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HEALTH STATUS OF OLYMPIA OYSTERS (*OSTREA LURIDA*) IN BRITISH COLUMBIA, CANADA

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ABSTRACT The Olympia oyster (*Ostrea lurida*) is the only oyster species native to British Columbia, Canada. Despite management regulations designed to protect this species, populations continue to decline in many locations. In an effort to determine whether parasites or disease are contributing to the population decline, oysters were collected from 5 locations and examined for the presence of pathogens and disease using histology and polymerase chain reaction (PCR) assays. Five parasite/symbiotic organisms were detected by histology, including Rickettsia-like prokaryotes, *Mytilicola* sp., Rhynchodida-like ciliates, encapsulated copepods, and an unknown protist. Six pathologies of unknown etiology were also detected, including hemocytic neoplasia, basophilic inclusions in the digestive gland epithelium, metaplasia of digestive gland tubules, diapedesis, and diffuse and focal hemocyte infiltration. Despite the variety of histological findings, most were detected at low prevalence and intensity, and are not believed to have a significant impact on the health of oysters at the individual or population levels. In addition, 7 different PCR assays were conducted for known bivalve pathogens, including *Mikrocytos mackini, Haplosporidium* spp., and *Bonamia* spp. Although all PCR test results proved to be negative, some assays did produce nonspecific amplification products, emphasizing the need for caution and validation when applying PCR assays to new hosts and new geographical regions. Although the current study did not identify any pathogens or diseases of concern, it provides important baseline data for future health assessments.

KEY WORDS: *Ostrea lurida*, Olympia oyster, parasite, disease, histology, PCR, British Columbia, Canada

INTRODUCTION

The Olympia oyster (*Ostrea lurida*) recently delineated from *O. conchaphila* by molecular studies (Polson et al. 2009), inhabits lower intertidal and shallow subtidal zones of weather-protected bays and estuaries on the west coast of North America from approximately southeast Alaska to Baja California. They are typically small, seldom exceeding 60 mm in shell length, have slow growth rates, and are the only naturally occurring oyster species in British Columbia (BC), Canada. Extensive declines of Olympia oyster populations occurred between 1930 and 1942, thought to be the result of overexploitation in combination with heavy frosts in 1929 (Quayle 1969). Commercial harvesting of this species ceased in the 1930s as a result of declining stocks and a shift toward introduced oyster species (initially *Crassostrea virginica,* and later *C. gigas*). Scattered remnant populations of Olympia oysters currently exist in BC, but only a few isolated sites have stocks that are characterized as abundant (Gillespie 2009). In 2003, Olympia oysters were listed as a species of special concern under the Canadian Species at Risk Act, and both commercial and recreational harvesting is currently prohibited.

The effect of disease on Olympia oyster populations in Canada is unknown and, in general, relatively little is known about diseases and parasite fauna of this oyster species. Haplosporidian parasite infections and a fatal neoplastic disorder were reported in Olympia oysters from Yaquina Bay and Alsea Bay, OR (Mix & Sprague 1974, Mix 1975). Farley et al. (1988) documented microcell disease, neoplasms, and *Mytilocola orientalis* also in Olympia oysters from Yaquina Bay, OR. More recently, 3 diseases—including a *Mikrocytos*-like protist, a haplosporidian, and hemic neoplasia—were reported among populations of Olympia oyster from San Francisco Bay estuary (Friedman et al. 2005). Previous laboratory and field exposure experiments have shown that Olympia oysters are susceptible to infection with *Mikrocytos mackini* (Bower et al. 1997), a microcell parasite of unknown taxonomic affiliations that causes mortality and reduced marketability in *C. gigas* from BC. However, field surveys have not been conducted to determine whether this parasite is naturally occurring in and affecting Olympia oyster populations in BC. The current study represents the first disease survey of Olympia oyster populations in BC. It was initiated to determine whether pathogens or parasites may be contributing to their low abundance and/or inability to rebound after historical overexploitation.

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MATERIALS AND METHODS

Collection and Processing

During May and July 2008, 251 Olympia oysters (estimated to be 3 y or older) were collected from 5 locations ($n = 25$–60 per site) in British Columbia, Canada (Fig. 1). Necropsy and tissue samples were obtained in the field at the Watt Bay and Port Eliza N. sites whereas oysters from the other 3 locations were transported live to the laboratory where tissue sampling was conducted within 3 days of collection. Each oyster was shucked via the hinge ligament; the shell length was measured and any abnormalities or unusual macroscopic observations were recorded (Table 1).

Histopathology

Tissues were preserved in Davidson's solution (Shaw & Battle 1957) for 24–72 h. Routine paraffin histological techniques were performed on 2 transverse cross-sections (2–3 mm thick) from each oyster: one from the anterior region through the labial palps and one from the midbody including gills,

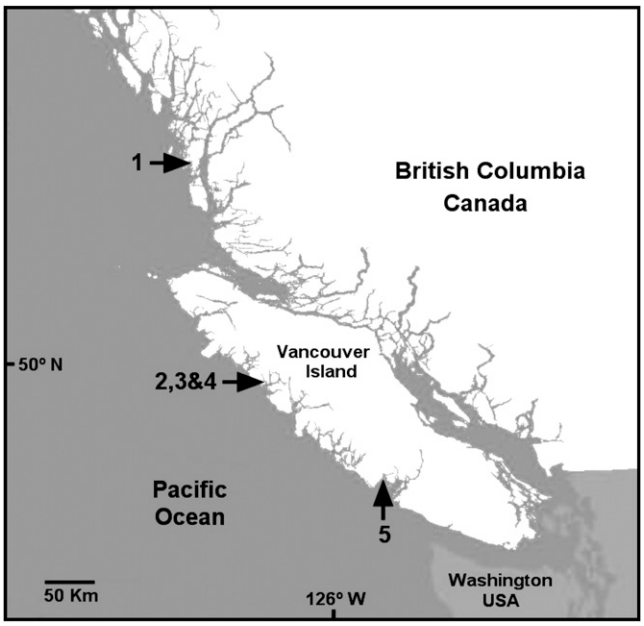

Figure 1. Coastal map of British Columbia, Canada, indicating the 5 sites where Olympia oysters were collected: 1, Watt Bay, Kildidt Sound; 2, Port Eliza N., Nootka Sound; 3, Port Eliza W., Nootka Sound; 4, Queen Cove, Nootka Sound; and 5, Snowden Island, Barkley Sound.

digestive gland, gut, and gonad. Deparaffinized 5-μm-thick tissue sections were stained with Harris' modified hematoxylin and 0.5% alcoholic eosin, and a small subset of samples were also stained with periodic acid Schiff and Feulgen DNA stain. Histology slides were examined via light microscopy (100–1,000× magnification) for the presence of pathogens known to cause disease in bivalve molluscs. All other observations pertaining to pathology, parasites, and symbionts were also recorded.

Polymerase Chain Reaction (PCR) Assays

For pathogen detection using PCR, a small piece of tissue from the anterior region of each oyster containing mantle and labial palps was preserved in 95% ethanol. For the 3 groups of oysters processed in the laboratory (Port Eliza W., Queen Cove, and Snowden Island), an additional sample was preserved in 95% ethanol that consisted of pooled tissues (gill, digestive gland, gut, and gonad) excised from the midbody region parallel to the second transverse histology body section.

DNA was extracted using a DNeasy Tissue Kit (Qiagen, Mississauga, Ontario, Canada) following the manufacturer's protocol except for a minor modification that involved incubation of the spin columns at 70°C for 3 min before the final spin to elute DNA. DNA concentration and purity was measured using a Nanodrop spectrophotometer.

Separate PCR assays designed specifically to amplify 18S ribosomal DNA of haplosporidians and *M. mackini* using primer pairs HapF1/HapR3 and Mikrocytos-F/Mikrocyots-R, respectively, were tested on all samples (primer information and sources are included in Table 2). Because an unknown protist was detected in samples from Snowden Island through histological examination, several other PCR assays targeting a variety of oyster pathogens were performed, as was an assay targeting nonmetazoan DNA (Table 2). PCR products were visualized on 1.5% agarose gels stained with Sybr-Safe (Invitrogen, Inc., Burlington, Ontario, Canada), subsequently purified using Exosap-IT (VWR, Mississauga, Ontario, Canada), and sequenced to confirm the source of the amplified product. Sequencing reactions used Big Dye Terminator v. 3.1 (Applied Biosystems, Inc.) and were run on a 3130x1 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). Sequencher v. 4.7 (Genecodes Corp., Ann Arbor, MI) was used for viewing and editing sequence data.

RESULTS

Macroscopic Observations

Shell length was similar across all oyster populations, ranging from an average of 34.7 mm for Snowden Island to 43.9 mm for Watt Bay (Table 1). Gross signs of shell disease, caused by the fungus *Ostracoblabe implexa* and typified by nacre-covered bumps protruding from the shell into the adductor muscle, were observed in 1 oyster from Snowden Island. This infection did not appear to be causing damage to adjacent tissues or impeding closure of the valves. Red "worms" were visible in the soft tissues of a different Snowden Island oyster, and further dissection revealed 4 parasitic copepods (*Mytilocola* sp.) inside the gut. In all other oysters examined, there were no gross lesions or other macroscopic indications of disease (Table 1).

TABLE 1.

Locations and macroscopic observations for Olympia oysters (*Ostrea lurida*) collected in British Columbia, Canada, during May and July 2008.

Site No.	Location	*n*	Mean Shell Length ± STD (mm)	Necropsy Observation/Abnormalities
1	Watt Bay (Kildidt Sound)	50	43.9 ± 6.9	1/50 with eggs; no larvae
2	Port Eliza N. (Nootka Sound)	60	38.5 ± 4.8	None
3	Port Eliza W. (Nootka Sound)	56	42.0 ± 3.4	3/56 were watery/emaciated 2/56 pale or unusual colored digestive gland 1/56 numerous-eyed larvae in mantle cavity
4	Queen Cove (Nootka Sound)	25	43.2 ± 2.8	None
5	Snowden Island (Barkley Sound)	60	34.7 ± 4.3	3/60 were watery/emaciated 1/60 had shell disease 1/60 *Mytilicola* sp. ($n = 4$) observed inside gut

TABLE 2.

PCR primers used for pathogen testing of Olympia oysters (*Ostrea lurida*) collected in British Columbia, Canada, during May and July 2008.

Pathogen	Primer	Sequence (5′-3′)	Source
Mikrocytos mackini	Mikrocytos-F	AGATGGTTAATGAGCCTCC	Carnegie et al. (2003)
	Mikrocytos-R	GCGAGGTGCCACAAGGC	
Mikrocytos-like parasite	MmLike (used with Mikrocytos-R)	CCTGTCCTATGTCTATGGGCAAGG	Gagne et al. (2008)
Haplosporidium sp.	HapF1	GTTCTTTCWTGATTCTATGMA	Renault et al. (2000)
	HapR3	AKRHRTTCCTWGTTCAAGAYGA	
Haplosporidium sp. (alternate)	16SA	AACCTGGTTGATCCTGCCAGT	Medlin et al. (1988)
	16SB	GATCCTTCCGCAGGTTCACCTAC	
Haplosporidium nelsoni	MSX-161-F	GGGCTAATACGTGATAAATGGTACG	Stokes and Burreson (1995)
	MSX-334-R	TTCCCCGTTACCCGTCAT	
Bonamia sp.	Bonamia-CF	CGGGGGCATAATTCAGGAAC	Carnegie et al. (2000)
	Bonamia-CR	CCATCTGCTGGAGACACAG	
Bonamia sp. (alternate)	BO	CATTTAATTGGTCGGGCCGC	Cochennec et al. (2000)
	BOAS	GGGGGATCGAAGACGATCAG	
Universal nonmetazoan	18SEUK-581-F	GTGCCAGCAGCCGCG	Bower et al. (2004)
	18SEUK-1134-R	TTTAAGTTTCAGCCTTGCG	

For each pair, the forward primer is listed first.

Histology: Diseases, Parasites, and Symbionts

None of the following bivalve pathogens or diseases were detected by histology: *Marteiloides chungmuensis*, *Haplosporidium* spp., *Perkinsus* spp., *Marteilia* spp., *Bonamia* spp., *M. mackini,* or nocardiosis. However, an unknown protist was detected in 5% of oysters (3 of 60) from Snowden Island (Table 3). This protist was spherically shaped (approximately 10 μm in diameter) and resembled haplosporidian-like multinucleate organisms, but also had characteristics of hypertrophied host connective tissue cells containing tightly packed microcells (Fig. 2).

TABLE 3.

Prevalence,* severity of pathology,† and intensity of parasites/symbionts‡ in Olympia oysters (*Ostrea lurida*) from 5 sites in British Columbia, Canada.

Parasite/Symbiont	Watt Bay (Kildidt Sound)	Port Eliza N. (Nootka Sound)	Port Eliza W. (Nootka Sound)	Queen Cove Nootka Sound)	Snowden Island (Barkley Sound)
Unknown protist	0%	0%	0%	0%	5% (2 L, 1 M)
Rickettsia-like prokaryotes	2% (1 L)	0%	1.8% (1 L)	0%	5% (2 L,1 M)
Gill ciliates (Rhynchodida-like)	0%	5% (3 L)	8.9% (5 L)	0%	3.3% (1 L)
Intestinal copepods (*Mytilicola* sp.)	0%	1.7% (1 L)	1.8% (1 L)	0%	0%
Encapsulated copepods in visceral mass	2% (1 L)	1.7% (1 L)	0%	0%	0%
Pathology of unknown etiology					
Basophilic inclusions in DG tubules	14% (4 L, 3 M)	3.3% (2 M)	0%	0%	0%
Hemocytic neoplasia	2% (1 L)	0%	0%	0%	1.7% (1 M)
Metaplasia of DG tubules	2% (1 L)	16.7% (8 L, 2 M)	5.4% (3 L)	8% (2 L)	0%
Diapedesis	6% (3 L)	0%	3.6% (2 L)	8% (2 L)	3.3% (2 L)
Diffuse hemocyte infiltration	16% (6 L, 2 M)	3.3% (2 L)	3.6% (2 L)	4% (1 L)	36.7% (21 L, 1 M)
Focal hemocyte infiltration	6% (3 L)	1.7% (1 L)	0%	8% (1 L, 1 M)	8.3% (4 L, 1 M)

* Prevalence recorded as the percentage of oysters either with pathology or infected with parasites/symbionts for each site.

† Severity of pathology recorded as the number of oysters with subtle to low-level (L) or easily recognizable moderate to severe (M) pathology in parentheses.

‡ Intensity of parasites/symbionts recorded as the number of oysters with light (L; <10 per tissue section) or moderate (M; 10–50 per tissue section) in parentheses.

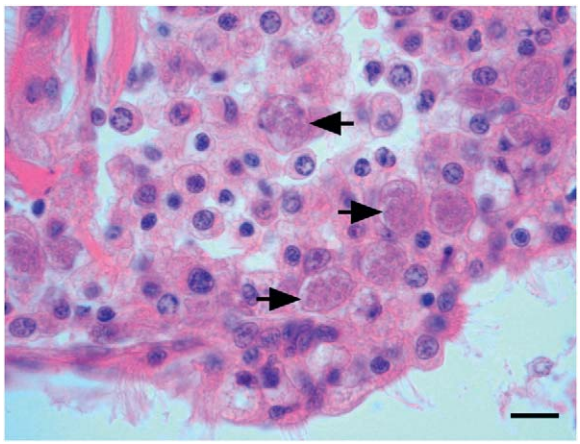

Figure 2. Histological tissue sections of *Ostrea lurida* showing the presence of unknown protistan parasites (arrows) with morphological characteristics similar to both haplosporidian plasmodia and microcells (H&E stain; scale bar, 10 μm).

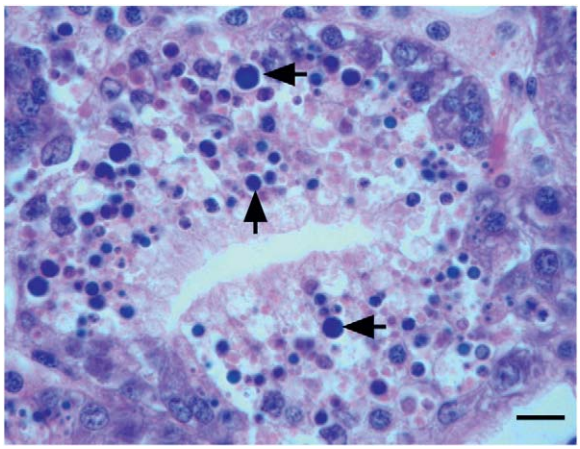

Figure 3. Histological tissue sections of *Ostrea lurida* showing abundant basophilic staining inclusions (arrows) within the epithelial cells of the digestive gland tubules (H&E stain; scale bar, 10 μm).

They were only detected in gill tissue and usually occurred in focal aggregations. One oyster had a moderate level of infection (approximately 50 per tissue section), however the other 2 specimens were light (<10 per tissue section). The low prevalence and intensity of these unknown infections made it difficult to assess them, but their tissue distribution and morphology of the parasites appeared atypical of infection with *Haplosporidium* spp., *M. mackini,* or *Bonamia ostrea*. PCR results also suggest that this unknown protist does not belong to any of these 3 groups (discussed later).

Additional parasites or symbiotic organisms detected at relatively low prevalence and intensity included Rickettsia-like prokaryotes in the digestive gland epithelial cells, ciliates in close association with the gills (Rhynchodida-like), intestinal copepods (*Mytilicola* sp.) and encapsulated/necrotic copepods within the visceral mass (Table 3).

Pathology of Unknown Etiology

A summary of the histological findings by site is given in Table 3. Hemocytic neoplasia was observed in a single oyster from each of Watt Bay and Snowden Island. Metaplasia of digestive gland tubules, diapedesis, and hemocyte infiltration were observed at low prevalence from most sites; the severity was usually low and no etiology was detected. Basophilic inclusions of varying severity (Fig. 3) were observed in the digestive gland of 14% of oysters (7 of 50) and 3.3% of oysters (2 of 60) from Watt Bay and Port Eliza N., respectively. These inclusions were sometimes associated with nuclear pyknosis and karyorrhexis, but again no etiological agent was detected. These inclusions were negative when stained with the Feulgen DNA reaction and weakly positive with periodic acid Schiff stain and they did not resemble those that are associated with Rickettsia-like prokaryotes.

PCR Assays

Separate PCR tests for *M. mackini* and *Haplosporidium* spp. did not yield positive results for any samples; however, nonspecific priming occurred. The *M. mackini* primers consistently produced a band 50–70 bp larger than the expected size of 544 bp, which was sequenced in multiple individuals. The resulting sequence could not be aligned with *M. mackini* 18S ribosomal DNA and did not generate any significant BLAST hits, thus there was no evidence that it represented specific amplification of the 18S target and was therefore considered uninformative. The haplosporidian PCR assay did not produce any products in the size range expected.

Additional PCR tests were performed in attempt to identify the unknown protist detected in 3 oysters through histology (see Table 2 for a complete list). All assays produced negative results.

DISCUSSION

Although no etiology was detected for the basophilic inclusions observed in the digestive gland of some oysters from Watt Bay and Port Eliza N., possible causes include viral infection or unusual metabolic or secretory activity. It is not surprising that hemocytic neoplasia was detected in the current survey, given its reported presence in Olympia oysters from Oregon and California (Mix 1975, Friedman et al. 2005) and in many other shellfish species encompassing worldwide geographical distribution (Elston et al. 1992, Barber 2004).

All other pathologies reported here have been similarly observed in other bivalve species from BC, and it is relatively common that no etiology is discernible, particularly given the subtle levels of pathology. Furthermore, it is thought that these observations in shellfish may result from environmental or physiological stress rather than from disease.

Although the identity of the protistan parasite described here is unknown, it was only detected in oysters from Snowden Island. Considering the low prevalence and focalized nature of these infections it is possible that the tissues selected for PCR simply did not contain any parasites, which might explain the negative PCR results. All other parasites and symbionts encountered during this survey are known to occur in other bivalve species from BC and are generally considered benign (Bower et al. 1992, Marshall et al. 2003).

The nonspecific amplification product that was routinely generated when the *M. mackini* PCR assay was applied to Olympia oysters is similar in length (about 50 bp larger) to the

expected product for *M. mackini*, which limits its usefulness as a screening tool in Olympia oysters. Future work on molecular diagnostic methods for *M. mackini* should consider this problem and ensure that any newly developed tests for this parasite do not amplify a product in this and other potential hosts.

Although the current survey did not identify any pathogens or diseases of concern, it provides important baseline health assessment data for a Canadian Species at Risk Act-listed species against which any future disease developments or significant changes in population health can be compared. Additional sampling including different locations and times of the year would lead to a more comprehensive overview of the health of Olympia oyster populations throughout its geographical range.

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