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PRESENCE OF *BONAMIA OSTREAE* AMONG POPULATIONS OF THE EUROPEAN FLAT OYSTER, *OSTREA EDULIS* LINNÉ, IN CALIFORNIA, USA

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ABSTRACT European Flat oysters, *Ostrea edulis* Linné, reared in Tomales Bay and the Santa Barbara Channel, California were examined to determine the possible causes of elevated mortality among these stocks. The protozoan parasite, *Bonamia ostreae* was found to be the most significant parasite in these oysters. A rickettsiales-like and a gregarine-like parasite were seen within gill tissues of a flat oyster and one bay mussel, respectively. *Bonamia ostreae* elicited an intense inflammatory reaction in affected flat oysters and is believed to be the cause of the elevated mortality observed in these stocks.

KEY WORDS: European flat oysters, *Ostrea edulis*, protozoan parasite, *Bonamia ostreae*, ricketsiales-like, gregarine-like, bay mussel

INTRODUCTION

Few studies have been conducted to ascertain the status of diseases in shellfish grown along the west coast of North America despite the recent increased demand for shellfish. This, in combination with a decline in natural bivalve mollusc abundance, has encouraged more intensive culture of marine bivalves (Elston and Leibovitz 1980, Chew 1984). Katkansky et al. (1969) examined the feasibility of rearing the European flat oyster, *Ostrea edulis* Linné, in California waters. Flat oysters were planted in Tomales Bay, Drakes Estero, Morro Bay, and Elkhorn Slough in 1963. By 1969 *O. edulis* reared in the latter three sites had suffered stunted growth and up to 100% cumulative mortality. All gapers and up to 57% of the live oysters sampled were found, upon routine histological examination, to be infected with an intrahemocytic microcell. Only the Tomales Bay site appeared to be free of both the microcell and elevated mortality (Katkansky et al. 1969, Katkansky and Warner 1974). In a later study, Elston et al. (1986) surveyed seven locations in Washington state and two sites in California to determine the incidence of the microcell parasite, *Bonamia ostreae*, in flat oysters, *O. edulis*. Bonamiasis was detected in flat oysters cultured in the four Puget Sound, Washington state sites sampled but not among oysters from California. Historically, this disease is associated with up to 80% cumulative mortality within 6 mo after initial introduction of oysters to waters known to contain *B. ostreae* (Poder et al. 1982, Balouet et al. 1983, Elston et al. 1986). Although the two California locations sampled, both in Humbolt Bay were free of the disease, Elston et al. (1986) traced the source of the infected Washington stocks to initial outplanting stock from Elkhorn Slough, California. Katkansky et al. (1969) previously determined that *O. edulis* from Elkhorn Slough were infected with an intrahemocytic "microcell" that Elston et al. (1986) later concluded were *B. ostreae*, the same parasite found in the Washington state oysters.

In response to elevated mortality of flat oysters grown in California, we have conducted an extensive survey of *O. edulis* grown in Tomales Bay and the Santa Barbara channel, two of the principal culture sites in California. The purpose of this study was to identify and enumerate pathogens of flat oysters and to determine the potential impact of such parasites on the commercial oyster industry in California.

MATERIALS AND METHODS

Samples of between 30–56 *O. edulis* were collected from five culture facilities in Tomales Bay between May

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16–June 16, 1986. Eighteen to 67 flat oysters were collected from the Santa Barbara channel site between May 21–July 28, 1986. In addition, samples of the following species were collected from three Tomales Bay culture sites during April, May and June 1986: bay (blue) mussels, *Mytilus edulis*, Olympia oysters, *O. lurida* and Pacific oysters, *Crassostrea gigas*. All animals were fixed in Davidson's solution (Shaw and Battle 1957) for 48 hr and transferred to 70% ethanol. A 4–5 mm wide section of each fixed oyster including heart tissue was removed ventral to the labial palps, and processed for routine histological examination. Paraffin sections (5 μm) were stained with hematoxylin and eosin and observed and photographed using an Olympus light microscope.

RESULTS

The mortality of certain oyster stocks listed in Table 1 had approached 80% in previous years as reported by the growers. Infected oysters collected in our survey did not show specific signs of disease but some were thin and watery, indicating their poor condition. Light microscopy of stained tissue sections showed massive infiltration of hemocytes into the vesicular connective tissue in certain oysters. These affected areas often surrounded the stomach, digestive diverticulae and to a lesser extent, the gonads (Fig. 1a). Hemocytic infiltration could also be detected within the gills of some individuals. Parasites which measured 2–3 μm in diameter with an eccentric nucleus appeared to be cytozoic within many of the hemocytes in areas of heavy infiltration. Up to 10 parasites could be observed within infected cells (Fig. 1b). *Bonamia ostreae* was found only in the European flat oyster, *O. edulis*. A rickettsiales-like intracellular parasite was observed within the gills of one brooding female *O. edulis* grown in Tomales Bay (TB-5) (Fig. 2a and 2b). There was no apparent host reaction to the intracellular parasite although many cells of the gill epithelium harbored developing bacteria within inclusions.

Gills of one bay mussel from the TB-2 site were infected with a gregarine-like parasite (Fig. 3). There were no parasites detected in any of the *O. lurida* examined during the course of this study.

DISCUSSION

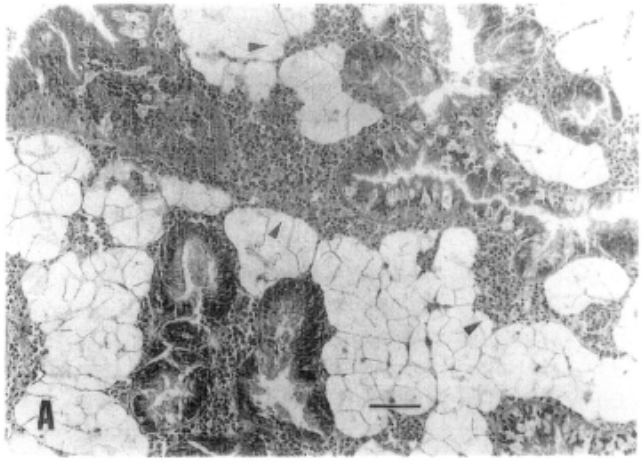
The most prevalent parasite affecting *O. edulis* reared in California coastal waters as determined by this survey was *B. ostreae*. Several species other than the flat oyster harbor intracellular parasites similar to *B. ostreae*. These include: *O. lurida*, the Sydney rock oyster, *Saccostrea commercialis* and *C. gigas* (Farley et al. 1988). In addition, a flat

TABLE 1.

Prevalence of *Bonamia ostreae* among populations of European flat oysters *Ostrea edulis* from Tomales Bay and the Santa Barbara Channel, California

Location	Date	Species	No. Positive / No. Examined	Percentage
TB-1	4-28-86	*M. edulis*	0/60	0
TB-2	5-16-86	*O. edulis*	4/42	9.5
	5-21-86	*O. edulis*	11/56	19.6
	5-30-86	*M. edulis*	0/30	0[1]
	5-30-86	*M. edulis*	0/30	0
	5-30-86	*M. edulis*	0/2	0
	6-16-86	*O. edulis*	1/30	3.3
	6-16-86	*O. edulis*	1/14	7.1
	6-16-86	*O. lurida*	0/8	0
TB-3	5-30-86	*O. edulis*	0/20	0
	6-2-86	*O. edulis*	0/30	0
TB-4	5-30-86	*O. edulis*	0/11	0
	5-30-86	*O. edulis*	0/26	0
	6-2-86	*O. edulis*	0/30	0
TB-5	6-1-86	*O. edulis*	0/41	0[2]
	6-2-86	*O. edulis*	0/24	0
TB-6	6-2-86	*O. edulis*	1/33	3.0
TB-7	6-3-86	*C. gigas*	0/30	0
SB-1	5-21-86	*O. edulis*	0/18	0
		O. edulis	1/12	8.3
		O. edulis	1/14	7.1
	7-28-86	*O. edulis*	2/60	3.0
	7-28-86	*O. edulis*	2/42	4.8
	7-28-86	*O. edulis*	0/67	0

[1] A single animal contained gregarine-like parasites within the gills.
[2] One animal contained rickettsiales-like parasites within the gills.



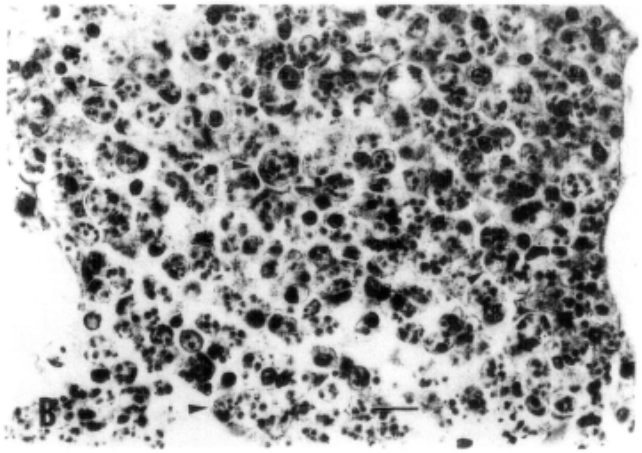
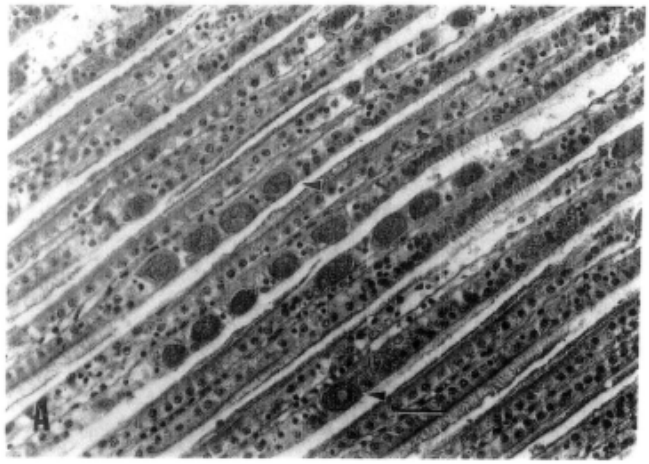


Figure 1. (a) Infiltration of granular haemocytes into the vesicular connective tissues surrounding the digestive diverticulae of a 2 yr old flat oyster (*O. edulis*) infected with *B. ostreae*. H and E stain, Bar = 40 μm. (b) Hemocytes of flat oyster (*O. edulis*) infected with *B. ostreae*. H and E stain, Bar = 10 μm.



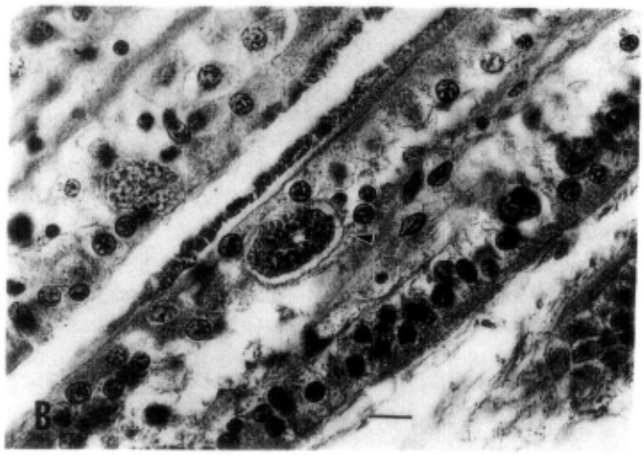


Figure 2. Gills of a female flat oyster (*O. edulis*) containing rickettsiales-like parasites (a) lower magnification (Bar = 30 μm) and (b) higher magnification (Bar = 10 μm). H and E stain.

oyster, *Ostrea lutaria* indigenous to New Zealand, reared in the U.K. in waters known to contain *B. ostreae* infected *O. edulis* have been shown susceptible to bonamiasis (Bucke and Hepper 1987). The diseases caused by "microcell" parasites in these hosts can range from acute to chronic (Farley et al. 1988).

Comparisons of the various "microcells" has been attempted in an effort to resolve their taxonomic relationships (Farley et al. 1988). A new genus (*Microkytos*) has been proposed which contains two of the species, *M. mackini* (g. n. sp. n.) and *M. roughleyi* (g. n. sp. n.), that are the causes of "Denman Island disease" of *C. gigas* and "Australian Winter disease" in *S. commercialis,* respectively (Farley et al. 1988). They can be distinguished from *B. ostreae* by morphological properties, host specificity and tissue tropism (Farley et al. 1988).

Of the various "microcells", *B. ostreae* has been studied in most detail and has been associated with significant losses of the European flat oysters in France (Pinchot et al. 1979, Poder et al. 1982, Balouet et al. 1983) and in Washington state (Elston et al. 1986) and California (Katkansky et al. 1969, Farley et al. 1988) in the United States. In our study, infestations with *B. ostreae* evoked an extensive inflammatory response absent in the few animals harboring rickettsiales-like or gregarine-like parasites. These observations, in conjunction with experimental results of other researchers in Europe and Washington state (Poder et al. 1982, Balouet et al. 1983, Elston et al. 1986), suggest that bonamiasis is a significant cause of *O. edulis* mortality and a hinderance to flat oyster culture in the state of California. In contrast to infection prevalences in other studies, the maximum 20% observed in oysters in our study is relatively low. Katkansky et al. (1969) recorded a higher prevalence of "microcell disease" in flat oysters grown in Morro Bay (29%), Drakes Estero (36%) and Elkhorn Slough (57%). The reason for the low prevalence of bonamiasis in our study compared to that reported by Katkansky et al. (1969) is unknown but may be due, in part, to differences in present culture methods and perhaps development

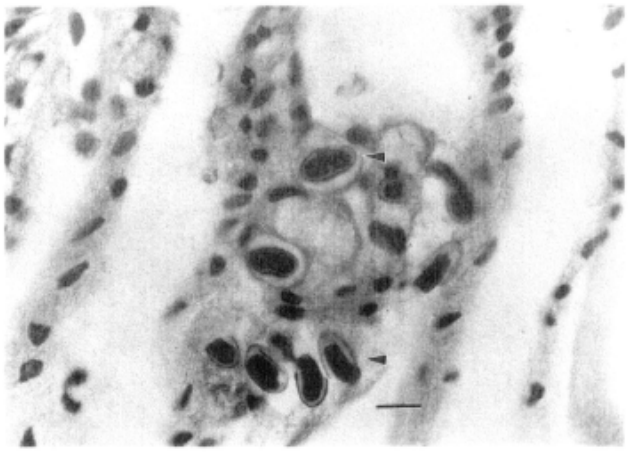


Figure 3. Gregarine-like parasites within the gills of a bay mussel (*M. edulis*). H and E stain, Bar = 10 μm.

of some resistance to the disease. Elston et al. (1987) has shown that certain stocks of European flat oysters are more resistant to bonamiasis than others. The existance of this resistance was demonstrated in experimental studies but the mechanisms involved are unknown. Although the disease can be transmitted directly from one oyster to another, further examination of bay-dwelling animals, including other molluscs, fishes, and worms, could reveal alternative host for *B. ostreae*.We did not detect evidence for such alternative hosts in the bivalve species present in the same waters with flat oysters examined in our study.

Attempts have been made to trace the source of the *B. ostreae* infections in the U.S. and France. Farley et al. (1988) observed "microcells" in *O. edulis* from Milford, Connecticut, the source for the California farms. Elston et al. (1986) further reconstructed the transplantation history of bonamia-infected flat oyster stocks from Elkhorn Slough, California to France, where bonamiasis was first reported in 1979 and subsequently devastated the *O. edulis* culture industry (Elston et al. 1986, Balouet et al. 1983, Poder et al. 1982). The movement of seed from the California brood stock was also believed to have been the source of the *O. edulis* in Washington state (Elston et al. 1986). Although "microcells" observed in *O. lurida*, a native west coast oyster, from Yaquina Bay, Oregon might suggest a west coast source of *B. ostreae* for *Ostrea* spp., these parasites resemble more closely *M. mackini* in their tissue specificity (leydig cells not hemocytes).

Inspections and certifications help but may not prevent the spread of oyster diseases. The oysters shipped to France and Washington state were examined prior to shipment but detection of the parasite in seed is difficult. Perhaps if the oysters had been reared for some period in quarantine and examined a second time the disease would have been detected before they were out planted. Certification combined with controlled rearing of potential imported species of fish and shellfish may be one method to reduce or prevent introductions of exotic diseases to new geographical regions.

In contrast to *B. ostreae*, there was little to no host response to the rickettsiales-like microorganisms in our study. The enlargement of infected host cells is similar to the responses of other bivalve molluscs, such as sea scallops, *Placopecten magellanicus* Gmelin (Gulka and Chang 1984a), Japanese littleneck clam, *Tapes japonica* and Japanese scallop, *Patinopecten yessoensis* (Elston 1986), to similar infestations. In contrast, Gulka and Chang (1984b) reported that blue mussels, *M. edulis*, from Rhode Island were infected with rickettsiales-like microbes which evoked encystment of the parasites. Gulka and Chang (1984a) and Elston (1986) noted that the potential effects of rickettsiales-like infections may not become apparent until the animals are held under stressful conditions, such as intensive culture and certain field environments.

Life stages of several gregarines (Phylum Apicomplexa, Class Sporozoea) have been documented in marine bivalves. Nematosial gregarines use marine pelecypods as intermediate hosts. Oocysts of *Nematopsis schneideri* have been found within the gills of *M. edulis, Spisula solida, Tapes pullastra, Cardium edule, Macoma balthica,* and others (Lauckner 1983). The pathogenicity of gregarine infestations in these bivalves and those in our study has not been determined. Parasites similar to the gregarine observed in *O. edulis* in our study have been observed within tissues of approximately 10% of *M. edulis* collected from Bodega Bay and from black abalone (*Haliotis cracheriodii*) and red abalone (*H. rufescens*) from the central and southern California coast (unpublished observations). These parasites were observed within many of the tissues of *M. edulis* and *Haliotis* spp. and may reach these tissues via the circulatory system. Examination of large numbers of gregarine-parasitized bivalves has revealed little or no inflammatory changes associated with these invaders. Further examination is needed to determine the effect of the rickettsiales-like and gregarine-like parasites on mussels and other bivalves to properly assess the significance of these microorganisms to the health of bivalve molluscs and the culture industry.

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