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A comprehensive oyster disease survey in California

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Project Hypotheses
This project was a snapshot disease survey of cultured and wild native oysters in California.

Project Goals and Objectives
The goal of this project was to investigate and document the presence of disease agents in cultured and wild oyster populations throughout California. This is the first comprehensive survey to be conducted since that of Katkansky and Warner in the late 1960s to early 1970s (Katkansky and Warner, 1974). Particular emphasis was placed on detection of the agent of Denman Island Disease (*Mikrocytos mackini*, Farley et al., 1988). This pathogen of the Pacific oyster (*Crassostrea gigas*) was detected in the State of Washington in 2002, and many California oyster growers receive seed from Washington State. All major populations of farmed and feral commercial oysters and wild native oysters *Ostrea lurida* were targeted for sampling. Sufficient numbers from each population were collected so that relatively rare pathogens should have been detected when they are present.

Briefly describe project methodology
Adult oysters (n = 60 per population, when possible) were collected from major oyster populations, including farmed and feral Pacific oysters (*Crassostrea gigas*), farmed Kumamoto oysters (*C. sikamea*), Atlantic oysters (C. virginica), and European flat oysters (*Ostrea edulis*), and wild native oysters (*Ostrea lurida*). The sample size of 60 allows for detection of a pathogen with 95% confidence if its prevalence in the population is at least 5% (American Fisheries Society, 2005). Samples of labial palp (20mg per oyster, in pools of four oysters) were preserved in absolute ethanol and archived for potential future molecular detection of pathogens. Two cross-sections of each animal 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 hrs. Hematoxylin and eosin-stained 5μm tissue sections were prepared and examined for the presence of pathogens and evidence of disease.

Describe progress and accomplishments toward meeting goals and objectives
We collected 28 separate samples totaling 1676 oysters from major growing areas and some unusual locations. The samples included four farmed oyster species (*Crassostrea gigas*, *C. sikamea*, *C. virginica*, *Ostrea edulis*) in addition to the native oyster, *O. lurida*. Commercial oysters were collected from farms located in Santa Barbara, Morro Bay, Drakes Estero, Tomales Bay and Humboldt Bay, and

from an experimental planting of *C. gigas* and *C. sikamea* at Crescent City. A feral population of *C. gigas* at Ports of Call, San Pedro was also sampled. Native oysters (*O. lurida*) were collected from wild populations in Elkhorn Slough, Sailing Lake (an enclosed lagoon in Mountain View), Drakes Estero, Tomales Bay and Humboldt Bay. Most samples consisted of adult oysters (e.g. *C. gigas* shell height 70+mm) although one sample of *C. gigas* seed (10-20mm) from Humboldt Bay was included.

A variety of disease conditions, symbionts and potential disease-causing agents including virus, neoplasm, bacteria, protozoa and metazoa were detected and the prevalence in each sampled population was recorded. Essentially all symbionts of potential pathological significance occurred at very low prevalence and were not associated with signs of disease. There was no evidence for the presence of *Mikrocytos mackini*, the agent if Denman Island Disease. Below is a brief synopsis of the findings. Details on the specific sample sizes, sample locations, animal sizes, and prevalence of each condition or organism encountered will be published separately.

Low densities of Trichodina-like ciliate organisms were observed in association with the gill or mantle epithelium in a minority of individuals in 20 of the 28 populations sampled. Similarly, in 15 of the 28 samples a minority of individuals had low numbers of Ancistrocoma-like ciliates in digestive gland tubules. No host response was observed in association with the presence of either ciliate.

Larval tetraphyllid cestodes residing in the gut lumen were the most abundant metazoan commensal observed, at prevalences up to 15%. One to several individuals were present per oyster, with occasional tissue damage and localized infiltration of hemocytes. The organisms appeared to be one of the cestodes reported in various clams in California as Echeneibothrium spp. (Sparks and Chew 1966, Katkansky and Warner 1969, Katkansky et al. 1969, Warner and Katkansky 1969a,b).

Copepods were observed within the intestine of numerous oysters. Although these organisms are typically identified to species by examination of whole animals rather than histological sections, their appearance was consistent with being of the genus Mytilicola (probably *M. orientalis*; Odlaug 1946, Katkansky et al. 1967, Katkansky and Warner 1968, Katkansky and Warner 1974). Copepods associated with the gill and mantle occurred at all locations and in most oyster species. As with Mytilicola spp., the identification in histological sections is tenuous but their morphology and location was consistent with assignment as a member of the genus Pseudomyicola, and probably *P. spinosus* (Caceres-Martinez et al. 2005).

Gregarines are a taxonomically uncertain and poorly understood group of organisms. We observed gregarine-like protozoa in one out of 60 individuals of each of two populations of *C. gigas* from Humboldt Bay, one of 60 *O. lurida* from Humboldt Bay, and one of 60 *C. gigas* from Tomales Bay. None were associated with host response or other signs of disease.

Large basophilic inclusions were observed in male gonadal cells in one individual each of the *C. gigas* collected from Crescent City Harbor and Santa Barbara. These appeared identical to those described by Meyers et al. (2009) for *C. gigas* from Alaska, for which they demonstrated the presence of viral

particles within the inclusions, and as reported in males and females of this species around the world (e.g. Cheslett et al. 2009, Waterman et al. 2008). Prokaryotic inclusions were observed residing in the digestive gland or other gut epithelium in 1-2 individuals each among the *C. gigas*, *C. sikamea* and *C. virginica* sampled. These are most likely rickettsial bacteria. Although members of these groups can cause catastrophic disease, such as the rickettsial agent of abalone withering syndrome, they are very commonly found at low prevalence and low intensities in many marine organisms, and more often not associated with any signs of disease.

The leukemia-like disease known as disseminated neoplasia (Elston et al. 1992) was observed in one native oyster (*O. lurida*) in Tomales Bay and at relatively high prevalence (43%) at Drakes Estero. Numerous studies of this disease in this and other species have failed to establish linkages between disease prevalence and degraded environments or the presence of carcinogens.

A 'microcell' protozoan was identified within hemocytes in one European flat oyster (*O. edulis*) from Tomales Bay. Using molecular methods with the material collected from this study we have demonstrated that the organism is *Bonamia ostreae* (J. Moore, R. Carnegie, K. Hill, unpublished studies).

Although present in low numbers in oyster populations at nearly every location, the boring polychaete *Polydora websteri* was unusually abundant among oysters from Sailing Lake, Mountain View. This is likely related to its unique environment (an enclosed saltwater lagoon) and the presumed low presence of key predators.

Project modifications
In order to more thoroughly capture different populations at each major growing area, the scope of the sampling was nearly doubled from 15 samples to 28 samples, all but two of which included at least 58 individuals. Additional funding to process these samples was provided by the California Department of Fish and Game.

Project outcomes
This study represents the first comprehensive California oyster disease survey in more than three decades. No evidence of the agent of Denman Island Disease, *Mikrocytos mackini*, was detected in any of the populations sampled. Populations of cultured oysters from all of the major growing areas appeared healthy with no evidence of significant disease and low prevalences of potential pathogens. Native oyster populations also appeared healthy and with low prevalence of potentially significant disease agents. Disseminated neoplasia occurred at high prevalence at Drakes Estero, ironically in a population with consistently high density. Subsequent sampling of *O. lurida* in San Francisco Bay demonstrated similar prevalences of the disease at some locations (Grosholz et al. 2008).This is similar to distribution of the same disease in bay mussels *Mytilus trossulus* in Puget Sound, Washington, where it is present in both contaminated and pristine environments (Elston et al. 1992).

Impacts of project
Knowledge of pathogen distribution is necessary for rational decision-making with respect to shellfish importations, transfers and disease management. It also provides a proper background to understand and interpret the onset of any significant diseases that may occur in the future.

Benefits, commercialization and application of project results
The survey supports continuation of the current practices regarding oyster importations and transfers, enabling oyster farming to continue to thrive in the major growing areas, providing jobs to local economies, many of which are depressed due to fishing industry declines. Due to findings of oyster disease agents in Washington State, assessment of the disease status of California oyster populations was essential to allow continuation of national and international oyster seed exports from California, as well as transfers of oysters between farms within California. This project accomplished that objective and has been useful in the permitting process for oyster transfers by the California Department of Fish and Game. The results will also be used by national and international authorities when reviewing the status of California oysters in their own importation decision-making. In addition to the data obtained by histological examination of oyster tissues, the archived tissue samples stored in ethanol can be used for future molecular diagnosis of specific pathogens. Collectively, the sampling effort provides a baseline dataset that may indicate the apparent absence, in 2004-5, of specific disease agents that are later identified within the state. Such baseline data is essential to understanding the introduction, movement, and management of emerging diseases.

Issue-based forecast capabilities
This study provides baseline information that will be critical to interpret and respond to future disease outbreaks.

Tools, technologies and information services developed
N/A

Publications
Workshops/presentations
Denman Island Disease Risk Assessment and Risk Management Workshop, Tacoma WA, October 2004. Participated in development of guidelines to manage spread and impact of Denman Island Disease in western states.

Dissemination of results
Conversations with registered California aquaculturists, shellfish industry regulators in Washington and Alaska, and members of the native oyster restoration community.

Cooperating organizations
Local and state
California Department of Fish and Game, Marine Region, provided matching funds for personnel expenses and histology processing service.

Nongovernmental
Registered oyster aquaculturists within the State of California.

International implications
Up-to-date knowledge of pathogen distributions in California oyster populations should facilitate continuity and development of oyster seed export markets to other states and countries, particularly from Humboldt Bay, which has a long history of oyster and clam seed exports.

Keywords
oyster, disease, pathogen, California, survey