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A histopathology survey of California oysters

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Pathogen surveys form an essential component of effective aquatic animal disease surveillance programs. We report the results of a histological examination of farmed (*Crassostrea gigas, C. sikamea, C. virginica, Ostrea edulis*) and wild Olympia (*O. lurida*) oysters collected from locations throughout the state of California during 2004-2005. Most of the symbionts encountered among 1676 oysters examined are commonly observed in oyster populations worldwide and are usually of negligible or minor significance as pathogens. These include ciliates, copepods, cestode larvae, gregarine protozoa, and Rickettsiales-like prokaryotes. Conditions or agents more commonly associated with disease include the protozoan *Bonamia* sp. in *O. edulis* and disseminated neoplasia in *O. lurida*. No organisms potentially pathogenic to humans were identified. Farmed and wild oyster populations in California generally appeared healthy in accordance with a lack of reports of significant mortality events with the exception of summer mortality of juvenile *C. gigas* in Tomales Bay which is associated with herpesvirus infection.

Key words: oyster, disease, histopathology, surveillance, *Crassostrea*, *Ostrea*

Oyster culture is the most economically productive sector of California marine aquaculture. Statewide oyster production in 2004 totaled approximately 450,000 kg shucked weight valued at $7.2 million (California Department of Fish and Game, Marine Region, unpublished data). The most common methods of culture include suspended seed on oyster shell cultch (containing about 20 spat per shell) and as cultchless single oysters held in mesh bags attached to racks (Conte and Moore 2001). The primary species cultured is the Pacific oyster (*Crassostrea gigas)*, with lesser amounts of the Kumamoto oyster (*C. sikamea)*, the Eastern (or Atlantic) oyster (*C. virginica)*, and the European flat oyster (*Ostrea edulis)*. Due to a lack of oyster hatcheries in California, growers purchase larvae or small seed from California Department of Fish and Game (CDFG)-approved hatcheries

in Washington, Oregon, and Hawaii, and raise them to market size over a 2-3 year production cycle.

The native Olympia oyster (*Ostrea lurida)* historically was locally abundant in bays and estuaries throughout the state. It was heavily fished in the mid-19th century, particularly in San Francisco Bay (Conte and Moore 2001). Declines in this embayment and at many other locations were exacerbated by industrialization and habitat modification (Baker 1995). In recent years, there has been growing interest in restoring Olympia oyster populations to recover their functions as ecosystem engineers that provide complex habitat and improve water quality (McGraw 2009).

Health monitoring of oysters relies heavily on histopathology, or the identification of pathogens and diseases by examination of stained sections of tissues fixed in formalin. No comprehensive health assessment of farmed California oysters had been conducted since the surveys of Katkansky and Warner in the mid-1960s to early 1970s (Katkansky and Warner 1974). The work reported here is a snapshot survey of potentially parasitic symbionts and pathologic conditions in oyster populations throughout the state. Sufficient numbers from each population were collected so that relatively rare symbionts or conditions should have been detected, if present. We use the term 'symbiont' after Cheng (1967) to include all organisms that are 'living together' with a recognition that many microscopic organisms associated with animals can be benign under certain conditions and pathogenic under others.

Materials and Methods

We conducted a histological survey of oyster populations during 2004-2005. Most collections targeted adults and a sample size of 60, which allows for detection of a symbiont or condition with 95% confidence if its prevalence in the population is at least 5% (American Fisheries Society 2005), assuming 100% efficiency of the diagnostic method. Oysters were transported in insulated containers with gel ice to the CDFG Shellfish Health Laboratory located at the University of California, Davis (UC Davis) Bodega Marine Laboratory in Bodega Bay, California, USA. The range in shell height was recorded for each sample set. Each oyster was shucked and examined for macroscopic lesions. Samples of labial palp (20 mg per oyster, in pools of four oysters) were preserved in absolute ethanol and archived for potential molecular detection of pathogens and population genetic studies. Two cross-sections together containing heart, kidney, adductor muscle, digestive gland, stomach, intestine, mantle, and gill tissues were excised and placed in Davidson's invertebrate fixative for 24 hours (Shaw and Battle 1957). Hematoxylin and eosin-stained 5 µm tissue sections were prepared and examined for the presence of symbionts and evidence of disease.

Results

Description of collections.—We sampled 1,676 oysters from major growing areas and several non-traditional locations (Table 1). The samples included four farmed oyster species (Pacific, Kumamoto, Eastern, and European flat) in addition to wild Olympia oysters. Oysters were collected from farms located in Santa Barbara, Morro Bay, Drakes Bay, Tomales Bay, and Humboldt Bay, and from an experimental planting of Pacific and Kumamoto oysters at Crescent City, California. A feral population of Pacific oysters at

Ports of Call, San Pedro, California was also sampled. Olympia oysters were collected from wild populations in Elkhorn Slough, Sailing Lake (an enclosed lagoon in Mountain View connected to San Francisco Bay), Tomales Bay, Drakes Bay, and Humboldt Bay, California.

Description of symbionts and conditions.—The prevalences of the most common potentially pathogenic or parasitic endosymbionts identified in oysters from each location are shown in Table 1, while Table 2 lists less common observations at locations where they were found. A variety of disease conditions, symbionts, and disease-causing agents were detected, including virus, neoplasm, bacterium, protozoan, and metazoan. In general we observed low numbers of symbionts, most of which are commonly observed in oyster populations and considered of minor pathologic significance although, at sufficient numbers, each may have the potential to be harmful. We also observed low numbers of several pathogens or conditions more commonly associated with disease.

Table 1.—Oyster collections and the prevalence (%) of common symbionts from California, USA, 2004-2005.

Oyster Type	Location	Date	N	Shell Height (mm)	*Trichodina*-like Ciliate at Gill or Mantle	*Ancistrocoma*-like Ciliate in Digestive Gland Tubule	Cestode Larva in Intestine	Copepod in Intestine	Copepod at Gill or Mantle	Gregarine in Gut Epithelium
Pacific	Crescent City Harbor	Mar 05	60	22-39	3.3	0	0	0	1.7	0
Pacific	Crescent City Harbor	Oct 05	150	48-95	0	11.3	0	0	0	0
Pacific	Humboldt Bay	Feb 04	60	142-281	3.3	3.3	5	8.3	0	0
Pacific	Humboldt Bay	Feb 04	60	117-165	1.7	5	3.3	3.3	0	1.7
Pacific	Humboldt Bay	Mar 04	60	137-230	0	3.3	0	3.3	0	0
Pacific	Humboldt Bay	Feb 04	60	75-110	20	3.3	1.7	11.7	0	1.7
Pacific	Humboldt Bay	Feb 04	60	10-20	10	0	0	1.7	0	0
Pacific	Tomales Bay	Apr 04	60	122-266	1.7	1.7	0	0	0	1.7
Pacific	Tomales Bay	Mar 04	60	103-192	11.7	5	8.3	10	0	0
Pacific	Drakes Bay	Jul 04	60	77-99	0	1.7	1.7	0	0	0
Pacific	Morro Bay	Aug 04	59	75-235	15.2	0	3.4	0	0	0
Pacific	Morro Bay	May 05	32	39-108	6.3	0	0	18.8	0	0
Pacific	Morro Bay	Aug 04	60	35-64	8.3	3.3	1.7	1.7	0	0
Pacific	Santa Barbara Channel	Jul 04	60	90-150	0	11.7	0	0	0	0
Pacific	Ports of Call, San Pedro	Jan 05	60	73-129	0	1.7	0	0	0	0
Kumamoto	Crescent City Harbor	Oct 05	38	12-55	5.3	0	0	0	2.6	0
Kumamoto	Crescent City Harbor	Mar 05	60	16-31	3.3	3.3	0	0	0	0
Kumamoto	Tomales Bay	Jul 04	60	46-69	10	0	3.3	0	1.7	0
Kumamoto	Morro Bay	May 05	16	33-56	37.5	0	0	0	0	0
Eastern	Tomales Bay	Jul 04	60	63-92	0	0	15	1.7	1.7	0
Eastern	Tomales Bay	Oct 05	60	57-93	1.7	0	8.3	1.7	0	0
European flat	Tomales Bay	Oct 05	58	54-105	3.5	13.8	0	0	0	0
Olympia	Humboldt Bay	Feb 04	60	46-65	3.3	0	0	3.3	0	1.7
Olympia	Tomales Bay	Apr 04	60	36-64	3.3	1.7	1.7	0	0	0
Olympia	Tomales Bay	Aug 04	60	38-55	0	0	0	0	5.0	0
Olympia	Drakes Bay	Jul 04	63	39-58	1.6	0	3.2	0	1.6	0
Olympia	Sailing Lake	Jan-Feb 05	60	17-86	0	0	0	0	0	0
Olympia	Elkhorn Slough	May 04	60	38-71	1.7	13.3	3.3	0	0	0

Table 2.—Less common symbionts and conditions observed among the oysters listed in Table 1; prevalence (%) is shown in parentheses.

Oyster Type	Location	Organism or Condition
Pacific	Crescent City Harbor	Male gonad hypertrophy (0.7)
Pacific	Humboldt Bay	Prokaryote inclusion in digestive gland (1.7)
Pacific	Humboldt Bay	Unidentified ciliate at base of gill (1.7)
Pacific	Morro Bay	Unidentified protozoan at gill (3.1)
Pacific	Santa Barbara Channel	Male gonad hypertrophy (1.7)
Kumamoto	Crescent City Harbor	Prokaryotic inclusion in digestive gland (2.6)
Kumamoto	Tomales Bay	Prokaryotic inclusion in digestive gland (1.7)
Eastern	Tomales Bay	Bacterial focus (1.7)
Eastern	Tomales Bay	Prokaryotic inclusion in epithelium of gut (1.7) and digestive gland (1.7)
European Flat	Tomales Bay	*Bonamia* sp. in hemocytes (1.7)
Olympia	Humboldt Bay	Unidentified gill ciliate (3.3), unidentified metazoan in vessel (1.7)
Olympia	Tomales Bay	Disseminated neoplasia (1.7)
Olympia	Drakes Bay	Disseminated neoplasia (43)
Olympia	Sailing Lake	Unusually abundant Polydora boring polychaetes in shell

In 20 of the 28 populations sampled, 1.6-37.5 % had *Trichodina*-like ciliated protozoa in association with the gill or mantle epithelium (Figure 1A). Similarly in 15 samples, 1.7-13.8 % contained *Ancistrocoma*-like ciliates in the digestive gland tubules (Figure 1B). No host responses to the presence of any ciliates were observed.

Larval tetraphyllid cestodes (Figure 2) were the most prevalent metazoan symbiont with a prevalence range of 1.7-15 % for the 13 samples in which they were present (Table 1). The cestodes appeared to be identical to those reported in various clams in California as *Echeneibothrium* sp. (see Discussion). The cestodes caused localized tissue trauma where attached to gut epithelium (e.g., Figure 2A); otherwise, no host response was present.

Copepods were observed within the intestine of oysters (Figure 3A) at prevalences of 1.7-19 % for 11 samples in which they were present. Their morphology and location were consistent with the genus *Mytilicola,* most likely *M. orientalis,* although a definitive identification could not be made with the material available. Damage to the gastrointestinal epithelium, with focal inflammation and wound repair, was often apparent, albeit with highly variable intensity. Copepods associated with the gill and mantle (Figure 3B) were present at six locations with a prevalence range of 1.7-5.0 %. Their morphology and location were consistent with the genus *Pseudomyicola* or closely related genera, although a definitive identification could not be made with the material available. No host response to gill or mantle copepods was seen.

We observed putative gregarine protozoa in gut epithelium of one out of 60 individuals from two populations of Pacific oysters and one of Olympia oysters from Humboldt Bay (Figure 4A) and in one Pacific oyster from Tomales Bay (Table 1). A "microcell" protozoan with characteristics of the genus *Bonamia* was identified within hemocytes throughout the circulatory system of one European flat oyster from Tomales Bay (Figure 4B, Table 2).

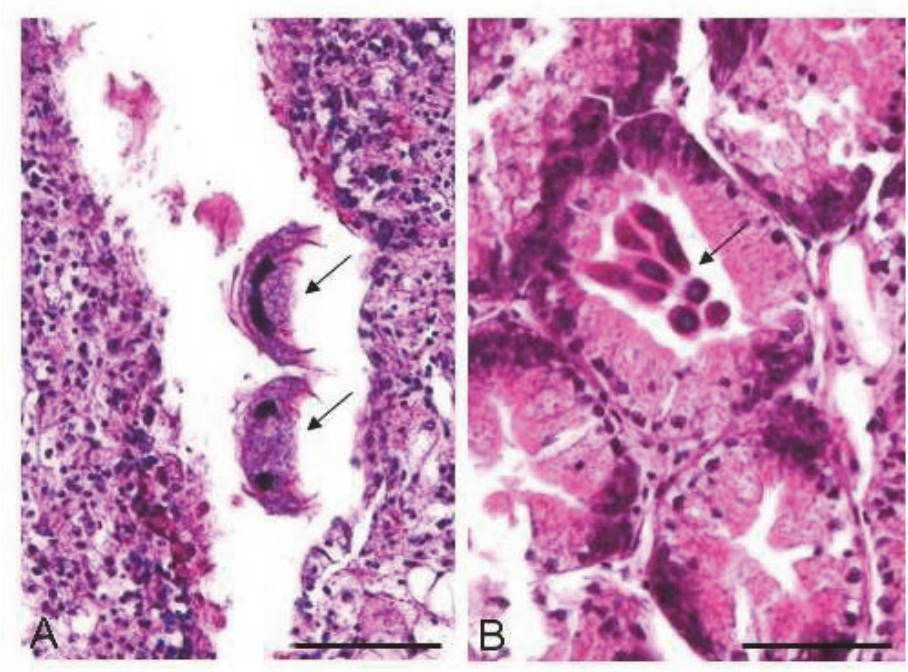

Figure 1.—Ciliates. A: *Trichodina*-like ciliates (arrows) in gill tissue of Pacific oyster, Morro Bay, California, August 3, 2004. B: Cluster of *Ancistrocoma*-like ciliates (arrow) in digestive gland lumen of Olympia oyster, Elkhorn Slough, California, May 26, 2004. Scale bars are 50µm.

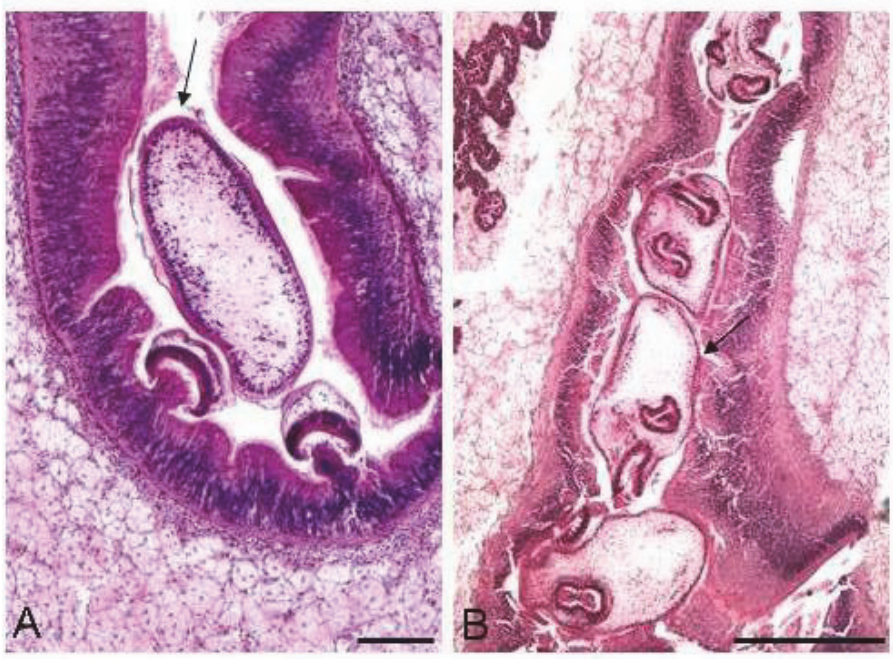

Figure 2.—Cestode larvae (arrows) in gut tissue. A: Kumamoto oyster, Tomales Bay, California, July 8, 2004. B: Pacific oyster, Tomales Bay, California, March 14, 2004. Arrow points to one of four individuals present. Scale bars are100µm in A and 500µm in B.

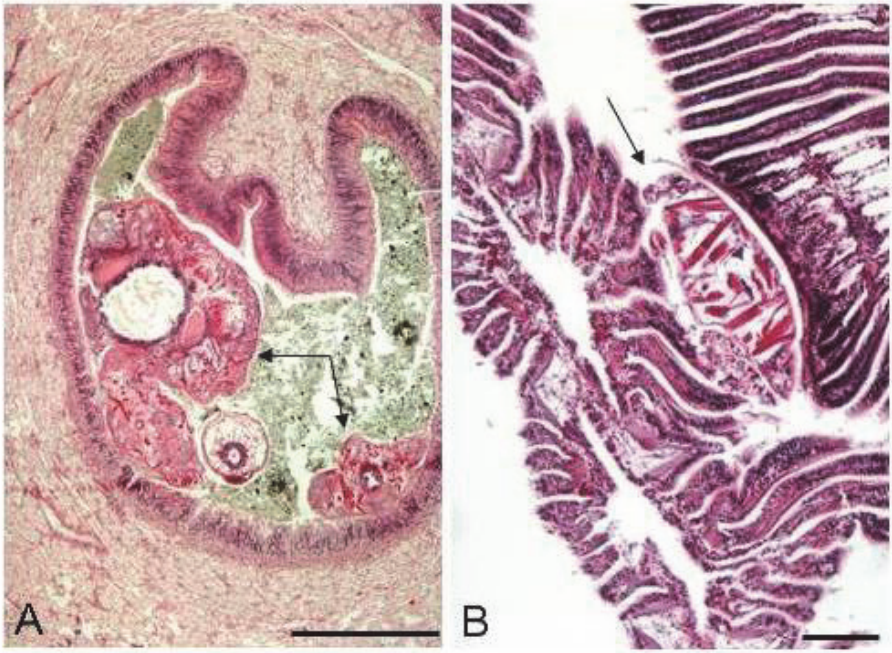

Figure 3.—Copepods (arrows). A: Pacific oyster intestine, Humboldt Bay, California, February 15, 2004. B: Olympia oyster gill, Tomales Bay, California, August 31, 2004. Scale bars are 500µm in A and 100µm in B.

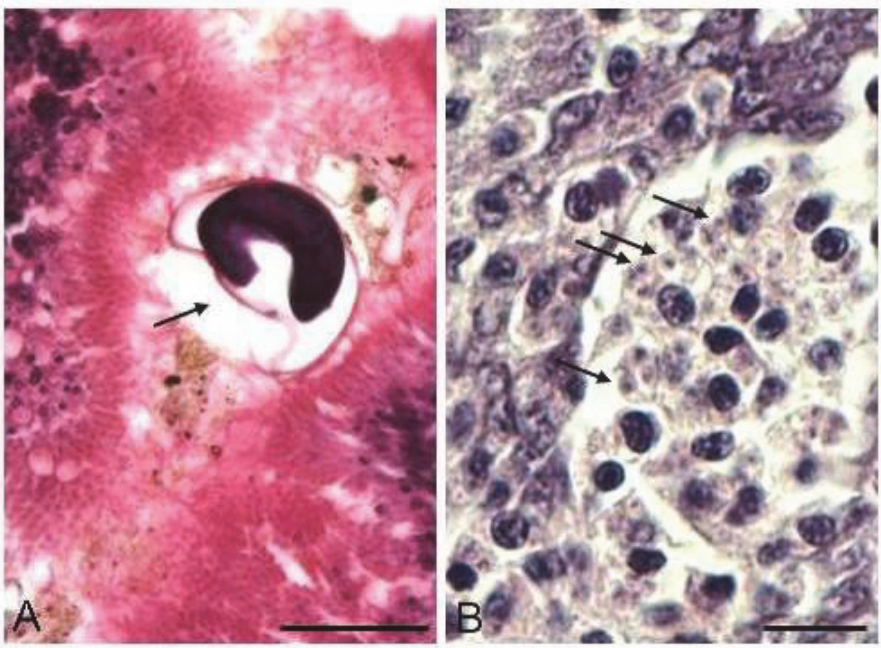

Figure 4.—A: Gregarine-like protozoan (arrow) in gut epithelium of Olympia oyster, Humboldt Bay, California, February 16, 2004. B: *Bonamia* sp. (arrows) in hemocytes within gut epithelium of European flat oyster, Tomales Bay, California, October 7, 2005. Scale bars are 50µm in A and 10µm in B.

Prokaryotic inclusions in the epithelium of the digestive gland or other gut epithelium (Figure 5A) were observed in 1-2 individuals of Pacific, Kumamoto and Eastern oysters. Large basophilic inclusions, consistent with the condition known as viral gametocytic hypertrophy, were present in male gonad of single Pacific oysters from Crescent City Harbor and Santa Barbara (Figure 5B). None of these conditions elicited a host response.

The leukemia-like disease known as disseminated neoplasia (Elston et al. 1992) was observed in Olympia oysters from Tomales Bay and Drakes Bay (Figure 6A and Figure 6B), where it was the most prevalent condition (43%) observed in this study. Although present in low numbers in oyster populations at nearly every location (data not recorded), boring polychaetes *(Polydora* spp.) were unusually abundant among oysters from Sailing Lake in Mountain View.

Discussion

The extensive histopathological survey of California oysters conducted by Katkansky and Warner (1974) was initiated in response to increasing reports of widespread losses of Pacific oysters in various West Coast locations, and was supported by funding from the National Marine Fisheries Service. Since then, various pathologists from the California Department of Fish and Game and other organizations have examined oysters from California, but most studies remain unpublished while others report a specific condition without description of the suite of symbionts present. In the present study, all major populations of farmed oysters were targeted for sampling along with wild Olympia oysters from central to northern California. For logistical reasons Olympia oyster populations in south-central to southern California were not included in this study.

Sufficient numbers from each population were collected so that relatively rare pathogens should have been detected if present. Nevertheless, it is important to note that pathogen prevalence is typically dependent on a myriad of factors such as season, temperature, host life-stage, host density, and stage of episodic outbreak at the population level. When possible, it is highly informative to sample on a monthly basis for at least one year, or quarterly for several years.

Populations of cultured oysters from all of the major growing areas appeared healthy with no evidence of significant disease. When present, recognized oyster pathogens occurred at low prevalence and with apparently negligible population impact. This is in agreement with the lack of reports by oyster farmers of mortalities that appear to be caused by infectious agents, with the exception of 'summer mortality' in Pacific oyster seed in Tomales Bay that is associated with a herpesvirus, and is discussed below.

In our survey, endosymbionts generally considered of minor significance from a health management perspective were variably present in farmed oysters, but usually in a small minority of individuals and rarely with apparent harm to hosts. Olympia oyster populations also supported low prevalences of potentially significant disease agents, although an important neoplastic condition was observed in Olympia oysters at Drakes Bay and Tomales Bay. Subsequent investigations have described this condition in Olympia oysters from San Francisco Bay (Friedman et al. 2005a). A general discussion of each of the symbionts and conditions observed in this study follows.

Ciliates are motile, single-cell eukaryotic organisms common in marine environments. Ancistrocomid ciliates in digestive gland tubules and trichodinids associated with mantle or gill are both commonly reported in oyster histopathology surveys. Although

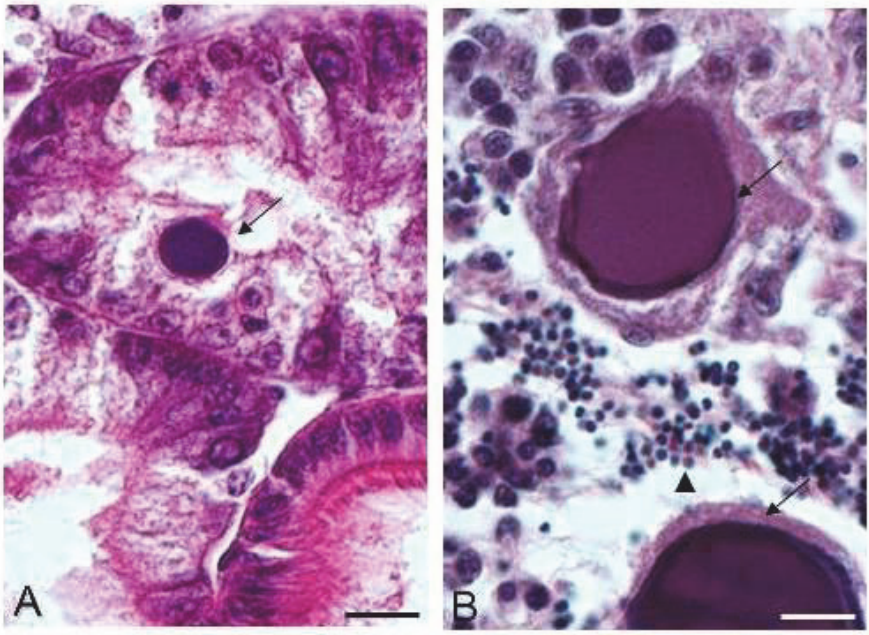

Figure 5.—A: Prokaryotic inclusion (arrow) in digestive gland tissue of Kumamoto oyster, Crescent City, California, October 7, 2005. B: Male gametocytic hypertrophy, Pacific oyster, Santa Barbara Channel, California, July 21, 2004. Arrows point to infected cells, arrowhead points to normal sperm. Scale bars are 10µm.

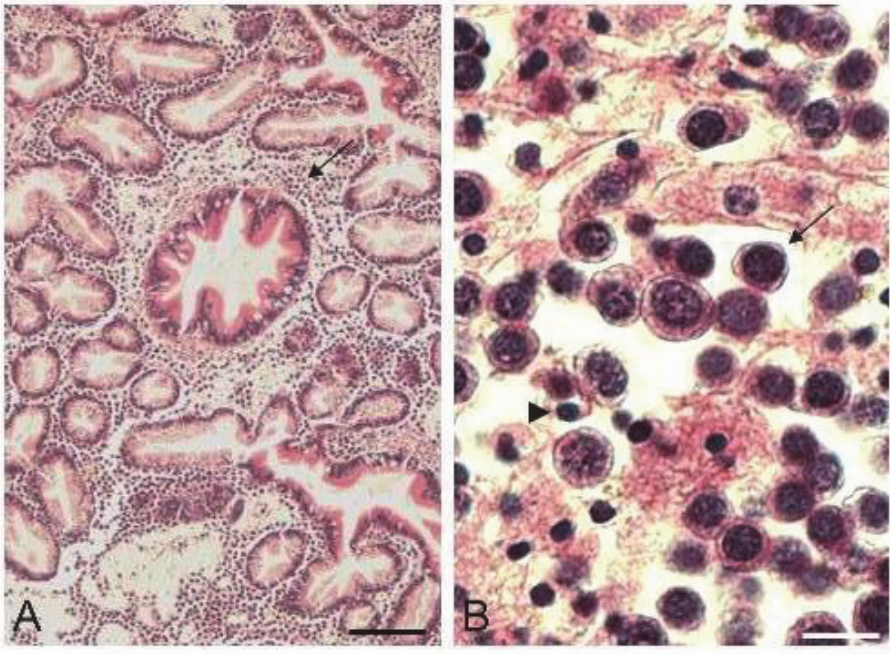

Figure 6.—Disseminated neoplasia in Olympia oyster, Drakes Bay, California, July 8, 2004. A: Digestive gland with neoplastic cells filling connective tissue spaces between tubules (arrow). B: Higher magnification, arrow points to one example of the large neoplastic cells, arrowhead points to nucleus of normal hemocyte. Scale bars are 100µm in A and 10µm in B.

cases of ciliates harming bivalve hosts have been documented, they generally become abundant only during adverse conditions. Indeed, a high density of ciliates associated with mantle or gill may be a useful indicator of physiologic stress in molluscs (J. Moore, unpublished observations). The ciliates observed in our study are morphologically distinct from those described by Elston et al. (1999) as pathogenic to Pacific and Kumamoto oyster nursery stocks in Washington.

Cestodes are parasitic flatworms (Phylum Platyhelminthes), also known as tapeworms. Cestodes in bivalves occur as larval stages, with the subsequent definitive stage developing in elasmobranchs (Lauckner 1983). In California, larval tetraphyllid cestodes *(Echeneibothrium* spp.) have been reported encysted in tissues of native littleneck clams *(Venerupis [Protothaca] staminea)* (Sparks and Chew 1966, Warner and Katkansky 1969a), rough-sided littleneck clams *(Protothaca lacinata)* (Katkansky and Warner 1969), Pismo clams *(Tivela stultorum)* (Warner and Katkansky 1969b), Washington (or California butter) clams (*Saxidomus nuttalli*) and gaper clams *(Tresus nuttallii)* (Katkansky et al. 1969a). *Echeneibothrium* has subsequent life stages in adult rays (Cheng 1967). The morphology of the larval cestodes we observed was consistent with those in the reports for clams cited above, although a definitive assignment requires examination of intact larvae (as opposed to histological sections). While the cestodes reported in clams were nearly always encysted within host tissue, we observed them in oysters only within the gastrointestinal lumen, suggesting that oysters may be inappropriate hosts.

Copepods form a large and diverse subclass of crustacean arthropods. Most are free-living, but parasitic forms have been described from a wide variety of marine animals. The copepods that we observed in the gut lumen of oysters are likely of the genus *Mytilicola*, members of which specifically inhabit the gastrointestinal tract of bivalves and can be of pathological significance (Odlaug 1946, Sparks 1962, Chew et al. 1964, Cheng 1967). Katkansky et al. (1967) reported reduced condition index of Pacific oysters infested with *M. orientalis* in Humboldt Bay and locations in Oregon and Washington, although shell growth and survival were not affected. Sparks (1962) demonstrated that potentially significant tissue alterations can occur in Pacific oysters infested with *M. orientalis*.

Katkansky and Warner (1974) reported a *Mytilicola orientalis* prevalence of 25-40% in Pacific oysters in Morro Bay, as compared with 0-19% in the current study. Katkansky and Warner (1968) reported prevalence of this copepod ranging from 10.5-23 % in Pacific oysters in Humboldt Bay in 1966, while Katkansky et al. (1967) reported incidences of "around 25 %" during 1963-1965, as opposed to 3-12% in the current study. Friedman et al. (2005a) reported very low prevalence of *Mytilicola* in Olympia oysters from San Francisco Bay (present in two of eight individuals sampled at one location, while absent in 287 from other locations). The copepods associated with gill tissue may be members of the genera *Myicola, Pseudomyicola*, or *Ostrincola* that often are found in the mantle or branchial cavity of bivalves and cause variable degrees of harm to the host (Cheng, 1967; Caceres-Martinez et al. 1996; Caceres-Martinez et al. 2005), although we observed no host damage in infested individuals.

Gregarines are a taxonomically uncertain and poorly understood group of protozoans that are commonly observed in oysters with little or no pathologic effects (Lauckner 1983). The low prevalence and lack of association with any signs of disease suggest that the organisms do not pose a significant threat to the oysters examined in this study. We did not observe the gregarine-like organisms that Elston et al. (1998) described in Pacific oysters in nursery beds in Washington.

Bonamia sp. that we identified in European flat oysters from Tomales Bay is the only pathogen encountered that is regulated by the Department of Fish and Game (and is listed as a "Serious Disease"; Title 14, Section 245, California Code of Regulations) or a regulatory agency of the European Union (infection with *B. ostreae* or *B. exitiosa*; World Organisation for Animal Health/OIE). Members of the genera *Bonamia* and the morphologically similar *Mikrocytos* are very small intracellular protozoans that infect oysters and are collectively referred to as microcells. A microcell was detected during the 1960s in European flat oysters imported from Connecticut and placed in Morro Bay, Drakes Bay and Elkhorn Slough (Katkansky et al. 1969b). Elston et al. (1986, 1987) proposed, and Cigarria and Elston (1997) later confirmed, that the microcell was *B. ostreae*, and that the (now defunct) oyster hatchery at Elkhorn Slough was the source of *B. ostreae* that was spread to Europe with European flat oyster seed, and that continues to devastate that species throughout much of the region. Friedman et al. (1989) reported the presence of *B. ostreae* in 1986 in European flat oysters from Tomales Bay and the Santa Barbara Channel.

We detected no evidence of *Mikrocytos mackini*, the causative agent of Denman Island Disease described in Pacific oysters from British Columbia, Canada (Farley et al. 1988). While never reported in California, it has recently been reported from areas in Washington State including locations near hatcheries that export seed to California. However, disease expression and consistent histological identification of the pathogen typically occur only in older animals held at low tidal levels and only following cold water periods (Bower 1988); few oysters are cultured under that combination of conditions in California. If *M. mackini* is present in California, it is cryptic and benign.

Prokaryotes that form inclusions in the bivalve gastrointestinal tract and other epithelia are typically described as Rickettsiales-like or Chamydiales-like organisms based on the histological and ultrastructural morphologies characteristic of these orders of bacteria (Fryer and Lannan 1994). These infections are generally described as benign with no host response in bivalves, although some can be highly pathogenic, such as the Rickettsiales-like prokaryotes associated with mortalities of scallops *(Pecten magellanicus)* in Rhode Island (Gulka et al. 1983), scallops *(Pecten maximus)* in France (Le Gall et al. 1991), clams *(Venerupis rhomboides)* in Spain (Villalba et al. 1999), and oysters *(Crassostrea ariakensis)* in China (Sun and Wu 2004).

The cases of gametocytic hypertrophy we observed in male Pacific oysters appeared identical to those described in various populations of that species worldwide (Meyers et al. 2009 and references therein). The condition occurs both in males and females. Those studies that include electron microscopy reported the occurrence of non-enveloped 40-60 nm icosahedral particles within hypertrophied nuclei consistent with being members of the Papillomaviridae or Polyomaviridae (Garcia et al. 2006). As in our study, most occurrences were at low prevalence, in a small proportion of gonad cells, and with little or no host response.

Disseminated neoplasia is a proliferative leukemia-like condition that has all of the hallmarks of cancer and occurs in numerous species of bivalves (Elston et al. 1992). It has been described in Olympia oysters from Oregon (Mix et al. 1977) and British Columbia (Meyer et al. 2010). Friedman et al. (2005a) reported very patchy distribution of the condition in Olympia oysters from San Francisco Bay, California, being absent at most sites while at an average prevalence of 37% at one site (Candlestick Point). Disseminated neoplasia occurred at a similar prevalence at Drakes Bay, California, and these high prevalences have been confirmed with additional sampling (J. Moore, unpublished

observations). It is somewhat paradoxical that the Candlestick Point and localized Drakes Bay populations are dense and thriving despite the presence of a clearly fatal disease. This is similar, however, to occurrence of disseminated neoplasia in bay mussels *(Mytilus trossulus)* in Puget Sound, Washington, where it is has been shown to cause significant mortality but consistently occurs at high prevalence in dense populations (Elston et al. 1992) and shows no relationship with anthropogenic carcinogens (Krishnakumar et al. 1999).

Infestation of oyster valves with shell-boring polychaetes (Phylum Annelida) of the genus *Polydora,* or related genera, is common worldwide. Although problematic in certain species in certain regions, in California they generally do not occur at densities sufficient to affect condition or appearance. However, we observed that Olympia oysters within Sailing Lake, an enclosed saltwater lake connected to San Francisco Bay, harbored an unusually high density of these polychaetes, possibly the result of diminished predators or lack of tidal influence in that unique environment.

Friedman (1996) and Burreson et al. (2000) reported the presence of the protozoan *Haplosporidium nelsoni* in Pacific oysters from Drakes Bay, California. *H. nelsoni* is the causative agent of the devastating MSX disease in Eastern oysters along the east coast of North America, although it has little effect on Pacific oysters. Although not detected in the current sample set, we found *H. nelsoni* in a subsequent sample of Pacific oysters from Drakes Bay (J. Moore, unpublished observations). Katkansky and Warner (1970a) reported a sporulating haplosporidian among Pacific oysters from Humboldt Bay that had been transplanted from British Columbia three years earlier. As discussed by Elston (1993), that haplosporidian appeared to be distinct from *H. nelsoni*, but it has not been reported since the initial description. Katkansky and Warner (1970b) reported the presence of the haplosporidian *Minchinia costalis* (later designated *H. costale*) in Tomales Bay Eastern oysters that had been raised on the east coast and trucked to the bay to hold before marketing. *H. costale* has not been reported since its initial discovery, and more recent restrictions would not allow the importation of infected seed. We saw no evidence of the unique microcell and the haplosporidian that Friedman et al. (2005a) reported at low prevalence in Olympia oysters in certain areas of San Francisco Bay. We also detected no evidence of the ovarian parasite described in Pacific oysters in Humboldt Bay by Becker and Pauley (1968) at high prevalence (3/5 females in 1967 and 15/21 in 1968) and also reported by Katkansky and Warner (1974). A shellfish pathologist who has examined large numbers of Pacific oysters from Humboldt Bay confirmed that the condition has been extremely rare to absent in recent years (Ralph Elston, AquaTechnics, personal communication).

A phenomenon known as 'summer mortality' has been reported for several decades in numerous populations of Pacific oysters worldwide, and has been associated with herpesvirus infection in some locations (Elston 1999). In California, summer losses of Pacific oysters are observed in Tomales Bay as sudden, but very patchy, mortality events of young seed. It is the most significant disease problem in California oyster culture, yet also is difficult to document and characterize because of the high variation in occurrences over space and time. Growers have reduced economic damage by altering size and source of seed, and timing of planting. A consistent relationship between the mortality events and a herpesvirus has been demonstrated for Pacific oysters in Tomales Bay (Friedman et al. 2005b; Burge et al. 2006, 2007). Outbreaks followed warm-water events and tissue alterations, including the presence of hypertrophic cells with nuclear chromatin margination,

were typically observed only at that time. Thus it is not unexpected that we saw no evidence of summer mortality in our Tomales Bay samples, which were collected in April and May prior to summer warming events.

Throughout the latter half of the 20th century there was increasing awareness of the potential for transmission of pathogens and other unwanted species with aquaculture transfers. Recent practices of the California Department of Fish and Game allow only the importation of bivalve seed from sources with multi-year health histories and acceptable biosecurity coupled with other management practices. In-state transfers are also managed to prevent further spread of disease agents. An informal zone-based system (i.e., establishment of areas harboring or lacking a specific pathogen) is used to minimize spread through human movement of animals. Clearly, knowledge of pathogen distribution is necessary for rational decision-making with respect to shellfish importations and transfers and provides a proper background to understand and interpret the onset of any significant diseases that may emerge in the future. Data presented here represent a baseline dataset of potential disease agents in California oyster populations during 2004-2005. In addition to histology, we archived tissue samples in ethanol for potential future molecular diagnosis of specific pathogens. Such samples may also prove useful for potential population genetic studies on the hosts.

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