with infectious agents in other molluscs (e.g., *Perkinsus marinus* in American oysters[20] and *P. karlssoni* in bay scallops[21]). Six scallops showed evidence of RLOs in the gonadal epithelia, which is an unusual tissue location for this type of infection. Two specimens showed unidentified inclusion bodies in gill epithelial tissue. All these intracellular infections require ultrastructural investigation to confirm their identity. DNA probes developed for salmonid *Piscirickettsia* could also provide an interesting comparative study, especially for scallops from the Bay of Fundy where there is intense salmon culture, to clarify whether or not this is a host-specific rickettsial organism, or group of organisms.

All specimens of *P. magellanicus* examined were in relatively good health. The infectious agents recovered have been previously reported and did not pres-

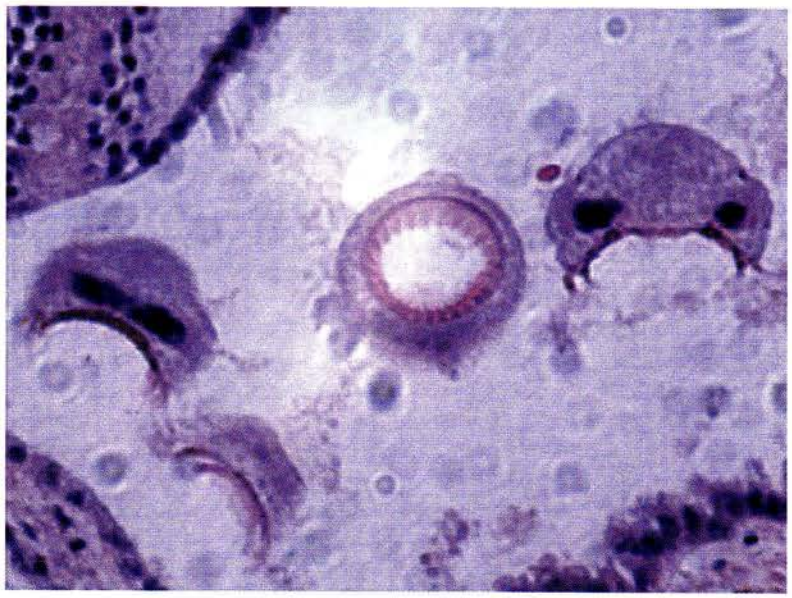

Figure 1. Photomicrograph of four (4) *Trichodina* sp. found in the vicinity of the gill of a giant sea scallop (*Placopecten magellanicus*). Haemotoxylin and eosin stain.

ent any apparent pathogenic threat to the wild specimens. However, none of these organisms have been properly identified (a common problem with surveys based on tissue sections) and their pathogenic potential under culture/enhancement conditions, as well as seasonal variation, is even more poorly understood. These questions are critical for accurate disease risk assessment and neither could be addressed within the study period supported by this program.

Argopecten irradians irradians (bay scallop)

Bay scallops collected from Ellerslie on November 21, 2000 were infected by *Perkinsus*-like organisms only, with a prevalence of 12% (7/60) (Table 1). Infections were characterized by intense swirl-like haemocyte aggregations, within which the parasite is frequently masked by heavy, ceroid cell aggregation. Transmission electron microscopy (TEM) of infected tissues was undertaken to confirm that the parasite was present within these lesions. Examination of the TEM material is ongoing.

Perkinsid-like organisms and their pathogenicity within bay scallops require extensive evaluation. This infectious agent has been in taxonomic limbo since its original description as *Perkinsus karlssoni*[22] (Apicomplexia: Perkinsidae). The validity of this description was drawn into question upon the discovery that certain aspects of this organism differed from typical *Perkinsus* spp. Biflagellates attributed to the zoospore stage of development showed molecular characteristics that related them to the thraustochytrid/labyrinthuloid complex, rather than the apicomplexa.[24] Obviously, in order to understand the biological nature of this organism, its taxonomy must be clarified. Recent development of a gene bank for thraustochytrids and related labyrinthulids at the National Research Council's Institute for Marine Biotechnology in Halifax, Nova Scotia has facilitated investigation of a related infection in hard-shell clams *(Mercenaria mercenaria)* known as quahaug parasite unknown (QPX).[25] These tools would provide useful taxonomic information on the tissue infections (via in situ hybridization) as well as the stages produced from infected tissues in fluid thioglycollate medium.

The Perkinsid-like organisms discovered in PEI stocks were shown, through extensive proximity challenges during the two last quarantine introductions, to be host specific for bay scallops. This reinforces results from subsequent investigations that showed the infection was introduced with the original bay scallop entry.[25] Since the quarantine procedure involved release of the F4 generation after multiple spawnings within quarantine, it indicates that infection of seed via broodstock is likely. Bay scallops have a low recovery rate after spawning and it is possible that this parasite may contribute to the host's inability to recover from spawning. Other *Perkinsus* spp. are known to influence mortality in oysters *(Crassostrea virginica)* from the eastern United States,[2] and abalone *(Haliotis ruber)* from Austra-

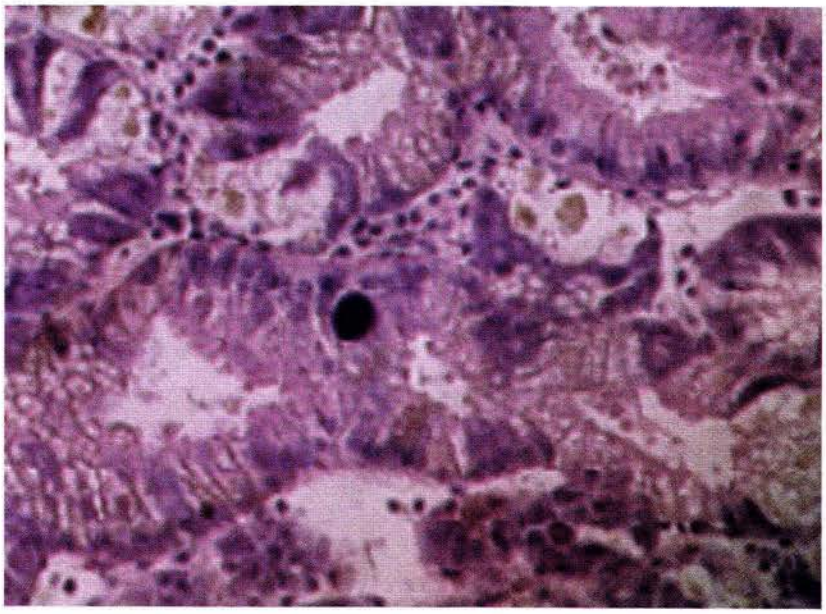

Figure 2. Photomicrograph of *Rickettsia*-like organism (RLO) in the digestive epithelium of a giant sea scallop (*Placopecten magellanicus*). Haemotoxylin and eosin stain.

lia.[26] Further research is therefore needed to clarify the pathogenesis of these organisms and their influence on post-spawning mortality in bay scallops. Infected tissues clearly show extensive disruption of the epithelial surfaces of the mantle, digestive gland and gonad. Based on the presence of the infection in open-water PEI bay scallops, however, it does not appear to have a significant adverse effect on survival.

Conclusions

During the winter conditions from which our scallop samples were obtained, the health profile of these populations does not appear to be of concern. The prevalence and intensity of the infectious agents did not show any direct evidence of a serious pathological threat. However, several of the agents found in this study are closely related to known pathogens of other bivalves, especially under culture conditions, and warrant further investigation.

This research will assess whether or not infective agents, which are common under wild conditions, could pose a serious threat to aquaculture stocks. Under containment/culture conditions, there is a strong likelihood (based on well-documented evidence from other culture species) that variation in handling and habitat characteristics, compared with wild growing conditions, could alter the pathogenicity of these agents. This could be a direct effect via pathogen proliferation, or indirect by suppression of scallop defense mechanisms. Either could have a serious impact on the development of a scallop culture industry. Scallops have well-documented physiological limits and do not demonstrate the wide tolerance ranges shown by mussels and oysters for temperature, salinity and air exposure. This research focuses on assessing the health hazards posed to scallop aquaculture. Without such data, future investment in the sector will be placed at unnecessary risk of uncontrollable losses.

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Haemic Neoplasia in Soft-shell Clams (*Mya arenaria*): Recent Outbreaks in Atlantic Canada and Discovery of a p53 Gene Homologue Associated with the Condition

Sharon E. McGladdery, Carol L. Reinisch, Gregory S. MacCallum, Raymond E. Stephens, Charles L. Walker and Jeffrey T. Davidson

In 1999, examination of soft-shell clam (*Mya arenaria*) mortalities at an experimental lease on Prince Edward Island (PEI) revealed a 95% prevalence of advanced haemic neoplasia (leukemia). Low prevalences of this condition had been documented previously in soft-shell clams from Nova Scotia and the Bay of Fundy, as well as the southern Gulf of St. Lawrence, but this was the first recorded case of mortalities in Atlantic Canada associated with the condition. An intensive survey of PEI clam beds revealed that both negative and positive sites for haemic neoplasia are linked to seed transfers as well as proximity to agriculture activity. An independent study investigating a similar condition in relation to industrial pollutants (e.g., PCBs) found high levels of leukemia in clams collected from Sydney Mines, NS, Kitimat Arm, BC, as well as sites along the east coast of the United States with high levels of anthropogenic substances. In an attempt to identify the role of industrial chemicals in inducing this condition, research focussed on the detection of a gene resembling the p53 gene family in leukemic clams. A similar gene has also been discovered in bar clams (*Spisula solidissima*). These findings suggest that clams may be ideal models for studying mechanisms of disease induced by chemical contaminants. Such molecular links to chemical exposure may have profound implications for many animals, including humans, and may prove useful for focussing on the triggers of neoplasia in clams from among the many possibilities (viral, genetic, environmental and anthropogenic).

Introduction

Soft-shell clams are a staple of the eastern seaboard seafood market. In Prince Edward Island (PEI), sales of clams generated over CDN$3 million in 1999. The total landed value of all species of clams in Atlantic Canada was over CDN$24 million. This solid market, along with fluctuating natural recruitment and successful enhancement initiatives along the east coast of the United States, has led to increased interest in soft-shell clam culture in Atlantic Canada. This aquaculture initiative is being spearheaded by the PEI Department of Fisheries and Environment and the New Brunswick (NB) Department of Fisheries and Aquaculture. In 1997, 15 experimental leases were issued for clam culture in PEI, in addition to the ongoing recreational and harvest fisheries. The culture leases are in areas where clam productivity has been historically high and the seed being relayed onto the leases are from conditionally-closed waters.

As with other molluscs, soft-shell clams are susceptible to several disease conditions. The best documented condition is haemic neoplasia, or leukemia, which transforms the blood cells of the clam from a functional state to a non-functional, often proliferative, state. The disease causes dedifferentiation of the nuclei of affected blood cells (haemocytes), which may or may not be accompanied by uncontrolled mitotic proliferation. Since normal blood cells are responsible for nutrient absorption, waste disposal, respiration, osmosis, and defense against trauma and invasive organsims in molluscs, such transformation, proliferation and necrosis of the blood cells weakens and may eventually kill the clam. Haemic neoplasia has been well documented in many marine bivalves since the early 1970s; in 1986, Peters[1] listed 15 affected species. Although most cases of haemic neoplasia occur at low prevalences[2] (< 5%) and are not associated with overt mortalities, some instances of higher prevalence in the United

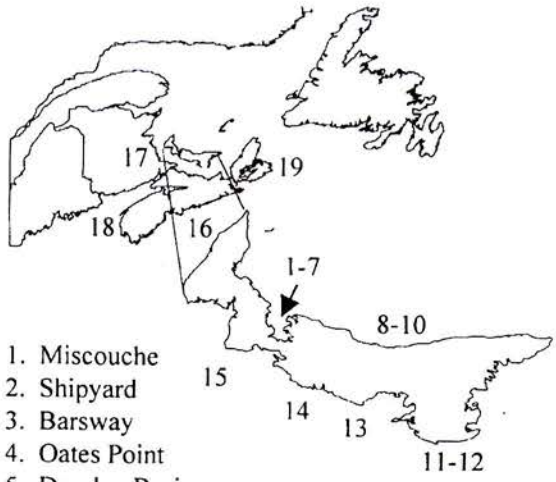
States have been associated with mass mortalities in mussels[3] and soft-shell clams.[4]

The factors associated with the occurrence of haemic neoplasia in soft-shell clams are not clearly understood. Multi-factorial and unrelated aetiologies have been documented,[3] including:

- anthropogenic substances such as chlordane and PCBs,[5-11]
- abnormal water temperatures,[12-14] and
- infectious triggers (e.g., viral).[15-16]

Regardless of the cause, disseminated sarcomas such as haemic neoplasia pose a significant threat to cultured and wild clam populations.[3,4,17-20]

Recent collections of soft-shell clams from Sydney Mines, NS, and Kitimit Arm, BC, have found significant levels of leukemia using a panel of monoclonal antibodies called the 1E10 series, which specifically recognize a protein on the leukemia cell surface.[21] This technique provides a more rapid and definitive diagnosis of leukemia than the traditional haematoxylin and eosin staining of fixed tissue sections or blood cell suspensions. The latter cannot readily distinguish cells undergoing early stages of leukemia transformation, since these do not manifest the classical enlarged and diffuse nuclei (compared to the condensed, granular, nuclei of functional haemocytes). Likewise, mitotic figures may be rare or absent in early stages of haemic neoplasia, compared to later stages of the condition, which also bear evidence of increased necrosis. The results obtained using these techniques may help provide a better understanding of the biochemical role(s) of anthropogenic agents in the manifestation of leukemia. Although most samples from the molecular label study were collected from wild populations, these scallops are inextricably linked to cultured populations, and the objective of the study is to enhance our understanding of this condition and its potential as both a bio-indicator and a threat to the development of clam culture. The technique may also provide a new mechanism for investigating the mass mortalities associated with this disease in the southern Gulf of St. Lawrence, for which a trigger is not clearly evident.



1. Miscouche
2. Shipyard
3. Barsway
4. Oates Point
5. Darnley Basin
6. Barbara Weit River
7. Wheatley River
8. Brackley Bay
9. Winter River
10. Tracadie Bay
11. Gascoigne Cove
12. Orwell River
13. West River
14. Victoria
15. Enmore River
16. Mill River
17. Richibucto River
18. Bay of Fundy
19. Sydney Mines

Figure 1. Main sample sites in the current and previous studies of soft-shell clam haemic neoplasia in Atlantic Canada.

Thus, the results reported in this paper are from two convergent studies:

1. Investigation of cultured clam mortalities in the southern Gulf of St. Lawrence, associated with high prevalences of haemic neoplasia; and
2. Concomitant investigations of the protein and genetic characteristics of the blood cells from clams (soft-shell and bar clams, *Spisula solidissima*) affected by leukemia at highly polluted sites in Canada and the United States.

Both studies aim to elucidate the factors associated with triggering the transformation of soft-shell clam blood cells and determining the factors that turn the condition from "normal" sub-clinical low prevalences to high prevalences associated with mass mortalities.

Materials and Methods

Mortalities associated with haemic neoplasia in cultured clams from the southern Gulf of St. Lawrence

Survey

Data from over 2000 soft-shell clams collected from histological material archived at the Gulf Fisheries Centre, along with tissues collected for inde-

pendent studies by the Atlantic Veterinary College, PEI Dept. of Fisheries and Environment, and St. Andrews Biological Station, were re-examined for evidence of haemic neoplasia. This was done to determine if the outbreaks observed in 1999 showed any relation to previously undocumented low prevalence sites, seasons, or size-classes of clams. In addition, an intensive survey was initiated in 1999 around Prince Edward Island to assess the geographic spread of the condition on both experimental culture leases and seed-source sites. The survey was continued in 2000, but the number of sites was reduced and the frequency of sampling was increased (Fig. 1).

Transmission experiments

Holding Conditions — Four recirculating tanks equipped with two biofilters and fed by water reservoirs (240 L) were used to hold the clams. Two tanks were used for the challenge experiments and the remaining two, located 2 m from the challenge tanks, were used for the controls. All tanks had splash covers and separate equipment was used to handle the clams in the the challenge and control tanks. Water temperatures were maintained at 20 ± 2°C. To prevent contamination from outside sources, access to the laboratory was restricted and foot dips were placed at the entrance to the lab.

The clams were held without a sediment substrate to facilitate observation of feeding, defaecation and weakening or mortality. Lack of substrate also enhanced stress-facilitated disease transmission, if transmissibility is possible. The clams were batch-fed cultured algae, with the recirculating pumps in the tanks turned off during feeding.

Pre-screening methods— Haemolymph (1-2 drops) was drawn from each clam, suspended in 3 mL artificial seawater, and allowed to adhere to microscope slides for approximately 30 minutes. The slides were fixed and stained for immediate examination. Another technique used was the direct removal of unsuspended haemolymph from the sub-cardiac sinus. An additional aliquot of the suspension was analysed using a Zahlkammer counting chamber (DYNATECH) to determine the number of cells per inoculation. A semi-quantitative scale was used to assess the stage/level of haemic neoplasia in both histological tissue sections and haemocyte preparations. Estimates of percentage of normal vs. neoplasm cells (Fig. 2a, b) were scaled as follows:

- Heavy = 51-100% of the haemocytes affected;
- Moderate = 11-50%; and
- Light = 1-10%.

Inoculation challenges — Approximately 0.2 mL of haemolymph from the anterior adductor muscle sinus of eight heavily infected clams (HN-positive) from Darnley Basin was pooled in 3 mL of filtered (0.22

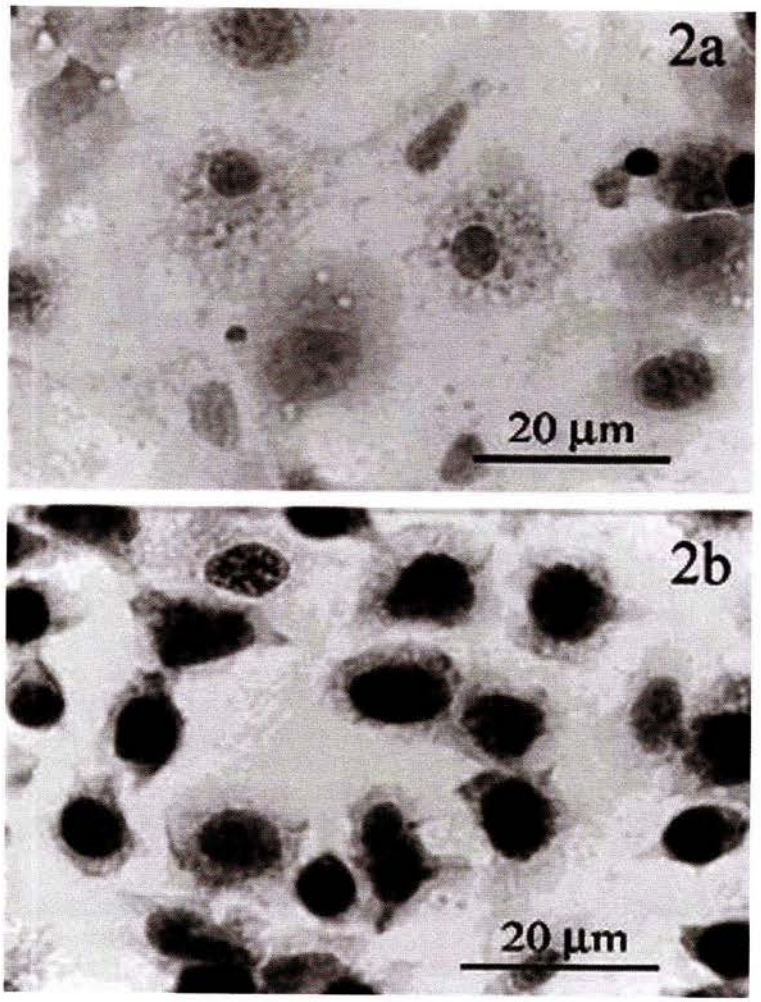


Figure 2. A — Normal haemocytes of a soft-shell clam collected using histocytology. B — Compare condensed, granular nuceli with haemocyte appearance.

μm) sterile seawater (FSS). Ten HN-negative Miscouche clams were inoculated with 0.2 mL of the HN-positive haemolymph suspension (1.85 x 10^5 haemocytes). Two control groups (n=5) were inoculated with: a) HN-negative haemolymph pooled from four Miscouche clams (4.2 x 10^4 haemocytes each); and b) 0.2 mL of cell-free FSS. All clams were monitored twice daily for morbidity (gaping) and mortalities (not responding to touch) for 38 days. Where possible, tissue from dead or dying clams was fixed for histological examination.

Inoculation challenges #2 — As described above, haemolymph from 9 HN-positive clams from Darnley Basin was pooled in 3 mL FSS. Fourteen pre-screened, HN-negtaive clams from Miscouche were inoculated with 0.2 mL of the suspension (5.1 x 10^4 haemocytes each). Fourteen control clams were inoculated with HN-negative haemolymph from three clams (2.3 x 10^4 haemocytes per clam) and another 14 HN-negative clams were inoculated with 0.2 mL of cell-free FSS. The clams were monitored as in experiment #1.

Immersion Challenge — 68 clams from Enmore were pre-screened and determined to be HN-negative. Thirty-four were used for immersion challenge and the remainder reserved as controls. Fourteen HN-positive clams from Barbara Weit River were used to prepare a tissue homogenate diluted 50:50 in FSS. Thirty-four uninfected clams were placed in a 40 cm x 30 cm x 10 cm high tray containing 150 mL of homogenate in 8 L of FSS for 3h and then returned to a holding tank. The 34 remaining clams from Enmore were placed in a separate tray containing FSS for 3 hrs, before being returned to a separate holding tank. Monitoring was conducted as for the inoculation experiments.

Proximity Challenge — 48 Miscouche and 48 Darnley Basin clams were pre-screened. Twenty-six were HN-positive (8 from Miscouche; 18 from Darnley Basin). Ten HN-negative clams (group A)

Table 1. Results of soft-shell clam survey for haemic neoplasia in the southern Gulf of St. Lawrence, 1997-2000.[a]

Site/Year	1997	1998	1999	2000
Miscouche	–	–	0%	4-13%
Shipyard	20%	–	7-33%	–
Barsway	55%	–	17-52%	–
Oates Point	0-20%	5%	9-80%	20-70%
Darnley Basin[b]	–	–	20-95%	–
Barbara Weit	–	–	17-63%	83%
Wheatley River	–	–	0%	–
Brackley Bay	75%	–	–	50%
Winter River	–	–	33%	13%
Tracadie Bay	–	–	6-29%	–
Gascoigne Cove	0-1%	–	–	40%
Orwell River	–	–	–	8%
West River	–	–	3-5%	0-25%
Victoria	–	–	–	0%
Enmore River	–	–	0%	0%
Mill River	–	–	5-13%	10%
Richibucto River[c]	–	–	4-34%	–

[a]1985-87 survey of soft-shell clams in the Bay of Fundy [24] < 6% prevalences, with the exceptions of 22-31% at the head of the bay in 1996. Samples from the Gulf of St. Lawrence (1990-1997) = <11%
[b]Site of original reports of mass mortalities. Beds crashed by November 1999.

were placed 60 cm downstream from the HN-positive clams and 30 HN-negative clams (group B), were placed 30 cm downstream of the HN positive clams (group C). Another 30 HN-negative clams were divided into two groups (groups D and E) in a separate tank to serve as controls. The clams were monitored as described above and the experiment ran for 36 days.

Protein and genetic characteristics of clams collected from highly polluted sites

Clams were collected from Sydney Mines, NS, and Kitimit Arm, BC, in 2000. Using monoclonal antibodies belonging to the 1E10 series, the clams were shown to have significant levels of leukemia. These clams form part of an extensive survey of North American sites to investigate the homogeneity/heterogeneity of haemic neoplasia/leukemia in soft-shell clams and related bivalves. Details of these collections can be found in Stephens et al.[22] and Jessen-Eller et al.[23]

Results

Mortalities associated with haemic neoplasia in cultured clams from the southern Gulf of St. Lawrence

Survey

The results from the 1997 and 1998 archived histology samples and fresh material collected between 1999 and 2000 are shown in Table 1. Place names correspond to locations given in Figure 1.

The data from over 2000 soft-shell clams from archived histological material at the Gulf Fisheries Centre, collected between 1990 and 1997,[25] revealed consistently low levels of haemic neoplasia (<11%). This was the same scenario for tissues collected from clams along the New Brunswick shore of the Bay of Fundy for gametogenic studies at the St. Andrews Biological Station; no samples were collected from the sites that had prevalences of 21.9-31.1% in 1986.[24]

Conversely, tissues collected for a parallel gametogenic study by the Atlantic Veterinary College [26] and the PEI Dept. of Fisheries and Environment [27] revealed high levels of haemic neoplasia at several sites on PEI since 1997 (Table 1). Three of these sites (Barsway, Oates Point and Shipyard) were located in the proximity of Darnley Bay, the site of the 1999 outbreak. Interestingly, a fourth area of high prevalence (75%) was detected in Brackley Bay, which corresponded to an area found to have high prevalences in 1999 (Table 1). In 2000, the number of sites examined was reduced and the emphasis shifted to tracking select negative and positive sites. Results showed that one site (Miscouche) located within the Malpeque Bay affected area, that had been negative in 1999, began to show low prevalences in 2000. Likewise, two sites with low prevalences (West River and Gascoigne Cove) in southeastern PEI showed increased levels of haemic neoplasia in 2000. Barabara Weit and Oates Point maintained high prevalences of the condition in 2000 (83.3% and 20-70%, respectively). All samples collected elsewhere in Atlantic Canada, as part of routine monitoring, for health check requirements by DFO Introductions and Transfers Committees, showed no evidence of the condition (despite prevalences of 34% being detected on the New Brunswick shore of the Gulf of St. Lawrence in 1999).

Transmission experiments

The initial pre-screening of clams resulted in significant mortality within 24 hours, especially for clams subsequently determined to be HN-positive. This reduced the number of heavily HN-positive clams available for the challenges, so pooled haemolymph and tissue homogenates were used.

Inoculation challenge #1 — by 3 days post-inoculation (DPI), 10.0% (1/10) of inoculated clams showed low level HN using histology. By 4 DPI, 20.0% (2/10) (cumulative prevalence) showed signs of HN. The five remaining clams died immediately thereafter and were too necrotic to permit histology. The total cumulative prevalence of haemic neoplasia 38 DPI was 20.0% (2/10 clams). All control clams were confirmed to be HN-negative using histology and haemolymph analysis.

Inoculation challenge #2 — by 13 DPI, 7.1% (1/14) of inoculated clams were confirmed to be HN-positive using histology. At 29 DPI, 14.3% (2/14) were HN-positive and at 30 DPI, 21.4% (3/14) were positive (cumulative prevalences). Interestingly, all the haemolymph samples were negative. By 42 DPI, all controls (seawater and negative haemolymph inoculations), were negative according to both histology and haemolymph screening.

Immersion challenge # 3 — At the end of this experiment (30 DPI), 9% of clams exposed to tissue homogenate from HN-positive clams, showed low levels of haemic neoplasia (by histology). All control clams were negative. As with the second inoculation challenge, both immersion challenge and control were found to be HN-negative using haemolymph screening.

Proximity challenge — Miscouche clams (group A), placed 60 cm from HN-positive clams, showed a cumulative HN of 20.0% by day 36 (end of the experiment). Darnley Basin clams (group B), pre-screened as HN-negative, showed a cumulative HN of 20.0% by day 36. Using histology, all control clams were determined to be negative at the end of the experiment.

As above, both challenge and controls were negative using haemolymph screening.

Protein and genetic characteristics of clams collected from highly polluted sites in Canada and the United States

Monoclonal antibodies belonging to the 1E10 series have been shown to react with soft-shell clam neoplasia cells, but not healthy cells, in clams collected from a wide variety of sites in North America, including recent collections from Sydney Mines, NS, and Kitimit, BC. Samples from PEI and other positive sites in Atlantic Canada have yet to be cross-tested using these antibodies.

Recent work on the biochemistry of these antibodies, has demonstrated cross-reactivity with a 252 kD transmembrane glycoprotein with spectrin/dystrophin-like characteristics. These proteins have a possible linkage to the p53 gene family.[22]

In another molluscan model, the bar (or surf) clam, *Spisula solidissima*, a new member of the p53 gene family, p120, is down-regulated with time if embryos of the clams are exposed to a low level of PCBs during *in vitro* fertilization. In contrast, levels of p53 remain constant. These results show interesting parallels with oncogenic studies in vertebrate models (including humans)[23] and details of the results from these studies are in press.

Discussion

The apparent recent and significant increase in levels of haemic neoplasia in Atlantic Canada is cause for concern on two levels. Over the last eight years, the soft-shell clam culture industry has shown strong potential for stabilising production of a staple and popular maritime seafood, as well as putting seed from conditionally closed sites to production use. The emergence of haemic neoplasia in the midst of this development poses a significant challenge — both in assessment of the risk of spreading the disease when seed is moved, as well as during the processing of market produce. In addition, identification of the trigger is essential for accurately identifying possible mitigative measures. The range of factors associated with haemic neoplasia in bivalves makes this a highly complicated process

An infectious aetiology was suspected in the Chesapeake clam mortalities and recent reverse transcriptase activity reinforces the possibility of a retrovirus trigger.[19] Challenge experiments designed to determine whether or not the condition in the southern Gulf of St. Lawrence could be transmitted directly from clam to clam, indicate the possibility of such transmission. Especially interesting is the result from the proximity experiment using HN-negative clams held in proximity to affected clams, but without exposure to or inoculation with haemolymph or tissue homogenate (mimicking tissue dispersal post-mortality). Both downstream groups developed evidence of neoplasia within the experimental period. Unfortunately, however, the negative histocytology screening, compared with histology results, renders the pre-screening process of dubious merit and it cannot be determined whether or not the time zero HN-negatives from Darnley Basin or Miscouche were truly negative. This means that the results can only be considered as preliminary indications of transmissibility and require repetition using conclusively negative controls, screened using both standard tools and a more sensitive technique such as the 1E10 monoclonal antibodies (discussed below).[21-23]

In addition to transmissibility, another possibility is a correlation with a broader environmental change (climatic or anthropogenic) and it is only the recent development of clam culture and closer monitoring of leased clam beds that has facilitated early detection of mortalities (as opposed to post-mortality detection of masses of empty shell, which negate disease screening). Comparison of HN-positive and HN-negative sites provide no obvious similarities, although the proximity of intense agriculture cannot be overlooked. There is little run-off protection from most field-based activities and some freshwater fish mortalities have been attributed to pesticide run-off. Notwithstanding this coincidental observation, much more work is required to prove or refute this correlation. Clam mortalities at Richibucto, NB, in the late spring and early summer of 1999 were also found to be caused by the same (or a closely related) haemic neoplasia condition, and these clams were not located near intense agriculture (although other human activities were present).

Although records back to 1997 suggest this condition may have been developing over the last 1-2 years, it should also be noted that mass mortalities of clam beds have been well-documented for years, but have lacked diagnostic follow-up. Most mortalities have been attributed to overfishing (clams disturbed to the point that they can not re-bury themselves) or smothering due to sediment or algal mat deposition. In Darnley Basin, as well as elsewhere in the southern Gulf of St. Lawrence, many shallow, warm-water estuaries have become inundated by sea lettuce (*Ulva lacteus*), which is known to cause sediment anoxia and subsequent clam mortalities. All these factors are threats not only to developing soft-shell clam aquaculture, but wild populations as well. To date, only soft-shell clams appear to be affected. A similar condition affects blue mussels and one case submitted for laboratory examination due to abnormal mortali-

ties in 1999, also showed an elevated prevalence of haemic neoplasia.[28] Mussels and oysters sampled from Darnley Basin at the same time as the original detection of soft-shell clam neoplasia, however, showed no signs of neoplasia or elevated mortalities.

Another interesting observation made during the tracking of heavily affected clam beds is the fact that not all clams appear to be dying from the condition. Clams from Oates Point also show high prevalences of haemic neoplasia, but the clams appear to be growing normally and bed production remains high. This suggests a further complication in the disease-host-environment interaction triad, i.e., the disease only becomes clinical under adverse growing conditions (possibly in the presence of sea-lettuce or another environmental variable).

The relationship among retroviruses, carcinogenic transformation and immunological suppression (regression) of molluscan neoplasias has yet to be clearly established.[3] Recent work on haemocyte dynamics, in association with both molluscan defense (phagocytosis) capabilities, as well as environmental contaminants, has improved knowedge in this area.[29-32] This, in addition to the immunoassays[33] and developing nucleic acid assays,[19] have enhanced our capability to pursue this question in greater depth. Given the emergent pressures for sustainable aquatic harvest (commercial and recreational fisheries, as well as aquaculture) along with increasing concern over inshore coastal quality degradation, these two components are well-situated to provide better answers to their interaction using the clam neoplasia epidemiological (ecological and man-made factors influencing the emergence and proliferation of a disease) model.

One major target of industrial chemicals appears to be the p53 gene family. Analysis of the p53 gene obtained from PCB-exposed soft-shell clams revealed a mutation in exon 6.[34] Recently, Kelley et al.[35] described the appearance of p73 and the disappearance of p97 coincided with leukemia-specific protein synthesis. In contrast, levels of p53 protein remain constant. These observations, along with consistent cross-reactivity by soft-shell clam leukemic haemocytes to the monoclonal antibody proteins 1E10, provide a promising avenue for determining the role of specific chemicals (and other measurable factors) on the triggering, development and regression of soft-shell clam and other molluscan neoplasias (e.g., gonadal neoplasia of soft-shell clams,[36] which was detected in 25% of soft-shell clams in Gascoigne Cove, along with 0-1.3% haemic neoplasia in 1999).

Conclusions

Both studies on haemic neoplasia in soft-shell clams indicate an acute need to refine our understanding of this condition. This is necessary to accurately assess the risks to developing culture of this species, as well as from the increased movement of seed and live market stock for processing. In addition, it is imperative to better understand the environmental factors that have a direct and indirect role to play in haemic neoplasia. Traditionally, mussels have filled the "environmental bio-monitor" niche for marine invertebrates. It seems likely, however, that soft-shell clams, in their sediment habitat, may be better environmental sentinels. Combining this possibility with the enhanced monitoring implicit in culture development, provides an opportunity to enhance results on two fronts — soft-shell culture productivity and environmental sentinels.

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The Efficacy of Clove Oil as an Anesthetic for Decapod Crustaceans

John Morgan, Coral Cargill and Erick Groot

Clove oil was evaluated as an anesthetic for use on three Pacific coast crab species: dungeness crab *(Cancer magister)*, hairy shore crab *(Hemigrapsus oregonensis)* and northern kelp crab *(Pugettia producta)*. Bath treatments of clove oil were successful in immobilizing all three species with no apparent distress. Induction times were dose dependent and varied considerably between the three species. Clove oil was most effective in kelp crabs with time to immobilization ranging from 54 to 2 min at 0.015 to 0.25 mL/L. Shore crabs demonstrated the longest induction times (188 to 87 min) and required the highest concentrations (1 to 3 mL/L) to achieve immobilization. Dungeness crabs showed an intermediate response with induction times ranging from 68 to 16 min at 0.5 to 1.5 mL/L. Recovery times for the three species averaged 14, 65 and 10 min for kelp, shore and dungeness crabs, respectively, and were independent of the dosage applied. The variation in dose responses to clove oil observed among the three crab species was likely due to differences in the ability to cease ventilation, reflecting different life history strategies (i.e., intertidal versus subtidal). The results suggest that clove oil is a useful crab anesthetic for teaching and research purposes, and it may also find application as a sedative to reduce stress and mortality during live transport of commercially important crustaceans (e.g., crabs, lobsters).

Introduction

Anesthetics are widely used in fish culture for routine husbandry procedures such as handling, transporting and harvesting.[2] Much less attention has been paid to marine invertebrates, but recently there has been an increased focus on animal welfare concerns, particularly in university teaching and research institutions, where anesthesia of laboratory animals is desirable to ease handling and reduce stress. Anesthetics also have application in commercial situations such as the transport and harvesting of species such as crabs and lobsters. Although numerous methods of immobilizing and euthanizing crustaceans have been developed (e.g., chilling, drowning), many are slow, inconsistent or appear to cause trauma.[2] According to the Canadian Council on Animal Care (CCAC),[3] the recommended anesthetics for cephalopods and crustaceans are tricaine methanesulfonate and benzocaine. However, these anesthetics are generally not effective for many crustacean species.[2,4] Effective, safe and cost-effective alternatives are therefore needed, both for teaching and research purposes, and for commercial applications.

Clove oil is a natural compound that is derived from the leaves, buds and stems of the clove tree. Its active ingredient is eugenol (4-allyl-2-methoxyphenol), which can comprise 90-95% of clove oil by weight. Clove oil has been used for many years as a food additive and a topical analgesic in dentistry, and also has antibacterial, antifungul and antioxidant properties.[5] Clove oil has recently become a popular fish anesthetic[5-8] and has also been used successfully for humanely immobilizing and euthanizing the Australian giant crab, *Pseudocarcinus gigas*.[2]

The objective of this study was to determine the efficacy of clove oil as an anesthetic for Pacific coast crabs. Three species were chosen based on their importance to teaching, research and commercial fisheries. Dungeness crabs *(Cancer magister)* are a commercially-important species that are transported live to market; hairy shore crabs *(Hemigrapsus oregonensis)* inhabit the intertidal zone and are routinely used in invertebrate zoology teaching labs; and northern kelp crabs *(Pugettia producta)* are a mainly subtidal species commonly found in the Pacific Northwest.[9] Trials were also conducted to compare the response to tricaine methanesulfonate with that to clove oil.

Table 1. Crab size-characteristics, chemical concentrations, and water temperatures used in the anesthetic trials.

	Dungeness crab	Shore crab	Kelp crab
Mean weight (g)	662	1.6	60
Mean carapace width (mm)	172	15	167
Clove oil (mL/L)	0.25, 0.5, 1.0, 1.5	1.0, 1.5, 2.0, 3.0	0.015, 0.03, 0.06, 0.125
Ethanol (mL/L)	9	27	0.6
TMS (g/L)	2.5	2.5	0.125, 0.5, 2.5
Water temperature (°C)	14-17	8-10	10-12

Materials and Methods

Test animals

Dungeness crabs were collected by trap from Cowichan Bay, BC in August 1999. Hairy shore crabs were hand-picked from the intertidal zone in Cowichan Bay in February 2000. Northern kelp crabs were collected by diving in the Ten Mile Point area near Victoria, BC in April 2000. The crabs were transported to Malaspina University-College (MUC) and acclimated in a saltwater recirculating system (salinity = 30‰) for at least 1 week before testing. Size characteristics of the crabs and water temperatures during acclimation and testing are given in Table 1.

Criteria for assessing anesthesia

Loss of equilibrium is a standard measure of anesthesia in fish,[1] but crabs typically do not lose equilibrium when anesthetized, so an alternative method for determining anesthesia and recovery for crabs was required. Gardner[2] considered the Australian giant crab to be anesthetized (paralyzed) when the claws could no longer be used for defense; however quantifying this response in some species can be somewhat subjective. A commonly-observed phenomenon with crabs is that when placed on their backs to expose the softer abdomen, they immediately attempt to right themselves and return to a less vulnerable position. With the onset of anesthesia, the crabs become immobilized and are unable to return to an upright position. Thus, for the purposes of this study anesthesia and recovery were defined as follows:

- Anesthesia — Crabs become immobilized and lose the ability to right themselves when placed on their back
- Recovery — Crabs regain the ability to right themselves and take up a defensive posture

Anesthetic trials

Dungeness and kelp crabs were exposed to anesthetics in bath treatments conducted in individual 20-L containers. Shore crabs were exposed in groups of six in 1-L containers. Continuous aeration was provided to the containers and the water in the containers was drawn from the larger holding tanks so that salinity and temperature were kept constant between acclimation and testing. Due to its incomplete solubility in water, clove oil (Xenex Laboratories, Coquitlam, BC) was first dissolved in ethanol at a ratio of 1:9 (vol/vol) before being added to the seawater. Separate trials with ethanol were also carried out to determine if the ethanol levels used to dissolve the clove oil had any anesthetic effect. Tricaine methanesulfonate (AquaLife TMS; Syndel Laboratories, Vancouver) was added directly to the containers and thoroughly mixed. The concentrations of clove oil, ethanol and TMS tested on the 3 species are given in Table 1.

For each trial, the crabs were added to the anesthetic solutions and the time to onset of anesthesia (immobilization) was recorded. The crabs were then removed from the anesthetic bath, weighed, placed into a similar container containing anesthetic-free seawater, and the time to recovery was recorded. The crabs were also observed for any signs of distress during testing and once recovered were kept overnight to check for delayed mortalities (none were observed). The number of crabs used for each treatment varied depending on availability of the species and the lack of response to some treatments (e.g., ethanol).

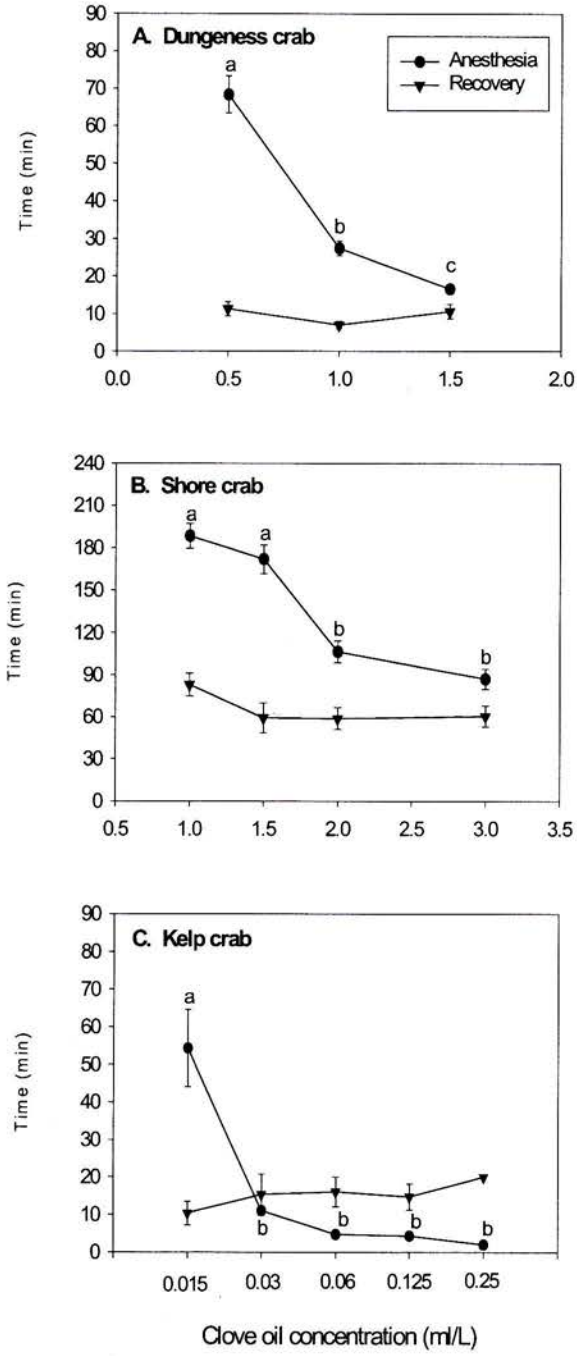

Figure 1. Effect of clove oil concentration on time to anesthesia and recovery for A) dungeness crab *(Cancer magister*; n=4-5), B) hairy shore crab *(Hemigrapsus oregonensis*; n=6), and C) northern kelp crab *(Pugettia producta*; n=3). Data are shown as means ± SE. Means with different letters are significantly different ($P < 0.05$, one-way ANOVA). There were no significant differences in mean recovery times for any species ($P > 0.05$).

Statistical analysis

Data are presented as means ± 1 standard error (SE) where appropriate. Results of the anesthetic trials were analyzed using a one-way analysis of variance (ANOVA). Significant treatment means were identified using Tukey's multiple comparison test ($P < 0.05$).

Results

Dungeness crab

Clove oil was ineffective with dungeness crabs at a concentration of 0.25 mL/L. Crabs were immobilized at clove oil concentrations of 0.5 to 1.5 mL/L. Mean time to induction varied from 68 to 16 min and decreased significantly with increasing dose (Fig. 1A). Mean recovery times ranged from 7 to 11 min and did not differ significantly between the doses of clove oil used. TMS and ethanol had no visible effect after 4 h (Table 2).

Shore Crab

Shore crabs required higher clove oil concentrations and had longer induction times than dungeness crabs, with mean times to immobilization ranging from 188 to 87 min at concentrations of 1 to 3 mL/L. Mean induction times at clove oil concentrations of 1 and 1.5 mL/L were significantly higher than those observed at 2 and 3 mL/L (Fig 1B). Mean recovery times ranged from 59 to 83 min and, comparable to dungeness crab, did not differ significantly between clove oil concentrations. Also in agreement with dungeness crabs results, TMS and ethanol had no visible effect on shore crabs during the observation period (Table 2).

Kelp Crab

Clove oil was most effective on kelp crabs with immobilization occurring at a concentration as low as 0.015 mL/L. Mean induction time was 54 min at this concentration and decreased substantially at higher doses (0.06 to 0.25 mL/L), ranging from 11 to 2 min (Fig. 1C). Mean recovery times ranged from 10 to 20 min and did not differ significantly between clove oil concentrations. In contrast with the other two crab species, recovery times for kelp crabs tended to be longer than induction times.

Similar to dungeness and shore crabs, ethanol had no anesthetic effect on kelp crabs. Unlike

Table 2. Results of anesthetic trials using tricaine methanesulphonate (TMS) and ethanol to assess responses of dungeness *(Cancer magister)*, hairy shore *(Hemigrapsus oregonensis)* and northern kelp *(Pugettia producta)* crabs. Times are given as means ± SE where appropriate.

Species/ Treatment	Concentration	Time to Anesthesia (min)	Time to Recovery (min)	Indication of Stress
Dungeness crab				
TMS (n=2)	2.5 g/L	No effect after 4 h	–	None
Ethanol (n=2)	9 mL/L	No effect after 4 h	–	None
Shore crab				
TMS (n=6)	2.5 g/L	No effect after 21 h	–	None
Ethanol (n=6)	27 mL/L	No effect after 21 h	–	None
Kelp crab				
TMS (n=3)	0.5 g/L	25.7 ± 0.9	8.7 ± 0.9	Agitated behavior, became rigid
	0.125 g/L	No effect after 4 h	–	No apparent distress
Ethanol (n=3)	0.6 mL/L	No effect after 4 h	–	None

the other two species, kelp crabs exposed to 2.5 g/L TMS were almost immediately immobilized. Exposure to a reduced TMS concentration (0.5 g/L) was accompanied by agitated behaviour and at the onset of immobilization the appendages curled in towards the abdomen and the body became rigid. Mean induction and recovery times at 0.5 g/L TMS were about 26 and 9 min, respectively (Table 2). A lower dosage of TMS (0.125 g/L) had no visible behavioural or anesthetic effects after 4 h.

Discussion

Clove oil was shown to be an effective anesthetic for the three crab species tested in this study, with no apparent distress or delayed mortalities. The times to anesthesia were generally inversely related to dose and recovery times were independent of dose for all three species. The crabs differed in their sensitivity to clove oil, with kelp crabs requiring the lowest doses and having the fastest induction times, shore crabs the highest doses/longest induction times, and dungeness crabs showing an intermediate dose response. For routine use, clove oil doses of 0.06 mL/L (kelp crab), 1 mL/L (dungeness crab) and 3 mL/L (shore crab) are recommended to give reasonable immobilization times (< 5 min, < 30 min, and < 90 min, respectively).

The variation in dose responses to clove oil observed among the three crab species was likely due to differences in life histories. Hairy shore crabs inhabit the intertidal zone and are required to cover their gills and cease ventilation during portions of the tidal cycle when they are exposed to air. As a result they have a high capacity for anaerobic metabolism that is shared by many intertidal organisms. It was noted during the trials that the shore crabs closed their mouth parts and showed infrequent gill ventilation when placed in the clove oil bath treatments. The higher concentrations and longer induction times observed in this study were therefore presumably a result of the shore crab's ability to withstand exposure to unfavourable conditions by ceasing ventilation. Kelp crabs, on the other hand, are typically subtidal and may be less able to respond to an unfavourable environment by regulating gill ventilation; active gill ventilation was observed in all trials with clove oil. The intermediate response of the primarily subtidal Dungeness crab may be due to its ability to bury in the sand, a behavior that would require some capacity to regulate gill ventilation.

TMS was not effective as an anesthetic for dungeness and shore crabs at a concentration (2.5 g/L) that is much higher than used for finfish.[1] TMS has also been shown to be ineffective with other species of decapods.[2,4,10] TMS was effective with kelp crabs at

concentrations < 0.125 g/L; however it appeared to cause distress. Given the inconsistent and generally ineffective responses of decapods to TMS, we recommend that it should no longer be listed by CCAC as an acceptable anesthetic for crustaceans.

Applications

The results of this study indicate that clove oil is an effective and humane crab anesthetic for teaching and research purposes where the animals need to be immobilized prior to handling. Higher doses and/or longer exposure times may also be used to euthanize crabs before carrying out dissections.[2] Clove oil has also been used as a relaxant with pearl oysters[11] and thus may have wider application with other aquatic invertebrates (e.g., bivalve molluscs, echinoderms). In addition to its anesthetic properties, clove oil is readily available, inexpensive and relatively safe to use compared to other crustacean anesthetics such as chloroform.[2]

Clove oil may also have application as a sedative to reduce stress and mortality during live transport of commercially-important crustaceans. Low concentrations of the anesthetic would decrease metabolic rates and reduce activity levels, especially in subtidal species such as lobsters and the edible crabs. These species are commonly transported long distances to live markets and sedation may therefore reduce the build-up of metabolic wastes and increase survival. Sedation would also decrease antagonistic behaviour between crabs that are often transported together in bulk containers. A potential drawback to the use of clove oil in commercial species is the strong smell of the oil, which may alter the taste of the meat.[2] Another factor to consider is that although clove oil has been classified by the US Food and Drug Administration as a GRAS (generally recognized as safe) substance,[5] it currently does not have American or Canadian regulatory approval for use with food fish or crustaceans. This limits its suitability to commercial situations but does not preclude its use for teaching or research purposes.

We would like to thank Gord Edmondson of MUC for providing the facilities and Jim Cosgrove of the Royal BC Museum in Victoria for collecting the kelp crabs. Funding for this project was provided by the MUC Bamfield Research Fellowship.

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Investigating the Cause of Episodic Mortalities in the Giant Scallop, *Placopecten magellanicus,* in the Gulf of St. Lawrence

Sonia Belvin, Réjean Tremblay, Marcel Roussy and Sharon McGladdery

Scallops from both natural beds and aquaculture farms along the Lower North Shore of Québec (northern Gulf of St. Lawrence) have experienced severe episodes of mortality in the past few years. Challenge tests and histopathological analysis were used to determine if a pathogenic agent or a virus could be responsible for the mortality. The challenge tests attempted to induce mortality similar to that observed in scallops from the Lower North Shore. The pattern of mortality in the first challenge test revealed that Lower North Shore scallops were more sensitive to stress than scallops from the Gaspé or the Magdalen Islands. An inoculation challenge into the muscle caused significant stress and mortality, regardless of the geographic origin of the scallops. The second challenge test showed that the injection of foreign material into scallops caused significant mortality. The results of the third challenge test seemed to suggest the presence of an epidemiological infection, but no pathogenic agent was found during histopathological analysis that might be responsible for the mass mortality. The cause of the episodic mortality is still unknown.

Les populations de pétoncles du Golf du St-Laurent ont été le siège d'épisodes de mortalité massive au cours des dernières années tant sur les gisements naturels que sur les structures d'élevage. Le Challenge Test a été entrepris afin de déterminer si un agent pathogène et/ou un virus pourrait être le facteur responsable de ces mortalités. L'expérience s'est déroulée en quatre parties, soit Challenge Test 1,2 et 3 suivi d'analyses histopathologiques. Le Challenge Test consistait à reproduire les épisodes de mortalité observées chez les pétoncles de la Basse-Côte-Nord. Les résultats de mortalité ont révélé que les pétoncles en provenance de la Basse-Côte-Nord sont plus sensibles au stress que les pétoncles de la Gaspésie et des îles-de-la-Madeleine. Une injection dans le muscle représente également un facteur de stress important pour les pétoncles peu importe l'origine de la population. Le Challenge Test 2 a permis de déterminer que l'injection d'un corps étranger a un impact significatif sur le taux de mortalité chez les pétoncles. Bien que les résultats de mortalité du Challenge Test 3 semblent révéler la présence d'une infection épidémiologique, aucun agent pathogène inconnu ou non-identifié pouvant être responsable des mortalités massives n'a été observé lors des analyses histopathologiques. Les causes exactes des mortalité restent encore à déterminer.

Introduction

In recent years, populations of giant scallops, *Placopecten magellanicus,* from the northern Gulf of St. Lawrence, particularly those along the Lower North Shore of Québec, have experienced mass mortality. The mortality episodes have occurred in both scallop beds and aquaculture facilities[1] and have had

a significant impact on the scallop stocks. Mortality of up to 80% of the stock occurred in 1993, 1997 and 1998.

The exact cause of the mortality episodes is unknown. Histopathological studies conducted in 1993[2] revealed a high level of infection, but it did not seem to be related to the observed mortality, as the same infection has been observed in other stocks in the Gulf that have not experienced heavy mortality.

Challenge tests were conducted to determine if an unknown or an unidentified pathogen could be responsible for the mortality. The tests were conducted in four steps: three challenge tests were followed by histopathological analysis of tissues extracted from the challenged scallops.

The objectives of this study were: 1) to determine the effect of inoculating healthy scallops with homogenized scallop tissue assumed to be infected with a pathogen, and 2) to identify the infectious agent.

Methodology

Challenge test 1

In the first challenge test, healthy scallops (total N of 270) from three stocks (Magdalen Islands, La Tabatière on the Lower North Shore, and Percé on the Gaspé coast) were challenged with tissue extracted from moribund scallops. The moribund scallops originated from Shekatika, an aquaculture facility in Jacques Cartier Bay on the Lower North Shore of Québec. The kidney, digestive gland, mantle and gills were removed from the scallops and homogenized in filtered sterile seawater. Half the mixture was centrifuged. Apparently healthy scallops from the three stocks were challenged in one of three ways with either centrifuged or uncentrifuged homogenate. The homogenates were injected into the mantle cavity or the muscle, or the scallops were exposed to an immersion challenge by mixing the homogenates with the sea water in which the scallops were being held. Healthy scallops inoculated with sterile sea water served as controls.

The treated and control scallops were held in running seawater (10°C) and fed with 10 000 cells/mL of TISO/MONO. Mortality was recorded over the next 50 days.

Challenge test 2

Tissues were extracted from healthy scallops and the homogenates were centrifuged and injected into scallops from the Magdalen Islands and the Gaspé. As in the first challenge test, the scallops from the two stocks (total N of 144 scallops) were challenged in one of three ways: inoculation challenges in the muscle and the mantle cavity, and an immersion challenge. Untreated scallops were used as controls. The scallops were fed with 10 000 cells/mL of TISO/MONO and kept in running sea water at 10°C for 50 days.

Challenge test 3

In the third challenge test, 108 healthy scallops from the Gaspé were challenged with a homogenate made from a single type of tissue extracted from moribund

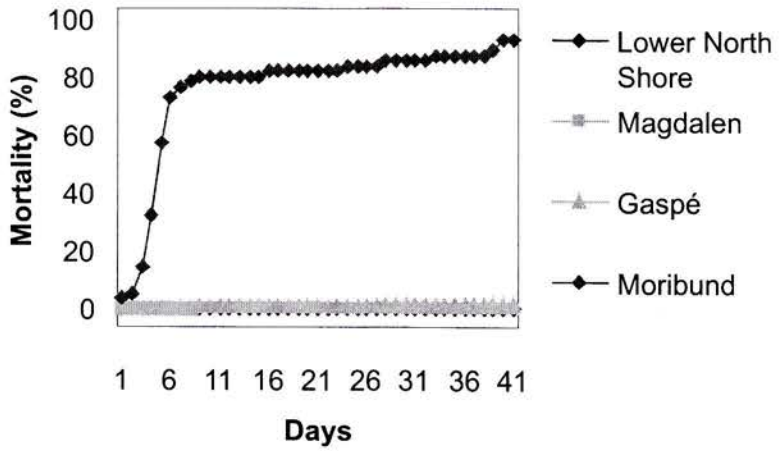

Figure 1. Mortality (%) in untreated scallops from three Gulf of St. Lawrence stocks held in the laboratory. In stocks that had been experiencing high mortality, up to 80% of the scallops died within 6 days of being brought into the laboratory.

scallops. Tissue from the gills and the mantle, the digestive gland and kidney, and the muscle were homogenized. Half of each of the three homogenates was sterilized, resulting in 6 homogenates (3 sterilized and 3 unsterilized). Each of the 6 homogenates was injected into the muscles of groups of scallops. The remaining portions of the homogenates were combined and mixed with the seawater in which a few scallops were being held. Some scallops were injected in the muscle with sterile sea water. Untreated scallops served as controls.

Histopathological analysis

Scallops that died in the three challenge tests were fixed in a solution of Davidson's for histopathological analysis. Scallops were also selected from each treatment of each challenge test for histopathological analysis. Tissue sections were also taken from moribund scallops from Shekatika.

Results and Discussion

Challenge test 1

During the experiment, untreated scallops from each of the three stocks and scallops from areas suffering heavy mortality were held in storage baths. Mortality of up to 80% was observed during the first six days of storage in the stocks that had been experiencing high mortality (Fig. 1).

Scallops from the Lower North Shore seemed to be more affected by the stress of being injected with homogenized tissue than the scallops from Gaspésie or the Magdalen Islands. Mortalities of 90% to 100% were observed in the scallops that were injected in the muscle or the mantle cavity with centrifuged or uncentrifuged homogenates. The differing response among scallops from different geographical areas might be explained by the fact that the Lower North Shore population is at the northern limit of the range of the giant scallop.

The scallops from Gaspésie and the Magdalen Islands responded differently to injections of homogenate into the muscle and the mantle. Scallops injected in the muscle with homogenate that had not been centrifuged, suffered high mortality (100% and 90%, respectively, for scallops from Gaspésie and the Magdalen Islands). Those injected in the mantle cavity had less mortality: 75% for the Gaspésie scallops and 24% for the Magdalen Islands scallops. Exposing the scallops to an immersion challenge had no significant effect on mortality. Thus, challenge test 1 provided no evidence of any lethal pathogen.

Challenge Test 2

The second challenge test similarly showed that the scallops from the Lower North Shore were more sensitive to stress than scallops from the two other areas. Inoculation of uncentrifuged homogenate into the muscle caused 100% mortality even when the homogenate was extracted from healthy scallops.

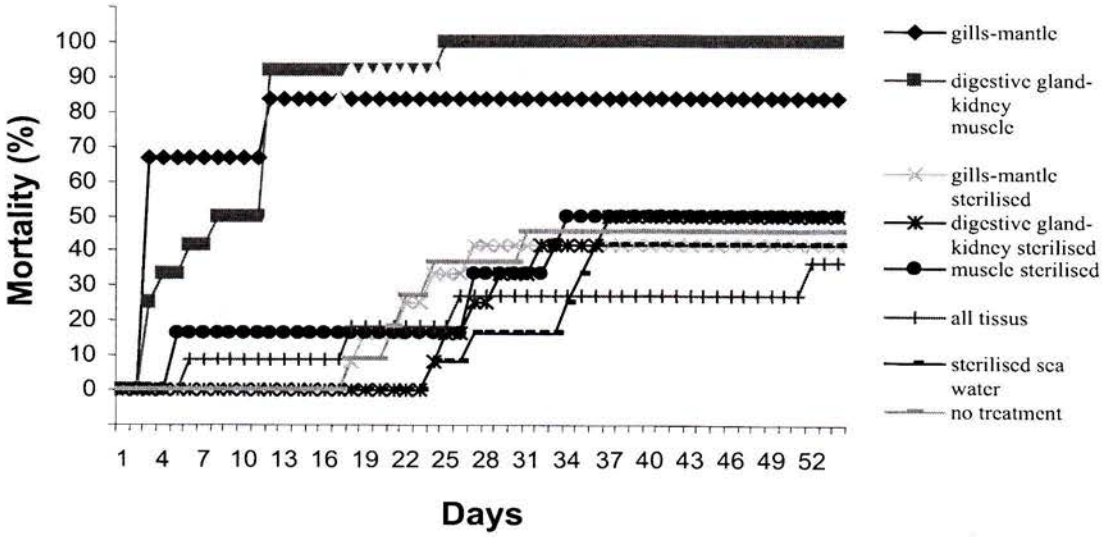

Figure 2. The mortality rate in scallops was higher when the scallops were inoculated with non-sterile homogenate than with sterile homogenate (challenge test 3).

The scallops from Gaspésie were more sensitive to the stress of the inoculation challenge than the scallops from the Magdalen Islands, which had mortality rates of less than 50 to 60%.

Injection of homogenate into the mantle cavity, exposing the scallops to an immersion challenge, and mixing the homogenate into the sea water had little effect on mortality (mortality < 40%) except for the population from the Lower North Shore.

Statistical analysis also revealed that the response of the scallops varied depending on whether or not the homogenized tissue was centrifuged prior to being injected into the scallops. The mortality rate was higher when the homogenate was not centrifuged, and the difference was independent of the site of the injection (muscle or mantle cavity) or whether the challenge was by immersion or inoculation.

The mortality results confirmed the hypothesis that the presence of a foreign body had an effect on the scallops. The mortality observed after the inoculation of homogenized extracts from healthy scallops is less significant than the mortality observed after injecting homogenate extracted from moribund scallops. The mortality rate in the control scallop population (average of 15%) indicated that the stress of handling (washing, labelling, and injection) caused mortality.

Thus, the second challenge test could not confirm the presence of a pathogenic agent.

Challenge test 3

The mortality rate was distinctly higher when the scallops were inoculated with non-sterile homogenate than with sterile homogenate (Fig. 2). Mortality of up to 100% occurred when the homogenate was extracted from the digestive gland and the kidney (90% when extracted from the muscle, and 85% when the homogenate was extracted from the gills or the kidney). However, these differences in the mortality rates are not significant.

Mortality in the control scallops and those treated with sterilized homogenates ranged from 25% to 50%; these mortalities were associated with the stress of manipulation and the effect of foreign material.

The results from the third challenge test suggest the possible presence of a pathogenic agent since the mortalities induced by the non-sterile homogenates are higher than that induced by the sterilized homogenate. Sterilization eliminates all possible pathogenic agents.

Histopathological analysis

The pathological analysis revealed the presence of gill (12% incidence) and intestinal turbellarians (4%), *Rickettsia*-like pathogens (8%), gill ciliates (4%), etc. All these pathogens were present at low levels and are not considered a disease concern.

There was a high prevelance of tissue necrosis (60%) in the tubules and the connective tissues of the digestive gland. This tissue damage is due to the fact that the scallops were dead when collected, so enzymes had begun to destroy the tissues. The analysis also showed the presence of saprobiont bacteria that are usually associated with advanced necrosis.

Conclusion

The results of the three challenge tests did not reveal the presence of any pathogens or viruses that could be responsible for the episodes of mass mortality. Although the results from the third challenge test seemed to indicate the presence of an epidemiological infection, the pathological analysis of the treated and moribund scallops did not reveal any sign of a specific infection. At present, the exact cause of the mortality is still unknown.

More advanced histopathological analysis will be done. Scallop samples are being sent to Tristan Renault at IFREMER in France to determine if a herpes virus could be responsible for the mortality. Bacterial cultures from moribund scallop tissues will also be done to isolate and identify the causal agent.

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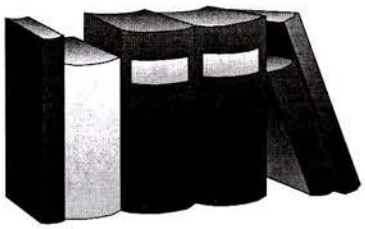

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The Diatoms: Applications for the Environmental and Earth Sciences, 1999, by E.F. Stoermer and J.P. Smol. Cambridge University Press, 482 p. (Hardcover: ISBN 0-521-58281-4, US$120.00; Paperback: ISBN 0-521-00412-8, US$44.95). This volume is an up-to-date summary of the expanding field of the uses of diatoms in environmental and earth sciences. The major emphasis in this volume is the use of diatoms in analyzing ecological problems such as climate change, acidification, and eutrophication. Cambridge University Press (tel 800 872-7423).

Seagrass Ecology, 2000, by M. Hemminga and C.M. Duarte. Cambridge University Press, 310 p., ISBN 0-521-66184-6, hardcover, US$80.00. Cambridge University Press (tel 800 872-7423).

The Changing Ocean Carbon Cycle — A Midterm Synthesis of the Joint Global Ocean Flux Study, 2000, R.B. Hanson, H.W. Ducklow, and J.G. Field. Cambridge University Press, 528 p. (Hardcover ISBN 0-521-65199-9, US$150.00; Paperback ISBN 0-521-65603-6, US$54.95). The Joint Global Ocean Flux Study is the first multidisciplinary program to directly address the interactions among the biology, chemistry, and physics of marine systems, with emphasis on the transport and transformations of carbon within the ocean and across its boundaries. Cambridge University Press (tel 800 872-7423).

Crustacean Farming: Ranching and Culture, 2nd ed, April 2002, by J.F. Wickins and D.O'C. Lee. Iowa State University Press. 480 p., hardcover, ISBN 0-632- 05464-6, US$94.95. Topics include: ranching and restocking, culturing ornamental shrimp and other small crustaceans used as live food, crustacean diseases, genetics, nutrition and advances in research. Iowa State University Press (tel 515 292-0140, fax 515 292-3348, website isupress.com).

Management and Ecology of Lake and Reservoir Fisheries, May 2002, I.G. Cowx, ed. Iowa State University Press. 384 p., hardcover, ISBN 0-85238-283-9, US$129.95. Chapters include submissions from the acclaimed University of Hull International Fisheries Institute (HIFI) Symposium, including the potential to control community fish structure, management of fisheries in a large lake, and use of a split beam echosounder. Iowa State University Press (tel 515 292-0140, website isupress.com).

Fishes in Estuaries, April 2002, M. Elliott and K.L. Hemingway, eds. Iowa State University Press. 352 p., hardcover, ISBN 0-632-05733-5, US$129.95. Contents include data analysis and interpretation, environmental quality of estuaries, estuarine development/habitat loss, recruitment processes of commercial species, and estuaries and associated habitats. Iowa State University Press (tel 515 292-0140, fax 515 292-3348, website isupress.com).

Proceedings from "Environmental Impacts of Marine Aquaculture — A Meeting of Stakeholders of the Northeast" held in January 2001 at the University of Massachusetts. The workshop brought together members of industry, government, academia, and NGOs to open lines of communication and strive towards consensus on how to develop aquaculture in an environmentally sustainable manner. The main theme areas were environmental impacts, impacts on wild stocks, impacts on other wildlife, and consensus building. Published by Cape Cod Press. Free copies can be downloaded from the workshop web site (www.es.umb.edu/mae01). Hard copies can be obtained for US$30 (payable to Univ. Mass Boston: Aq/Env Workshop) from Ms. Florence Wurzel, ECOS/ Workshop, Univ. Mass Boston, 100 Morrissey Blvd., Boston, MA 02125-3393.

Scientific Method for Ecological Research, 2000, by E.D. Ford. Cambridge University Press, 584 p. (Hardcover ISBN 0-521-66005, US$130.00; Paperback ISBN 0-521-66973, US$49.95). The book provides a valuable source of information for those seeking to strengthen the methodology underlying their studies or who have an interest in the analysis of research methods in ecology. Cambridge University Press (tel 800 872-7423, fax 914 937-4712).

Aquaculture News

CAIA's Herb Dhaliwal Award Presented to Ron Kilmury

The Canadian Aquaculture Industry Alliance (CAIA) presented the first annual Herb Dhaliwal Sustainable Aquaculture Award to Ron Kilmury, Managing Director of Nutreco North America, Marine Harvest Canada. Mr. Kilmury was one of six finalists considered for the award.

The award was presented by the Honourable Herb Dhaliwal, Minister of Fisheries and Oceans, at a gala on Parliament Hill on 27 November 2001. Mr. Dhaliwal noted that Kilmury and Nutreco have contributed to the tremendous strides made in the Canadian aquaculture sector, saying that "The initiatives he has developed and implemented have played a pivotal role in working toward the environmentally sustainable development of the aquaculture industry". Nutreco and Marine Harvest Canada employ more than 300 people in Canada at 18 salmon farms and two feed mills.

The other finalists for the Dhaliwal award were the Huntsman Marine Science Centre's Atlantic Salmon Broodstock Program; Odd Grydeland, President of the BC Salmon Farmers Association; Bill Vernon, General Manager of Creative Salmon Ltd.; Keith Reid, President, Odyssey Shellfish; and Fraser Walsh, CEO of Heritage Aquaculture.

Prior to the awards, the CAIA president, Anne McMullin, stated that "All of the finalists are exemplary in their belief in environmental stewardship and vision of cooperative problem solving. They are also united in the goal of ensuring an economically successful and environmentally sustainable aquaculture industry in Canada".

AAC was one of the many associations and producers that contributed to the CAIA Gala. In attendance at the gala were many of AAC's officers and directors, including Cyr Couturier, Sharon McGladdery, Linda Hiemstra, and John Bonardelli.

L to R: Anne McMullin, President of CAIA; Ron Kilmury of Nutreco North America, the first recipient of the Herb Dhaliwal Sustainable Aquaculture Award; the Honourable Herb Dhaliwal, Minister of Fisheries and Oceans; and David Rideout, the Executive Director of CAIA.

Calendar

conferences, workshops, courses and trade shows

- **International Boston Seafood Show,** 12-14 March 2002, Hynes Convention Center, Boston, USA. Information: Diversified Business Communications (tel 207 842-5504, fax 207 842-5505, e-mail food@divcom.com).
- **Aqua Sur 2002**, 18-23 March 2002, Puerto Montt, Chile. The conference theme is "Aquaculture in the Southern Hemisphere". Information: Claudia Carvajal, e-mail ccarvajal @aqua.cl, tel +56 2 756-5402, or Sue Hill, e-mail sue.hill@informa.com, tel + 44 20 7017 4501.
- **The Great Atlantic Shellfish Exchange 2002**, 21-23 March 2002, Delta Hotel, Charlottetown, Canada. Information: PEI Aquaculture Alliance (tel 902 368-2757, fax 902 368-5958, e-mail peiaqua@pei.sympatico.ca).
- **National Shellfisheries Association 94th Annual Meeting,** 14-18 April 2002, Hilton Mystic Hotel, Mystic, USA. Information: www.shellfish.org. For program information contact Carolyn Friedman at 707 875-2067 (cfriedman@ ucdavis.edu).
- **Aquaculture International 2002 and Coldwater Marine Farming Conference,** 18-20 April 2002, Scottish Exhibition and Conference Centre, Glasgow, Scotland. Information: Sue Hill, Heighway Events, London (fax +44 20 7831 2509, e-mail sue.hill@informa.com)
- **World Aquaculture 2002,** 23-27 April 2002, Beijing International Conference Centre, China. Annual meetings of the World Aquaculture Society and the China Society of Fisheries. Information: Director of Conferences (tel +1 760 432 4270, fax +1 760 432 4275, e-mail worldaqua @aol.com).
- **5th Congress of the International Society for the Study of Fatty Acids and Lipids 2002**, 7-11 May 2002, Montreal, Canada. The conference theme is "Dietary Fats and Health". For more information, check www.issfal.org.uk.
- **Asia-Pacific Conference on Marine Science and Technology 2002**, 12-16 May 2002, Port Dickson, Malaysia. Information: Prof. Phang Siew Moi (tel 603 79674610, fax 603 79674606, email h1phangs@umcsd.um.edu.my).
- **Feeding for Quality,** 10th International Symposium on Nutrition and Feeding in Fish, 2-7 June 2002, Rhodes, Greece. For information check the website http://www.fishnutrition 2002.gr.
- **Healthy Ecosystems, Healthy People: Linkages between Biodiversity, Ecosystem Health and Human Health 2002**, 6-11 June 2002, Marriott Wardman Park Hotel, Washington, DC, USA. The conference will focus on the links between biodiversity and ecosystem health, ecosystem health and human health, biodiversity and human health, and integration and policy. For more information contact Healthy Ecosystems, Healthy People, c/o International Society for Ecosystem Health (tel 519 661-2111 ext. 86223, fax 519 661-3797, e-mail hehp@ ecosystemhealth.com).
- **American Society of Limnology and Oceanography (ASLO) Summer Meeting 2002**, 10-14 June 2002, Victoria, BC, Canada. The main theme for the conference is "Inter-disciplinary Linkages in Aquatic Sciences and Beyond". There will be a session on assessing potential impacts of aquaculture. For information, check the website http://aslo.org/victoria2002/index.html.
- **AquaVision 2002**, 11-13 June 2002, Stavanger, Norway. For information send an e-mail message to vidar.julien@nutreco.com.
- **Atlantic Aquaculture Conference,** 15th Annual Trade Show and Fair, 12-16 June 2002, St. Andrews, NB, Canada. Information: Master Promotions at tel 506 658-0018, website www. masterpromotions.ca.
- **4th International Conference on Recirculating Aquaculture 2002**, 18-21 July 2002, Hotel Roanoke & Conference Center, Roanoke, Virginia, USA. Information: Terry Rakestraw (tel 540 231-6805, e-mail aqua2002@vt.edu website www.contd.vt.edu/recirc.aqua.htm).
- **10th International Congress of Parasitology,** 4-10 August 2002, Vancouver Conference and Exhibition Centre, Canada. Sponsored by the Canadian Society of Zoologists and the American Society of Parasitologists. Sessions: immunology, molecular biology, morphology and ultrastructure, biochemistry and physiology, systematics and evolution, ecology and epidemiology. Information: Conference Secretariat, Venue West Confer-

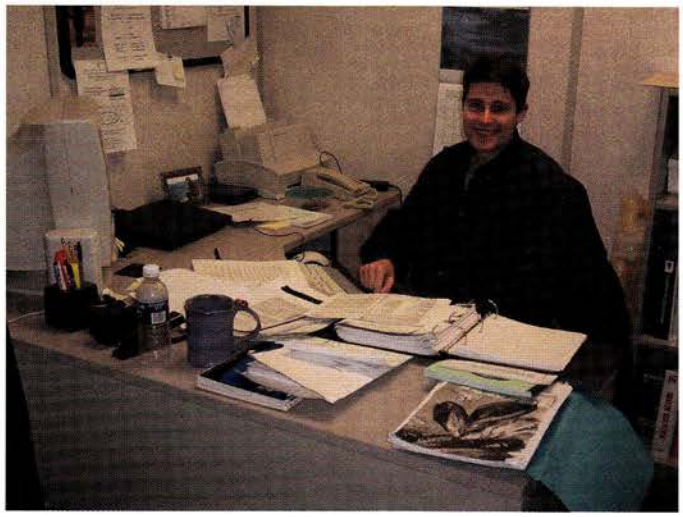
ence Services Ltd. (tel 604 681-5226, fax 604 681- 2503, e-mail congress@venuewest. com, website http:// www. venuewest.com).

- **4th Bioengineering Symposium,** 18-22 August 2002. The Symposium will be held in conjunction with the 132nd AFS annual meeting in Baltimore, Maryland, USA. For updates on the symposium, visit the website at http://biosys.bre.orst. edu/afseng/ bes4.htm or contact Mufeed Odeh (e-mail mufeed_odeh@usgs.gov, tel 413 863-3805), or Steve Amaral (e-mail amaral@ aldenlab.com, tel 508 829-6000 ext. 415).
- **2nd International Symposium on GIS and Spatial Analyses in Fishery and Aquatic Sciences 2002**, 3-6 September 2002, Sussex, Brighton, UK. Website address: www.esl.co.jp/Sympo/ sympo11.htm, e-mail itoh@esl.co.jp.
- **International Symposium on Low-lying Coastal Areas Hydrology & Integrated Coastal Zone Management 2002**, 9-12 September 2002, Bremerhaven, Germany. Website: www.bafg.de/welcome.html.
- **Aquaculture Canada 2002, 19th Annual Meeting of the Aquaculture Association of Canada,** 17-20 September 2002, Delta Prince Edward, Charlottetown. Theme: *Finding Solutions, Creating Sustainable Wealth*. Co-hosted by the PEI Aquaculture Alliance and the PEI Department of Fisheries, Aquaculture and Environment. Information: AAC Office (tel 506 529-4766, e-mail aac@mar. dfo-mpo.gc.ca, website www.mi. mun.ca/mi/aac).
- **Aquafest Australia 2002**, 19-22 September 2002, Wrest Point Convention Centre, Hobart, Tasmania. Information: Tom Lewis (TAGA), 73 Lansdowne Crescent, West Hobart 7000 Australia, (tel (03) 6231 9230, email tom_lewis@ biodevconsult.com
- **100th Anniversary Meeting of the International Council for the Exploration of the Sea (ICES) 2002**, 1-5 October 2002, Copenhagen, Denmark. The theme of the science conference is *Aquaculture: New Trends and Developments* in recognition of the important work being done in the North Atlantic region. If you are interested in participating, please contact the convenor by e-mail: I.R. Bricknell@marlab.ac.uk
- **Aquaculture Pacific Exchange Conference and Exhibition,** 3-4 October 2002, Strathcona Gardens, Campbell River, Canada. Consists of a 100-booth trade show and 2-day conference. Produced by Master Promotions Ltd. (tel 506 658-0018, e-mail show@nbnet.nb. ca).
- **Aquaculture Europe 2002,** 16-19 October 2002, Verona, Italy. Organized by the European Aquaculture Society. Theme: *Placing Aquaculture in Rural and Coastal Management.* Workshops: Applied solutions to health management in Mediterranean aquaculture, New technologies for Mediterranean aquaculture, and Certification in aquaculture (HAACP, ISO standards, ecolabelling and organic). Information: EAS (e-mail ae2002@ aquaculture.cc).

- **5th International Symposium on Flatfish Ecology 2002**, 3-7th November 2002, Port Erin Marine Laboratory, Isle of Man (UK). Symposium will address the role of flatfishes in benthic ecosystems under three themes: patterns, processes and management. For information, contact Richard Nash, Port Erin Marine Laboratory (e-mail flatfish@liv.ac.uk, website www.liv.ac.uk/peml/ flatfish.
- **Aquaculture America 2003**, 18-21 February 2003, Commonwealth Convention Center, Louisville, Kentucky. US National Aquaculture Conference and Exposition of the US Chapter of the World Aquaculture Society in conjunction with the National Aquaculture Association and the US Aquaculture Suppliers Association. Information: Director of Conferences (tel +1 760 432 4270, fax +1 760 432 4275, e-mail worldaqua@ aol.com).
- **World Aquaculture 2003,** 19-23 May 2003, Bahia Convention Center, Salvador, Brazil. Annual meeting of the World Aquaculture Society in conjunction with other associations, industry and government sponsors. Information: Director of Conferences (tel +1 760 432 4270, fax +1 760 432 4275, e-mail worldaqua@ aol.com).
- **Aquaculture Canada 2003 and Aquaculture Pacific Exchange Conference and Exhibition,** 28 Oct - 1 Nov 2003, Victoria Conference Centre, Victoria, Canada. For Aquaculture Canada information, contact Shawn Robinson (e-mail robinsonsm@mar.dfo-mpo.gc.ca, fax 506 529-5862). Trade show information, contact Master Promotions Ltd., (tel 506 658-0018, fax 506 658-0750, e-mail show@nbnet.nb.ca).
- **Aquaculture 2004**, 29 February - 4 March 2004, Hawaii Convention Center, Honolulu. Triennial meeting of the World Aquaculture Society, the National Shellfisheries Association, and the Fish Culture Section of the American Fisheries Society. Information: Director of Conferences (tel +1 760 432 4270, fax +1 760 432 4275, e-mail worldaqua@aol.com).

Message from the AAC Webmaster

I've recently taken over the responsibility of webmaster for the AAC web site from Jay Parsons and look forward to helping build and develop the web site over the coming months. In times of ever-increasing computerization, e-commerce and dot coms, web sites are playing an important role in our quest for information. When confronted with the need for information, my first response is no longer to go to the library, but to the world wide web. I believe it to be of great importance for AAC to have a strong presence on the web, not only to covey its message with authority and conviction, but also to act as a vehicle to promote the Association and the industry it serves.



Since becoming webmaster in November 2001, most pages on the website have been updated, including the links and the pages containing information on the Board of Directors and Standing Committees. A simple search feature has also been added. The abstracts from the Aquaculture Canada 2001 conference (Halifax, May 2001) have been posted. The pages on AAC's publications have been updated and now include photos of each of the publications as well as the tables of contents of each *Bulletin* published since 1993 and the four *AAC Special Publications.*

Future plans for the site include a complete redesign, which you can expect to see very shortly. The abstracts from Aquaculture Canada 2000 (Moncton, May 2000) will also be added, as will those for Aquaculture Canada 2002 in Charlottetown. A more advanced search function will be developed and the Aqua-L archive page will be redesigned and included under the search feature, so that searches can be done of the contents of the archives. An on-line membership application for new members will be added. I also hope to start an aquaculture photo album (full photo credits will be given and photos will not be reproduced without the written consent of the owner).

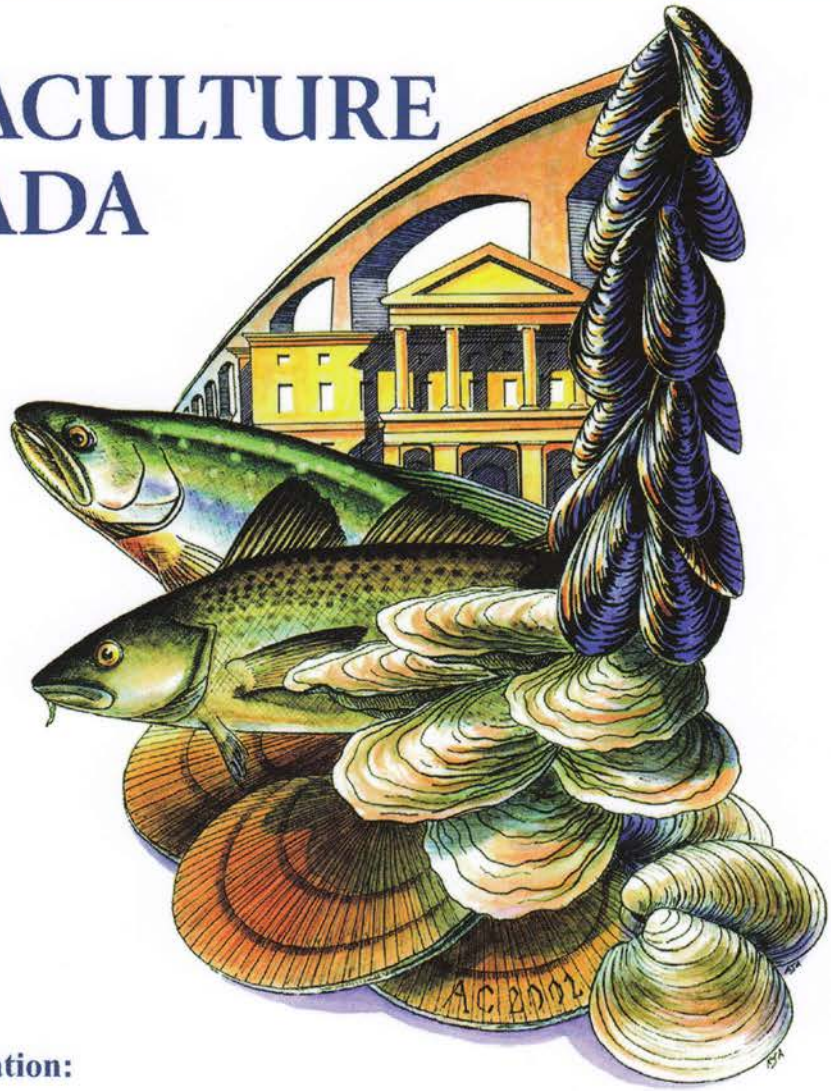
As this is the Association's web site, I would like to hear from AAC members concerning the web site. What did you like? What didn't you like? What would you like to see added or removed? I would also like to solicit photographs from members to add to the on-line photo album. In particular, photos dealing with the wackier side of aquaculture would provide a nice break from the usual images of long-lines and net-pens.

This is your site, so log on to www.mi.mun.ca /mi/aac and let me know what you think. Don't hesitate to contact me at Alistair.Struthers@ mi.mun.ca with comments and suggestions. I look forward to hearing your views and shaping the web site to suit everyone's needs.

— Alistair Struthers, AAC webmaster
Marine Institute
Memorial University, St. John's

Aquaculture Association of Canada
19th Annual Meeting

AQUACULTURE CANADA 2002



Conference Information:
Kim Shafer
Aquaculture Association of Canada ~ Home Office
16 Lobster Lane
St. Andrews, NB E5B 3T6
Tel: 506-529-4766
Fax: 506-529-4609
E-mail: aac@mar.dfo-mpo.gc.ca

Trade Show Information:
Jason Doucette
PEI Aquaculture Alliance
Tel: 902-368-2757
Fax: 902-626-3954
E-mail: peiaqua@aquaculturepei.com

www.mi.mun.ca/mi/aac

Finding Solutions, Creating Sustainable Wealth

Charlottetown, PEI **September 17-20, 2002**