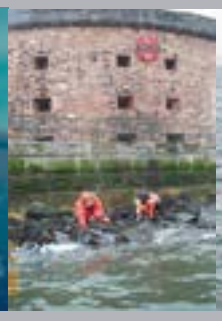
Occurrence of Parasites and Diseases in Oysters and Mussels of U.S. Coastal Waters National Status and Trends, the Mussel Watch Monitoring Program

NOAA National Centers for Coastal Ocean Science
Center for Coastal Monitoring and Assessment

D. A. Apeti
Y. Kim
G.G. Lauenstein
J. Tull
R. Warner




March 2014
N O A A T E C H N I C A L M E M O R A N D U M N O S N C C O S 1 8 2
NOAA NCCOS Center for Coastal Monitoring and Assessment

CITATION

Apeti, D.A., Y. Kim, G. Lauenstein, J. Tull, and R. Warner. 2014. Occurrence of Parasites and Diseases in Oysters and Mussels of the U.S. Coastal Waters. National Status and Trends, the Mussel Watch monitoring program. NOAA Technical Memorandum NOSS/NCCOS 182. Silver Spring, MD 51 pp.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Juan Ramirez of TDI-Brooks International Inc., and David Busheck and Emily Scarpa of Rutgers University Haskin Shellfish Laboratory for a decade of analystical effort in providing the Mussel Watch histopathology data. We also wish to thank reviewer Kevin McMahon for invaluable assistance in making this document a superior product than what we had initially envisioned.

Occurrence of Parasites and Diseases in Oysters and Mussels of the U.S. Coastal Waters. National Status and Trends, the Mussel Watch MonitoringProgram.

Center for Coastal Monitoring and Assessment (CCMA)
National Centers for Coastal Ocean Science (NCCOS)
National Ocean Service (NOS)
National Oceanographic and Atmospheric Adminstration (NOAA)

Authors
Dennis A. Apeti, Gunnar Lauenstein, and Rob Warner
NOAA Center for Coastal Monitoring and Assessment
Coastal and Oceanographic Assessment, Status and Trends Branch

Yungkul Kim
Assistant Professor
Department of Integrated Environmental Science & Department of Biology
School of Science, Engineering & Mathematics
Bethune-Cookman University

Jamila Tull
Florida A&M University
School of the Environment

March 2014

NOAA Technical Memorandum NOS NCCOS 182

TABLE OF CONTENTS

ACRONYMS AND TECHNICAL TERMS

Ag – silver
American oyster– *Crassostrea virginica* also known as Eastern oyster
As – arsenic
Blue mussel – *Mytilus edulis* (East coast), *M. galloprovinciallis* (California coast), M. trossulus (Northern Calif. to Alaska)
CCMA – Center for Coastal Monitoring and Assessment
Cd – cadmium
Cestode – a parasitic flatworm with a specialized organ for attachment
COAST – Coastal Ocean Assessments and Status & Trends Branch
Cr – chromium
Cu – copper
DDT – dichlorodiphenyltrichloroethane, a pesticide
Dermo – a common name for an oyster disease caused by *Perkinsus marinus*, a pathogen that affects oysters
Edema – tissue swelling from fluid internal to the organism
Hawaiian oyster – *Ostrea sandvicensis*
Hg – mercury
Infection intensity - is a measure of the severity of infection or occurrence of disease
Mn – manganese
MSX – multinucleated sphere x/unknown, an oyster disease caused by *Haplosporidium nelsoni*
MWP – Mussel Watch Program
NCCOS – National Centers for Coastal Ocean Science
Necrosis – tissue death
Nematode – a roundworm with an unsegmented body
Neoplasia – abnormal growth of cells forming a tumor
Neoplasm – new tissue growth serving no physiological function, commonly referred to as benign or malignant growth
Ni – nickel
NOAA – National Oceanic and Atmospheric Administration
NOS – National Ocean Service
NS&T – National Status and Trends
PAH – polycyclic aromatic hydrocarbons
Pb – lead
PCB – polychlorinated biphenyls
PIB – prokaryotic inclusion bodies
Prevalence - describes the proportion of individuals in the population that are infected or diseased
Se – selenium
Sn – tin
Tumor – a solid or fluid-filled lesion, commonly synonymous with neoplasia
Xenoma – a symbiotic complex formed by hypertrophying host cells and multiplying intra-cellular parasites; a cyst
Zebra mussel – two species of invasive Great Lakes mussels (*Dreissena polymorpha* (called zebra mussel)) and *D. bugensis* (called quagga mussel)
Zn – zinc

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

As a part of the National Oceanic and Atmospheric Administration's National Status and Trends Program (NS&T), the Mussel Watch Program (MWP) uses bivalve mollusks (oysters and mussels) as sentinel organisms to monitor the health of our nation's coastal and marine waters, including Alaska, Hawaii, Puerto Rico, and the Great Lakes. The program measures contaminant concentrations (organic compounds and metals) in bivalves collected from over 300 coastal locations around the U.S. In 1995, the program began surveying the histopathology conditions (diseases and parasites) of the sentinel mollusks. An array of parasite taxa (e.g. prokaryotic inclusions, cestodes, nematodes, trematodes), and diseases and certain tissue conditions (e.g. Dermo, MSX, tumor, neoplasia, edema and necrosis) are quantified using direct quantitative count or estimative semi-quantitative scales.

This report provides both national and regional patterns of parasites and diseases that occur in mussels and oysters, which hold some important commercial and recreational values in the U.S. This first-ever national assessment of Mussel Watch histopathology monitoring data provides coastal managers and the public with an overview of the most recent (2008-2009) conditions, and historical trends since collection of histopathology parameters began in 1995. Because contaminants, pathogens and parasite infection in bivalves have some linkages, correlations between contaminant body burdens and occurrence of histopathology parameters were also determined.

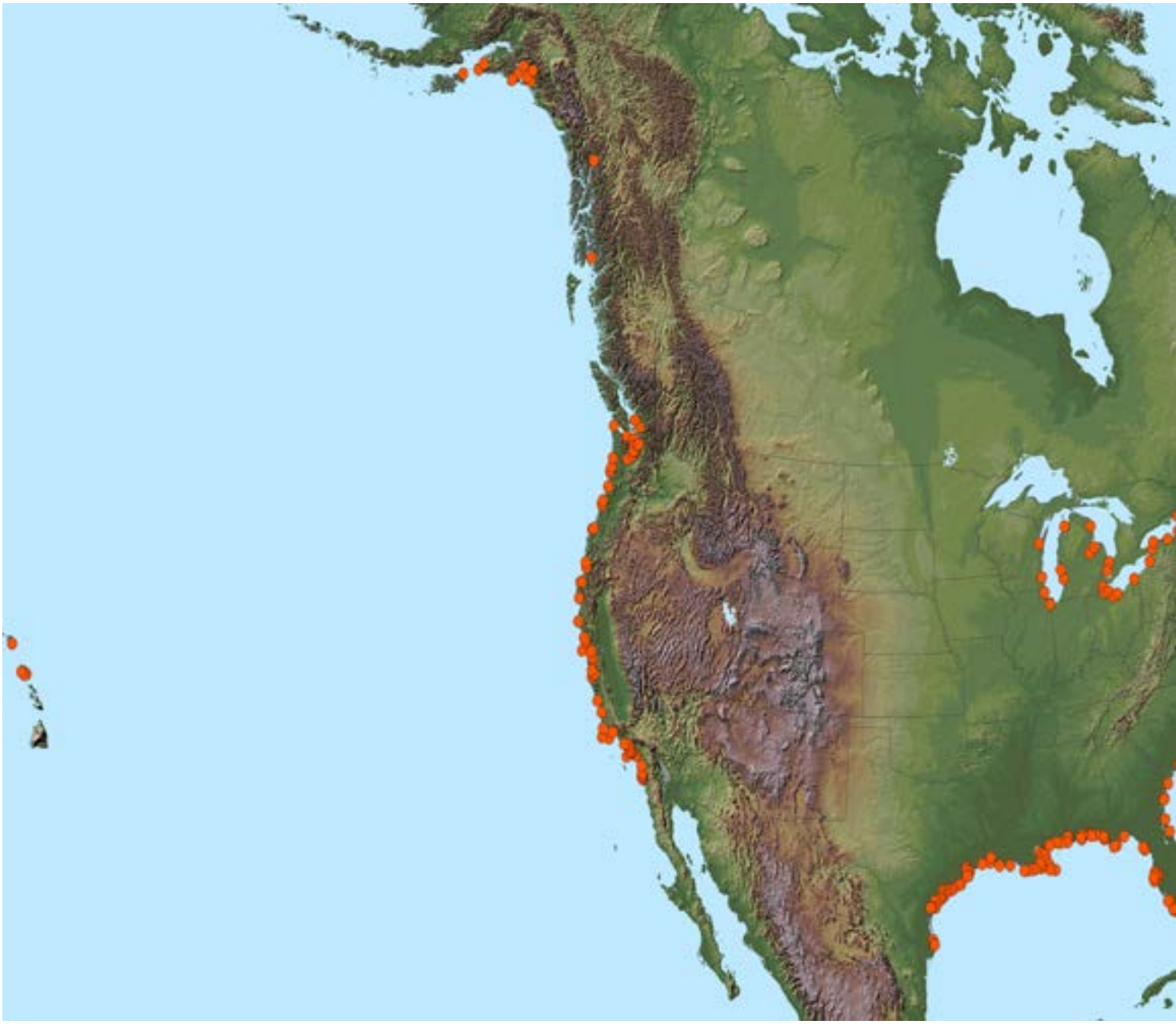
Occurrence as well as the severity of occurrence of parasite and disease were found to vary broadly among host bivalves and between geographic locations. Parasitic infections and diseases generally ranged from low to medium in the sentinel organisms. However, the presence of some hot-spots with elevated infection and disease occurrence were observed in some coastal areas indicating some regional differences. In general, American oysters from the Gulf of Mexico had significantly higher parasite taxa than the east coast American oysters, which in turn harbored more parasites than the mangrove oyster from Puerto Rico and the Hawaiian oysters. Among the mussels, the east coast blue mussels had significantly higher parasitic taxa richness than the west coast blue mussels, which harbored more parasites than blue mussels from Alaska. Zebra mussels were found to harbor few parasites and significantly lower cases of diseases than all the other sentinel bivalves measured. Many of the diseases and pathologies characterized as part of the MWP were only found in oysters and blue mussels. However, disease and tissue conditions, such as ceroid bodies, hemocytic infiltration, and digestive gland atrophy, were found to be pervasive among all of the bivalve types. The assessment of temporal trends indicated that occurrence of parasites and diseases were largely static and fairly minor in the sentinel organisms over the monitoring period.

The degree of parasitic infections and intensity of pathology in oysters and mussels are indicators of water quality. Our data showed that correlations between the histopathology parameters and the bivalve's contaminant body burdens were generally weak or nonexistent, indicating fairly healthy coastal conditions. Although some hot-spots of parasite infection and disease occurrence were observed at various levels across the monitoring sites, the lack or infrequency of correlation with high levels of contaminant body burdens suggests that the causes of infection hot-spots may lie elsewhere. These parasites and disease hot-spots may be the results of congruent environmental factors that cause intermittent changes in water quality.

In assessing the spatial distribution and temporal trends of some common parasites and diseases in bivalves from our coastal waters, this report provides valuable information on the histopathology conditions of the sentinel bivalve shellfish as indirect indicators of the health of our coastal waters. For management purposes, this report constitute an unprecedented baseline information against which histopathology measures could be weighed after unforeseen events of disease outbreak. This speaks to the importance of the NS&T monitoring data to federal, state and local coastal resource managers. As the only continuous coastal monitoring program that is national in scope, the MWP is unique at being capable of providing an unparalleled opportunity to de-

termine national, regional and local perspectives of the health of our coastal ecosystems. The need for sustained monitoring of our coastal waters at the national scale is therefore warranted as environmental stressors, including unforeseen natural and anthropogenic events, and climate change continue to impact coastal resources. This baseline information will be vital for early detection of degrading conditions, as well as providing a cost-effective approach for mitigation and protection of problem areas.

Mussel Watch Program National Monitoring Sites



INTRODUCTION

Coastal and estuarine environments contain diverse and unique habitats that support biologically diverse estuarine and marine species, which in turn support important recreational and commercial fisheries. However, coastal and marine waters are also a sink for organic and inorganic pollutants, which potentially have direct adverse effects on the habitats and biota, and indirect impacts on humans (through the food chain) who consume some of these marine resources.

The NS&T Mussel Watch program was designed to monitor the status and trends of chemical contamination of U.S. coastal waters and the health of the sentinel organisms as indicators of water quality. The program was established in response to a legislative mandate under Section 202 of Title II of the Marine Protection, Research and Sanctuaries Act (MPRSA) (33 USC 1442), which called on the Secretary of Commerce to, among other activities, initiate a continuous monitoring program. The Mussel Watch program began in 1986 and is one of the longest running, continuous coastal monitoring programs that is national in scope. The MWP is based on the collection and analysis of sediments and bivalve mollusks. The MWP currently includes nearly 300 sites (Figure 1) and measures more than 150 chemical pollutants, including polycyclic aromatic hydrocarbons (PAHs), chlorinated pesticides including DDT and its break-down products, tributyltin and its break-down products, polychlorinated biphenyls (PCBs), and trace elements (Table 1).

Mussels and oysters are sessile organisms that tolerate a variety of stressors, such as adverse physical conditions, and chemical and biological toxins of the surrounding water. Thus, they are known to be good integrators of contaminants in a given area (Berner et al., 1976; Farrington et al., 1980; Farrington, 1983; Tripp and Farrington, 1984).

Most bivalve species harbor an array of parasites and pathologies as a direct consequence of filter-feeding. Evidence of relationships between certain tissue pathologies and contaminant exposure, as well as influence of contaminant exposure on the bivalve immune system, has been established (Weis et al., 1995; Johnson et al., 1992; MacKenzie et al., 1995). Because pollutants, pathology, and parasite body burdens in bivalves have some linkage, the Mussel Watch program began surveying the sentinel organisms for histopathology parameters in 1995, with even earlier work performed on selected Mussel Watch samples. Parameters measured include an array of parasites (Table 2a), such as prokaryotes (e.g. rickettsia, chlamydia), tapeworms (e.g. cestodes), and roundworms (e.g. nematodes). Oyster diseases, like tumor, tissue necrosis and xenoma, and pathological conditions, such as digestive gland atrophy and tissue hemocytic infiltration (Table 2b), are also measured.

The NS&T Mussel Watch program is unique as being the only continuous coastal monitoring program that is national in scope; thereby providing an unparalleled opportunity to determine national, regional and local perspectives. Using the Mussel Watch program monitoring data from 1995 to 2009, this report provides both national and regional assessments of patterns of occurrence and distribution of parasites and diseases in mussels and oysters along the US coastal waters. This first ever national assessment summarizes more than ten years of Mussel Watch program monitoring data on occurrence of parasites and disease in mussels and oysters, and is intended for use by managers and concerned citizens for coastal shellfish and ecosystem health assessment.

Shellfish acquaculture (Image NOAA Fisheries)

METHODS

Monitoring Sites and Bivalve Species

Mussel Watch sites were selected to represent large coastal areas that can be used to construct a nationwide assessment. Mussel Watch started with 145 nationwide sites in 1986 and has operated continuously since. New sites were gradually added to this program, resulting in approximately 300 total sites today (Figure 1). Because one single species of mussel or oyster is not common to all coastal regions, several bivalve species are collected to gain a national perspective.

The introduced zebra mussel, *Dreissena polymorpha* (Pallas, 1771), and quagga mussel, *Dreissena bugensis* (Andrusov, 1897), were sampled at the Great Lakes. Zebra mussels were first discovered in the Great Lakes in 1988, and were likely introduced from Europe in ship ballast water (Hebert et al., 1989). Quagga mussels were found in 1991 (May and Marsden, 1992).

Mytilid mussels were collected from the Northeast and West coasts, including Alaska. According to Hilbish et al. (2000), mussels found on the East coast are predominately blue mussels (*M. edulis* Linnaeus, 1758). On the West coast, three mussel species were collected: *Mytilus californianus* (Conrad, 1837) and two species referable to the *M. edulis* complex, *Mytilus galloprovincialis* (Lamarck, 1819), and *Mytilus trossulus* (Gould, 1850). As mytilid populations in California may be the result from hybridization between *M. galloprovincialis* and *M. trossulus* (Hilbish et al., 2000), it's possible that some central and northern California sample sites yielded mussels that were hybrids. Therefore, for the purpose of this report, the west coast species complex were designated blue mussels (*Mytilus spp.*) while the blue mussels from Alaska were identified as *M. trossulus*.

Three oyster species were used. The American oyster, *Crassostrea virginica* (Gmelin, 1791) was sampled from coastal and estuarine areas of the mid-Atlantic, the Southeast coast and the Gulf of Mexico. The mangrove oyster, *Crassostrea rhizophorae* (Guilding, 1828), was collected in Puerto Rico and the Hawaiian oyster, *Ostrea sandvicensis* (Sowerby, 1871) were sampled in Hawaii.

American oyster (*Crassostrea virginica*)

Blue mussels *(Mytilus sp.*)

Zebra mussels (*Dreissena sp.*)

Figure 1. National distribution of shellfish species used as sentinel bivalves by the MWP. Except for instances where more detail is needed, in this report, the following inclusive terms will be used when referring to the different bivalve taxa: The name "zebra mussels" is used in reference to the *Dreissena bugensis* (Quagga mussel) and *Dreissena polymorpha* (zebra mussel) mussels. The general term "oysters" is used jointly for *Crassostrea virginica* (American oyster), *Crassostrea rhizophorae* (Mangrove oyster), and *Ostrea sandvicensis* (Hawaiian oyster). The common name "blue mussels" is used for *Mytilus edulis* (East coast blue mussel), *Mytilus californianus* (Californian blue mussel), *Mytilus galloprovincialis* (West coast hybrid blue mussel), and *Mytilus trossulus* (Northwest and Alaska blue mussel).

Sample Collection

The standard operational procedures for Mussel Watch bivalve collection are described in detail in Apeti et al. (2011) and Lauenstein and Cantillo (1998). Except in the Great Lakes, bivalve sampling occurs during winter months to minimize the influence of reproduction on contaminant body burden as changes in lipid levels may affect their contaminant body burdens (Jovanovich and Marion, 1987). In the Great Lakes, zebra mussels were collected in late August through mid-September; winter sampling in the Great Lakes is difficult because the lakes are frequently frozen. MWP sampling frequency for bivalves has varied over the years, but remained at a biennial frequency during the period of this study (1995-2009). Bivalves were dredged, tonged, or handpicked in intertidal or shallow subtidal areas. Great Lakes zebra mussels were also collected by free-diving. All samples were preserved on ice and shipped overnight within 24 hours of sampling to analytical laboratories.

Analytical Methods

Contaminant Analysis

The MWP analyzes a broad suite of pollutants, including: polycyclic aromatic hydrocarbons (PAHs); chlorinated pesticides, including DDT, and its break-down products; tributyltin and its break-down products; polychlorinated biphenyls (PCBs); and trace elements (Table 1). As a part of the program's quality control protocols, chemical analyses follow stringent procedures that are detailed in Kimbrough and Lauenstein (2006) for metals, and Kimbrough et al. (2007) for organic compounds. Lauenstein and Cantillo (1998) also provide a detailed account of the analytical methods quality assurance protocols. The NS&T reports contaminant concentrations on a dry weight basis and the data are available on the web at http://egisws02.nos.noaa.gov/nsandt/#.

Laboratory Sample Preparation for Histology Analysis

Zebra mussels

Due to their small size, zebra mussels were preserved whole in their shells in Davidson's fixative for one week without first cutting the adductor muscle. To speed shell decalcification, 20–30 ml of acetic acid was added to the fixative solution. When the shell became separated from the soft parts, the fixative was replaced by 70% ethyl alcohol for sample storage.

Blue mussels

The adductor muscles of organisms were cut with a sharp knife so that the valves remained open. The entire animal was placed in Davidson's fixative for one week and then transferred to 70% ethyl alcohol for storage. A sharp knife or scalpel was carefully run between the shell and the mantle to separate the meat from the shell. This procedure was repeated for the second shell to completely detach both sides of the mantle from the shell.

For both zebra and blue mussels, shell length of each animal was recorded and byssal threads were completely removed from the byssal gland to avoid problems when sectioning the tissue. A 3–5 mm thick cross-section, including digestive gland and gill, was removed using a scalpel and placed in a tissue capsule for immediate processing.

Oysters

For oysters, 12 animals from each site were randomly selected and opened with an oyster knife. A small section of mantle tissue (5 mm x 5 mm) was removed for the culturing of *Perkinsus marinus,* the Dermo disease pathogen. A 3–5 mm-thick transverse cross-section of tissue was removed from five of the animals using scissors. The cross-section was immediately transferred to a tissue capsule and placed in Davidson's fixative for two days, followed by storage in 70% ethyl alcohol.

Histological Analysis

For all bivalves, the chemically fixed tissue was embedded in paraffin after dehydration and cleaning. The tissue-paraffin block was then placed in a freezer overnight before sectioning. The paraffin-embedded tissue blocks were first sliced at 20 µm to expose an entire tissue cross-section, and then sectioned at 5 µm. Tissue sections were deparaffinized and hydrated using a xylene-ethanol series. Following hydration, slides were stained in a pentachrome series, dehydrated in a series of acetic acid dips, followed by acetone, cleared in xylene and mounted in Permount®.

Table 1. List of organic pollutants and metals analyzed by the NS&T Program.

Metals: Silver (Ag), Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), Mercury (Hg), Manganese (Mn), Nickel (Ni), Selenium (Se), Tin (Sn), Zinc (Zn)
Butyltins: monobutyltin, dibutyltin, tributyltin, tetrabutyltin
Chlordanes: alpha-chlordane, gamma-chlordane, oxychlordane, cis-nonachlor, trans-nonachlor, heptachlor, Heptachlor-Epoxide
Chlorpyrifos
DDTs: ortho and para forms of parent 2,4'DDT and 4,4'DDT and metabolites 2,4'DDE; 4,4'DDE; 2,4'DDD; 4,4'DDD
Dieldrins: aldrin, dieldrin and endrin
Chlorobenzenes: 1,2,3,4-Tetrachlorobenzene, 1,2,4,5-Tetrachlorobenzene, Hexachlorobenzene, Pentachlorobenzene, Pentachloroanisole
Hexachlorocyclohexanes (HCHs): Alpha-Hexachlorocyclohexane, Beta-Hexachlorocyclohexane, Delta-Hexachlorocyclohexane, Gamma-Hexachlorocyclohexane
Endosulfans: Endosulfan I, Endosulfan II, Endosulfan sulfate
PCBs: PCB8/5, PCB18, PCB28, PCB29, PCB31, PCB44, PCB45, PCB49, PCB52, PCB56/60, PCB66, PCB70, PCB74/61, PCB87/115, PCB95, PCB99, PCB101/90, PCB105, PCB110/77, PCB118, PCB128, PCB138/160, PCB146, PCB149/123, PCB151, PCB153/132, PCB156/171/202, PCB158, PCB170/190, PCB174, PCB180, PCB183, PCB187, PCB194, PCB195/208, PCB199, PCB201/157/173, PCB206, PCB209
Mirex

Parasite/Pathology Quantification

Tissue sections were examined under the microscope using a 10× ocular and a 10× objective. When necessary, a 25× or 40× objective was used for closer examination. Major tissue types examined included gill, mantle, gonad and gonoducts, digestive gland tubules, stomach/digestive gland, and connective tissue. For oysters, *P. marinus* was assayed by the more precise thioglycollate method (Powell and Ellis, 1998), rather than histology. All parasites and pathologies were scored for occurrence either quantitatively or on a semi-quantitative scale.

Conditions scored quantitatively (Table 3) were evaluated by keeping a running count of occurrences of the condition as the slide is scanned to avoid re-examining each incident multiple times. Quantitative scores were used for parasites, pathologies, and selected morphological conditions that could be tallied individually (Kim et al., 2006). Parasites counted quantitatively included prokaryotic inclusion bodies (rickettsia, chlamydia, etc.), various ciliates, gregarines, other protozoans, nematodes, encysted cestodes and metacercariae of trematodes, copepods and other unidentified organisms. Ciliates were quantified by tissue type (gill and digestive tract), as were the gregarines. Nematodes were also subjected to quantitative count based on their observed cross-sections. A number of tissue pathological conditions were also evaluated quantitatively, including the number of ceroid bodies, and cases of hemocytic infiltration that were scored separately as focal and diffuse.

Some conditions were assigned to a semi-quantitative scale relative to the intensity or the extent of the affected area (Table 3a). Definitions of scale values can be found in Kim et al. (2006). A semi-quantitative 0-to-4-point scale was used for invasive Trematode sporocysts (Fellodistomidae and Bucephalidae). *Perkinsus marinus*, an oyster parasite infection was also evaluated using the semiquantitative 0-to-5-point based on scale established by Craig et al. (1989). *Haplosporidium nelsoni* (MSX pathogen) infection was scored on a 0-to-4-point scale of Kim et al. (2006)

adapted from Ford and Figueras (1988). For each specimen examined, the presence of neoplasia and unusual digestive tubules was recorded. For digestive gland atrophy, a condition known to be caused by a variety of stressors most likely related to poor nutrition (Winstead, 1995), the average degree of thinning of the digestive tubule walls was assigned a numerical rating on a 0-to-4-point scale (Kim et al., 2006).

Table 2a. List of parasites measured by the MWP as part of the histopathology assessment of bivalves.

Parasite Category	Parasites
Cestodes	Body cestodes, Gill cestodes, Mantle cestodes
Copepods	Body Copepods, Gill Copepods, Gut Copepods
Ciliates	Digestive tract Ciliates, Large gill Ciliates, Small gill Ciliates, Gut Ciliates
Protozoans	Digestive tubule protozoan, Gut protozoan
P. marinus	
H. nelsoni	
Nematodes	Nematode
Trematodes	Trematode sporocyst, Trematode metacercaria, Proctoeces
Gregarines	Body Gregarines, Gill Gregarines, Mantle Gregarines
Prokaryotes	Digestive tubule rickettsia, Gut rickettsia, Chlamydia
Coccidians	Pseudoklossia
Pea crabs	Pinnotherid crab

Table 2b. List of diseases and tissue conditions/pathologies measured by the MWP as part of the histopathology assessment of bivalves.

Disease/tissue condition category	Parasites/Tissue Conditions
Digestive tubule conditions	Digestive gland atrophy, Unusual digestive tubule
Edema	Edema
Necrosis	Focal necrosis, Diffuse necrosis
Neoplasia	Neoplasm
Hemocytic infiltration	Focal hemocytic infiltration, Diffuse hemocytic infiltration
Tumor	Tumor
Xenoma	Xenoma

Table 3. List of quantitative and semi-quantitative categories of the histopathology conditions. In a number of cases (e.g. gregarines and ciliates), subcategories by bivalve type and organism morphology are individually tallied.

Quantitative Category			
	Oysters	Blue mussels	Zebra mussels
Prokaryote inclusions	X	X	
Gregarines	X	X	
Ciliates	X	X	
Xenomas	X	X	
Coccidians		X	
Cestodes	X		
Trematode metacercariae	X	X	
Nematodes	X		X
Copepods	X	X	X
Pinnotherid crabs	X	X	
Echinostomes		X	X
Ceroid bodies	X	X	X
Tissue hemocytic infiltration	X	X	X
Tissue necrosis	X	X	X
Semi-quantitative Category			
P. marinus (Dermo)	X		
H. nelsoni (MSX)	X		
Trematode sporocysts	X		
Digestive tubule atrophy	X	X	X
Unusual digestive tubules	X	X	X

Data Analysis and Statistical Approach

All data processing and analysis were evaluated using JMP® statistical software. Microsoft Excel and SigmaPlot® were also used for additional visual data analyses and graphing. Geographic information system ArcGIS® package was used for the spatial mapping of the results.

The contaminants used in this assessment include major and trace metals and the organic compounds as illustrated in Table 1. For this assessment, organic contaminants were grouped by compounds of the same class or by congeners. The concentration value of each class was defined by calculating the sum (total) of the concentrations of individual compounds in the class. Thus, total PAHs was defined as the sum of 24 polycyclic aromatic hydrocarbons compounds; the sum of DDT and its metabolites, DDE and DDD, as total DDTs; total dieldrins as the sum of aldrin, dieldrin and lindane; the sum of concentrations of all chlordanes as total chlordanes; and the sum of concentrations of 18 individual PCB congeners as total PCBs.

In this report, parasites or pathologies of the same taxa or group were pooled together by category as indicated in Tables 2a and 2b and the resulting total values were used to determine prevalence and intensity for parasitic infections, and occurrence and abundance for diseases. For instance, the class of Cestoda or tapeworms includes body cestodes, gill cestodes, and mantle cestodes (Tables 2a and 2b). For conditions measured semi-quantitatively, the scale rating replaced the number of occurrences in the following calculation.

Prevalence describes the degree of occurrence as the proportion of individuals in the population that are infected by a specific parasite or carried a particular pathology and was calculated as:

Prevalence= (Σ hosts with parasite or pathology)/(number of hosts analyzed)

Infection intensity was calculated as the average number of occurrences of the parasite or pathology in infected hosts. This is a measure of the severity of parasitic infection or occurrence of a pathology in the affected organisms.

Intensity = (Σ number of occurrences of parasite or pathology)/(number of hosts with parasites)

Site-specific prevalence and intensity values were used to assess a nationwide spatial distribution of parasite infection and disease occurrence, respectively. To evaluate the spatial distribution of parasite and disease occurrences in the U.S. coastal waters, the 2008-2009 Mussel Watch data, which represent the most recent and complete dataset, were used. A three-group category scheme (high, medium, low) was applied using ArcGIS 10 and was applied to the site-specific prevalence/intensity, and occurrence/abundance values. In ArcGIS, data classification was based on the Jenks grouping method, which uses natural break points inherent in the data. ArcGIS identifies break points that best divide the data into the specified number of classes. The resulting categories are made of values with significantly reduced variance, while the differences between the classes are maximized.

To determine site-specific temporal trends, nonparametric regression analysis based on Spearman rank correlation was applied to the long-term prevalence values for both parasite infection and occurrence of diseases. The assessment of temporal trends was based on data range between 1995 and 2009 of the monitoring years, which gave an n value of about 9 on average. With a significance probability level (p) < 0.05 and n value of 9, the critical value for Spearman Rho (ρ) was 0.7. The Spearman rank statistic was also used to characterize relationships between contaminants body burden and the parasite taxa and diseases respectively. When applicable, Wilcoxon or ANOVA were used to assess regional and species contrasts of the level of parasitic infection.

Table 4. Semi-quantitative scale for Haplosporidium nelsoni (MSX) infection modified from Ford and Figueras (1988).

Score	Description
0	Uninfected, no parasites found in the tissue cross-section
1	Parasites confined to gill or digestive tract epithelial tissue, ≤ 10 plasmodia per 100X field of either gill or body tissue
2	Parasites restricted to gill or digestive tract epithelial tissue, Very light infection, 11 ≤ plasmodia ≤ 100 per 100X field of either gill or body tissue
3	Parasites spreading into gill or digestive tract subepithelium, parasites restricted to epithelium and subepithelium area, > 100 plasmodia per 100X field of either gill or body tissue but < 1 per 1000X oil immersion field
4	Parasites more evenly distributed in gill or digestive tract subepithelium and scattered through somatic tissue, > 100 per 100X field of either gill or body tissue but 1 to ≤ 10 per 1000X oil immersion field

RESULTS

In this report, results of parasites are discussed first (with parasitic taxa organized from prokaryotes to eukaryotes) followed by diseases. Each section is introduced with a brief overview, with a descriptions of the parasites and diseases and their potential health impacts to both bivalves and humans. For each parasite taxa and disease, the prevalence and intensity (based on the 2008-2009 monitoring data) were used to assess national-scale spatial distributions (recent status) of the parasite taxa and disease conditions. Site-specific temporal trends were also mapped to illustrate, on the national scale, coastal zones where the measured histological parameters (parasites, diseases and tissue conditions) were increasing or decreasing. Any significant correlations between histopathology conditions and coastal contaminants are also presented. In order to provide a more synoptic perspective, parasitic taxa richness in the host bivalves was further discussed to address regional infection susceptibility.

PARASITES

Prokaryotic Inclusion Bodies (Chlamydia and Rickettsia)

Overview

Chlamydia and Rickettsia are two genera that are included together in this report under the heading of prokaryotic inclusion bodies (PIB) (Figure 2). Chlamydia is an obligate intracellular parasite to eukaryotic hosts. They are non-motile, coccoid bacteria that replicate within the cytoplasmic vacuole in the host. Chlamydia has two forms, a reticulate body and an elementary body, which is an infectious particle (spore-like) that is released when the infected cell ruptures. Rickettsia are non-motile, obligate, intracellular parasites. Rickettsia do not form spores but can take the form of cocci, rod, or thread-link forms. They can replicate either within the nucleus or cytoplasmic vacuole of the host. Several rickettsia bacteria have been linked to human diseases such as typhus, rickettsial pox and Rocky Mountain spotted fever. Prokaryotic inclusion bodies are usually observed in the duct and tubule walls of the digestive gland of bivalves (Kim et al., 2006).

Current Status and Temporal Trends

PIBs were present in blue mussels and oysters, but were not present in zebra mussels. The spatial distribution based on the 2008-2009 measurements indicated that the prevalence was low to medium in all bivalves, except in oysters from the East coast where the highest prevalence values were found (Figure 3). PIB infection intensity was generally low to medium, although sites in Gulf of Mexico and northern California have recorded of medium to severe infection (Figure 4). The prevalence and intensity data combined suggest a few hot-spots in the nation, including Cape Henlopen in DE, Cape May and Absecon Inlet in NJ, Charlotte Harbor in FL, Breton Sound in LA, and Gerstle Cove in CA.

PIB infection was fairly static over time except at locations in the Northeast Atlantic. Notable increasing trends were found in Brewster Island, Boston Harbor; and Lower Bay and Battery Park in Hudson Raritan estuary (Figure 5). A decreasing trend was observed at the Mamaroneck monitoring site in the Long Island Sound.

Correlation

No significant correlations were found between contaminant body burden of any of the sentinel bivalves and prevalence of PIB infection.

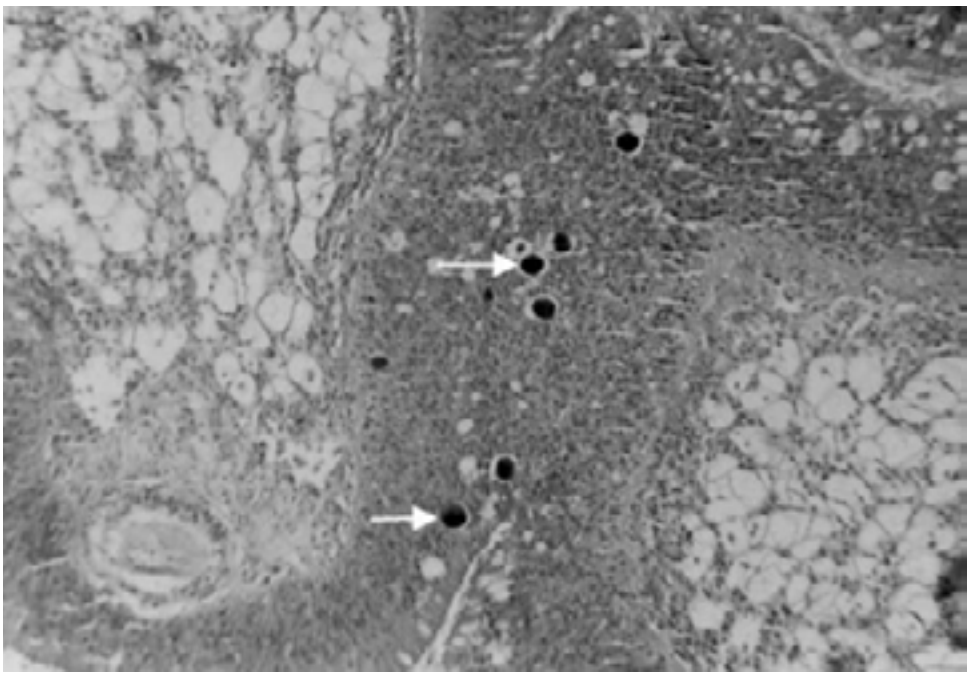


Figure 2. Prokaryotic inclusion bodies (PIB) present in digestive tract epithelium of an American oyster. Arrows indicate examples (Kim et al.,

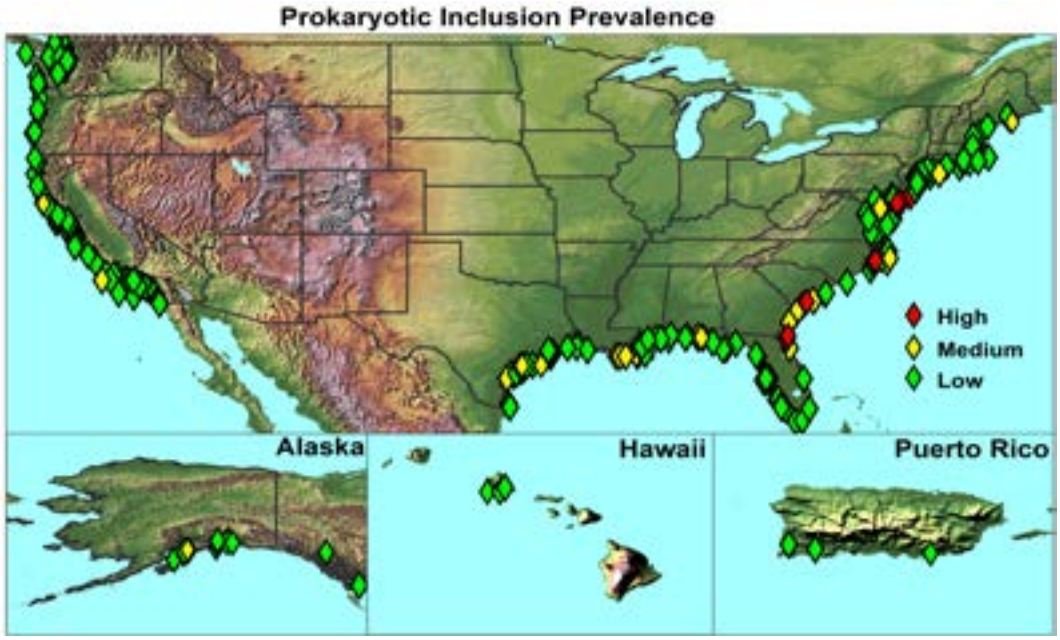


Figure 3. Spatial distribution of prokaryotic inclusion bodies (Chlamydia and Rickettsia) prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

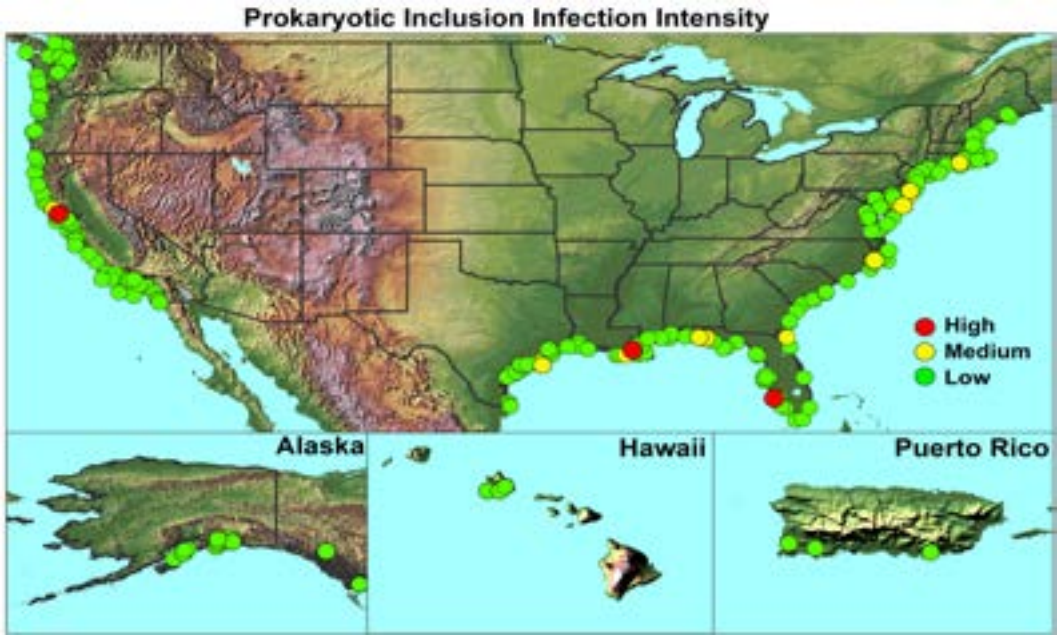


Figure 4. Spatial distribution of prokaryotic inclusion bodies (Chlamydia and Rickettsia) infection intensity in oysters and mussels, based on 2008-2009 Mussel Watch data.

Figure 5. Site-specific long-term temporal variation of prokaryotic inclusion bodies (Chlamydia and Rickettsia) prevalence in oysters and mussels

Gregarines (Protozoan Parasites)

Overview

The phylum Apicomplexa is a large group that contains numerous protozoan parasites (Padovan et al., 2003). Organisms within this phylum are characterized as having an apical complex structure involved in penetrating host cells. This group includes the Nematopsis species (Figure 6), which is a porosporid that develops into monozoic spores, and a gregarine species that develop into naked sporozoites (Snieszko, 1970). These parasites require two hosts: a mollusk for the intermediate stage, and an arthropod as the final host (Meyers and Burton, 2009). They usually invade the intestines, but are also found in other tissues such as the foot, mantle, and palps. Kim and Powell (2007) found gregarines in the connective tissue surrounding the visceral mass, as well as in the gills and the mantle connective tissues of blue mussels and oysters. Although heavy infections have been suggested to have some harmful effects on oyster physiology (Sindermann, 1990), Cheng (1967) concluded that, in general, gregarines infections have low pathogenicity in bivalves. According to Meyer and Burton (2009), the presence of gregarines in bivalves cause no human health concerns.

Current Status and Temporal Trends

Oysters from the Gulf of Mexico and the Eastern seaboard, from Florida to Virginia, had the highest infection prevalence (Figure 7). Elevated prevalence of gregarines was also found in blue mussels from coastal waters of Washington and southern California and in oysters from one site in southern Puerto Rico The intensity of gregarine infections were, however, low to medium, with the exception of the Gulf of coast oysters where more severe infections were found in Apalachicola Bay, FL, Nueces Bay, TX, and Pass Christian, MS (Figure 8). These findings corroborate results by Kim and Powell (2007), who found that gregarines are dominant parasites in oysters and are distributed along the entire range of the East coast and Gulf of Mexico.

While monitoring sites in Delaware, Louisiana, and South Florida showed decreasing trends in gregarine infection rates in oysters, gregarine infection is on the rise in oysters and blue mussels from several other coastal areas (Figure 9). Increasing occurrences of gregarines at monitoring sites in Alaska, California, Gulf of Mexico, West coast of Florida and the coast of Virginia may be indicative of environmental conditions that are conducive for the parasite in these areas.

Correlation

Gregarine infections were found to be significantly ($p < 0.001$), but weakly correlated ($\rho \leq 0.30$) with arsenic, lead and manganese body burden of blue mussels in the West coast (Appendix A). No correlations were found between gregarine infection and organic contaminant body burdens of the sentinel bivalves. A significant, but weak correlation, was also found between gregarines infection and *P. marinus* infection in American oysters (Appendix A)

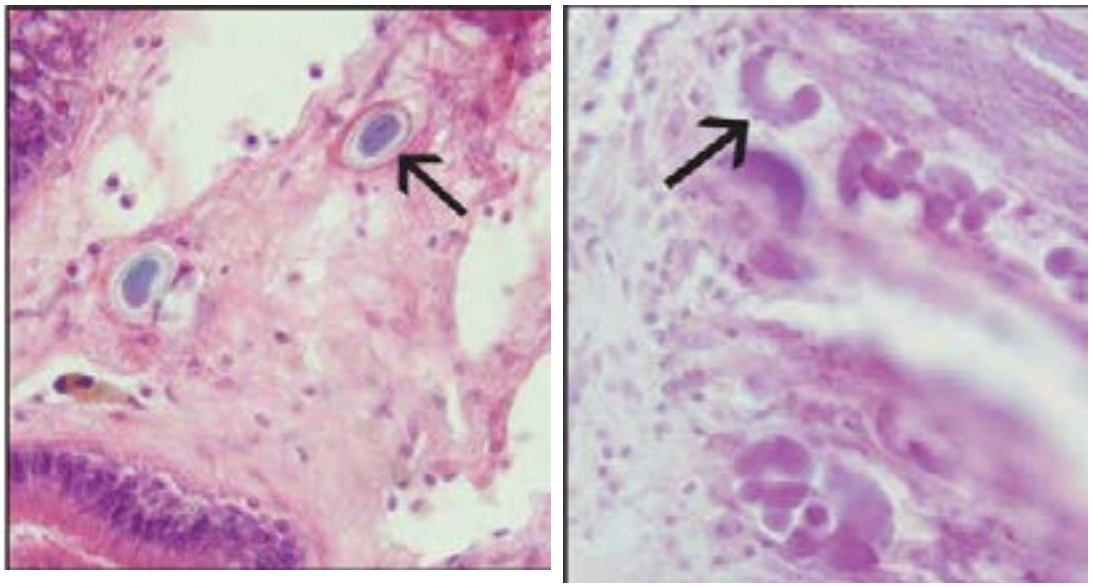


Figure 6. Section of oocyst (arrow) of gregarine Nematopis sp. in the connective tissues of scallop (left) and gregarine larvae in oyster (right) (Meyers and Burton, 2009).

Figure 7. Spatial distribution of gregarine prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

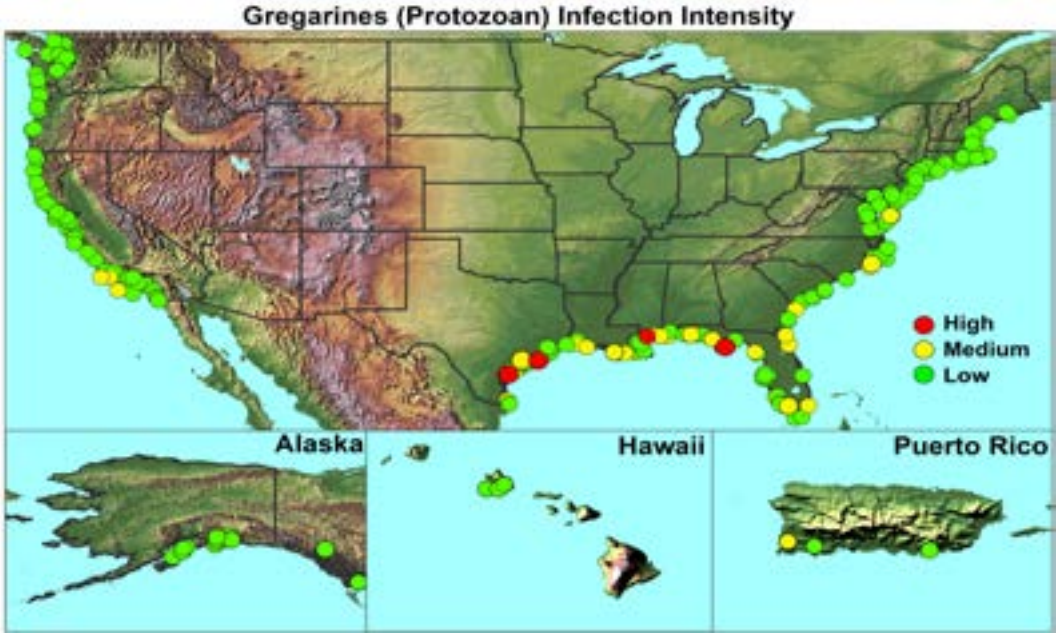


Figure 8. Spatial distribution of gregarine infection intensity oysters and mussels, based on 2008-2009 Mussel Watch data.

Figure 9. Site-specific long-term temporal variation of gregarine prevalence in oysters and mussels.

Haplosporidium nelsoni (MSX Pathogen)

Overview

Haplosporidium nelsoni is a protozoan that is the etiological agent of multinucleated sphere X/ unknown (MSX) disease in oysters (Figure 10). *Haplosporidium nelsoni* infections start in the gill epithelium, remain at light infection levels for months before the disease worsens and becomes systemic (Ewart and Ford, 1993). Infected oysters show mantle recession, gaping valves, watery emaciated tissues, and a pale digestive gland. The bivalves begin to die within a month. Death of infected oysters is so rapid that there is no loss of soft tissue (Haskin et al., 1965; Andrews, 1966; Couch and Rosenfield, 1968). MSX disease, which was reported in Chesapeake Bay in the 1950s, was a major contributor to the devastation of oyster fisheries in the Chesapeake Bay (Andrews, 1979) and along the East coast in the 1980s (Kemp et al., 2005). Although the parasite is lethal to oysters, it is said to be harmless to humans (Ewart and Ford, 1993).

Current Status and Temporal Trends

Prevalence results indicated that *H. nelsoni* occurs in oysters in the Chesapeake Bay region more frequently than the southeast or Gulf of Mexico(Figure 11). Kim et al., (2006) indicated that the East coast of the U.S. has historically had the highest prevalence and infection intensities for *H. nelsoni* infection. Current hot-spots for *H. nelsoni* infection in Chesapeake Bay include the Choptank River, MD, Dandy Point, VA and Upshur Bay, VA. (Figures 11 and 12). Although infections of *H. nelsoni* have been historically more severe at sites in the Chesapeake Bay (Andrews, 1979), current data showed other hot-spots observed in coastal waters of Apalachee Bay, FL, and Chaleston Harbor, SC (Figures 11 and 12).

The presence of *H. nelsoni* in oysters from the mid-Atlantic coastal waters was generally static at low infection rates. Only two sites—Ben Davis Point Shoal of Delaware Bay, DE, and John Creek in Roanoke Sound, NC (Figure 13)—had significant decreases in *H. nelsoni* infections.

Correlation

The current data showed no correlation between *H. nelsoni* infection and the measured chemical contaminants in oysters.

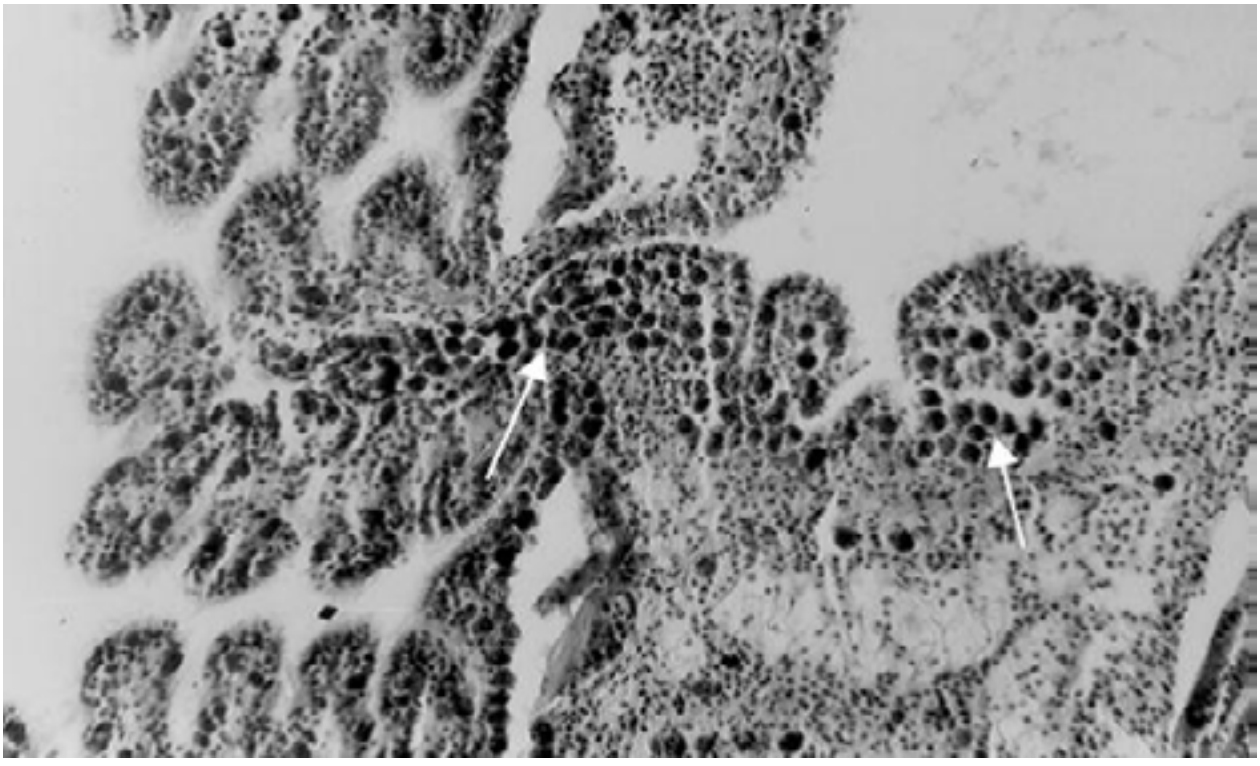


Figure 10. Numerous multinucleated plasmodia of *Haplosporidium nelsoni* in the gills of an American oyster. Arrows point to example parasites (Kim et al., 2006).

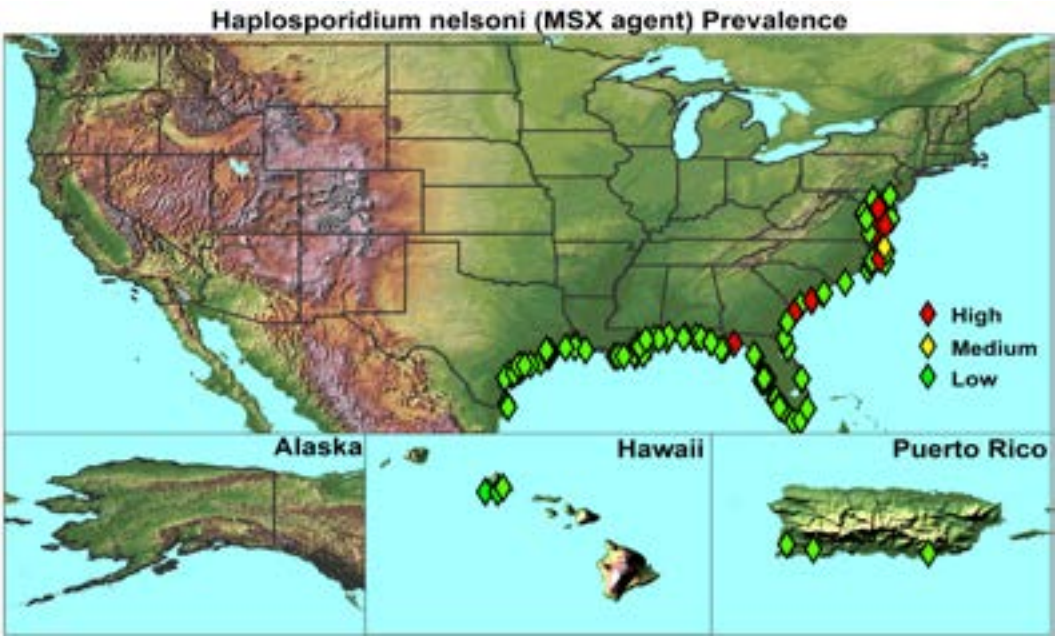


Figure 11. Spatial distribution of *H. nelsoni* prevalence in oysters, based on 2008-2009 Mussel Watch data.

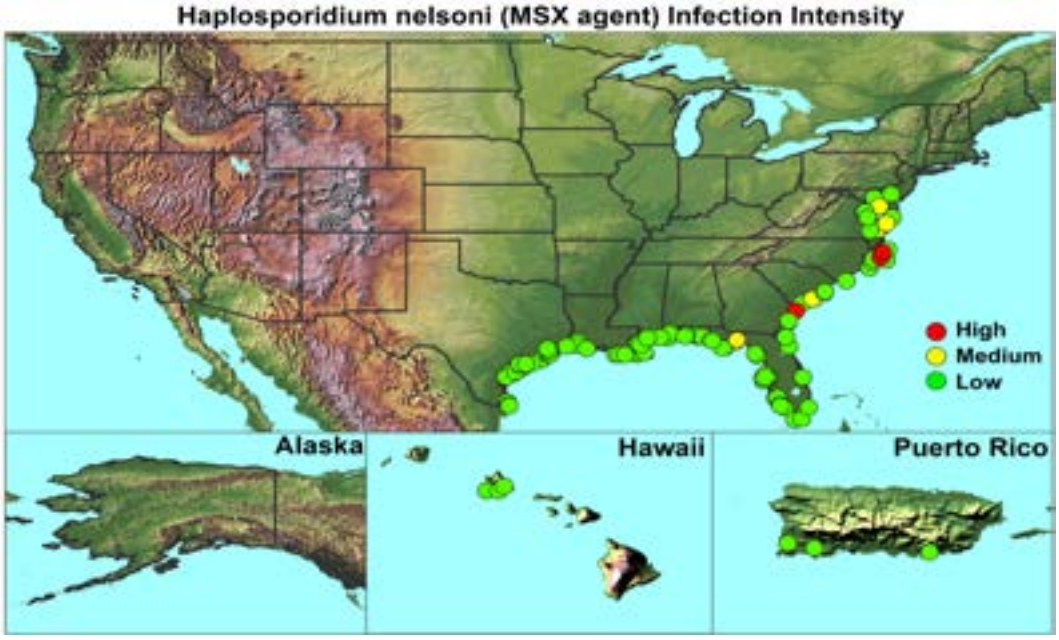


Figure 12. Spatial distribution of *H. nelsoni* infection intensity in oysters, based on 2008-2009 Mussel Watch data.

Figure 13. Site-specific long-term temporal variation of *H. nelsoni* prevalence in oysters.

Perkinsus marinus (Dermo Pathogen)

Overview

Dermo (Perkinsosis) is a major disease of oysters that causes high mortality. The disease (Figure 14) is caused by the protozoa, *Perkinsus marinus*. The infection occurs through water column (Elston, 1990). Proliferation of the parasite within the host causes systemic disruption of connective tissue and epithelial cells and is correlated with warm summer water temperatures (higher than 20 °C), when pathogenicity and associated mortalities are highest (ICES, 2012). Kennedy et al. (1996) explained that *P. marinus* stunts a bivalve's ability to produce new shell deposits and impairs adductor muscle strength, which leaves the host weak and prone to gape (dead but with soft tissue still present) when removed from water. American oysters tend to be most susceptible to infection, although some other bivalves can be infected. Mussels tend to be immune to *P. marinus*. Historically, infection is primarily along the Gulf of Mexico and the East coast, although the organism was inadvertently introduced in Hawaiian waters (Ford, 2011).

Current Status and Temporal Trends

High infection of *P. marinus* was recorded in American oysters from coastal waters of the East coast and the Gulf of Mexico (Figure 15). The current distribution of the prevalence data mirrored the historic description of the dermo pathogen along the Atlantic seaboard and the Gulf of Mexico (ICES, 2012). Prevalence of the dermo pathogen were high at more than 41% of the oyster sites. The infection intensity was low to medium in the Chesapeake Bay, but hot-spots high intensity were observed at locations in the Gulf of Mexico and along the southeast coastline (Figure 16). The hot-pots for *P. marinus* infection in American oyster include Panama City Municipal Pier, Joes' Bayou and Cedar key in FL, Matagorda Bay, Lower Laguna Madre and Nueces Bay in TX. Bahia de Boqueron in Puerto Rico was also shown to be a high infection intensity location for *P. marinus* (Figure 16).

The only increase in the prevalence of *P. marinus* was observed in Pamlico Sound, NC (Figure 17), while about 30 % of sites in the Gulf of Mexico showed declining trends in the dermo pathogen infection.

Correlations

No significant correlations were found between contaminant body burden of oyster and prevalence of *P. marinus* infection.

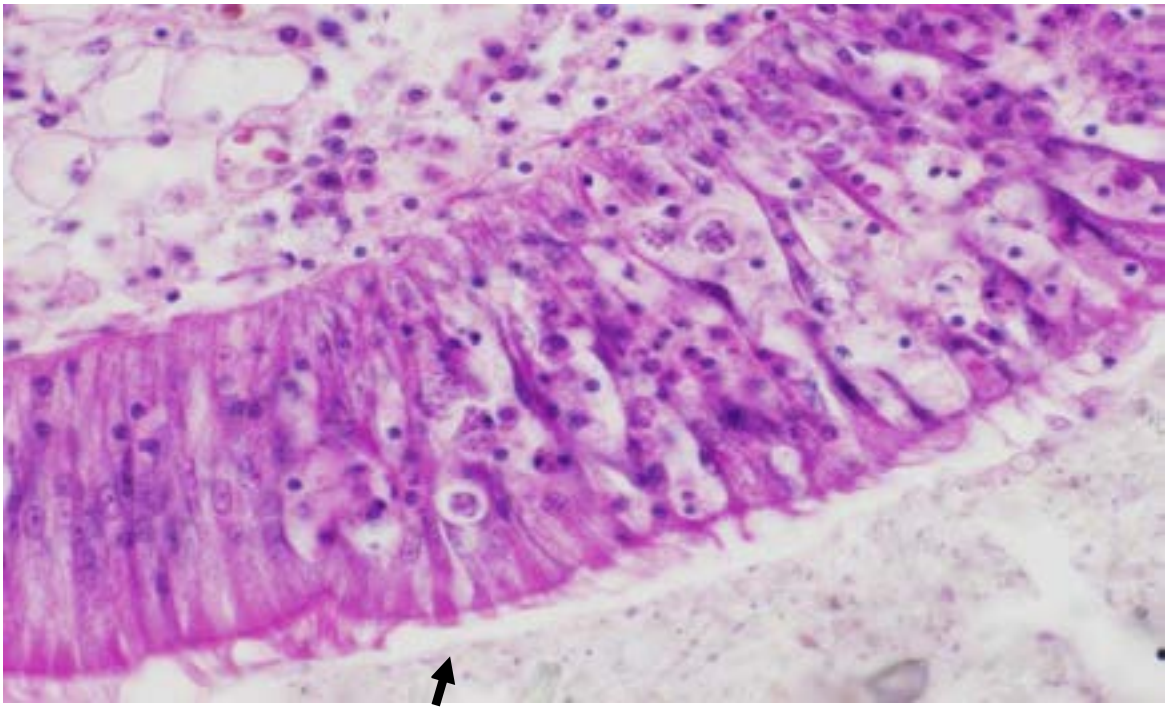


Figure 14. Several *Perkinsus marinus* cells in the intestine of an American oyster (40x mag, MHE stained). Photo by Joe Marcino, Maryland Department of Natural Resources.

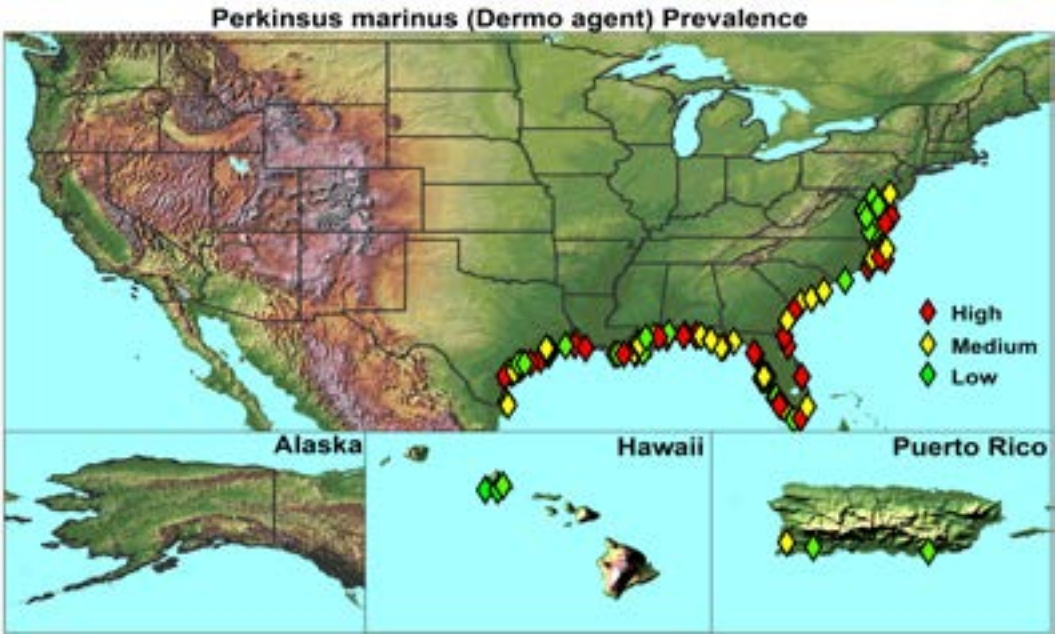


Figure 15. Spatial distribution of *P. marinus* prevalence in oysters, based on 2008-2009 Mussel Watch data.

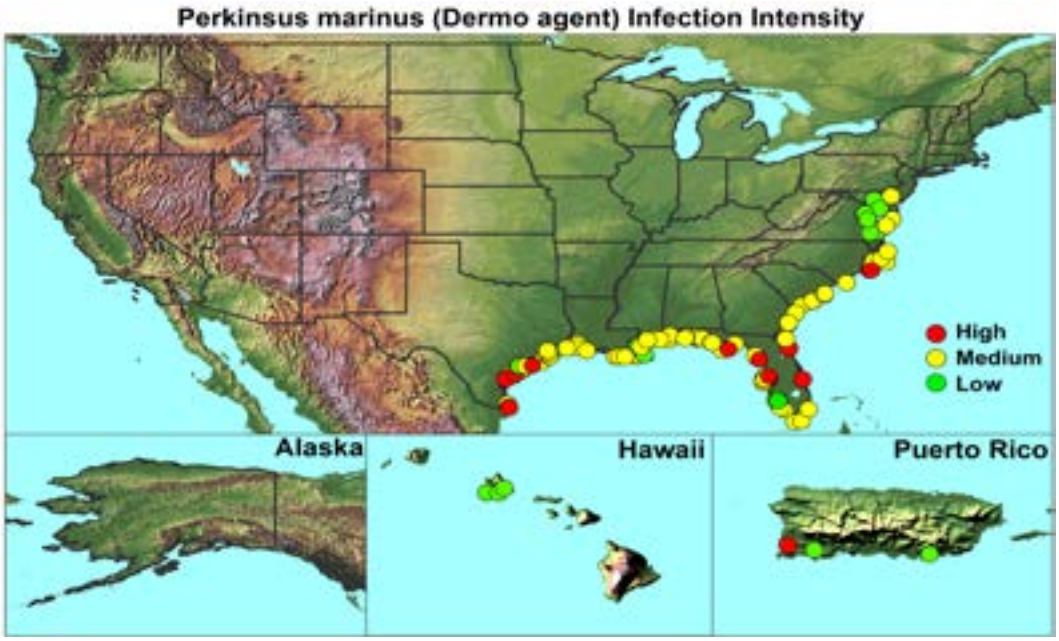


Figure 16. Spatial distribution of *P. marinus* infection intensity in oysters, based on 2008-2009 Mussel Watch data.

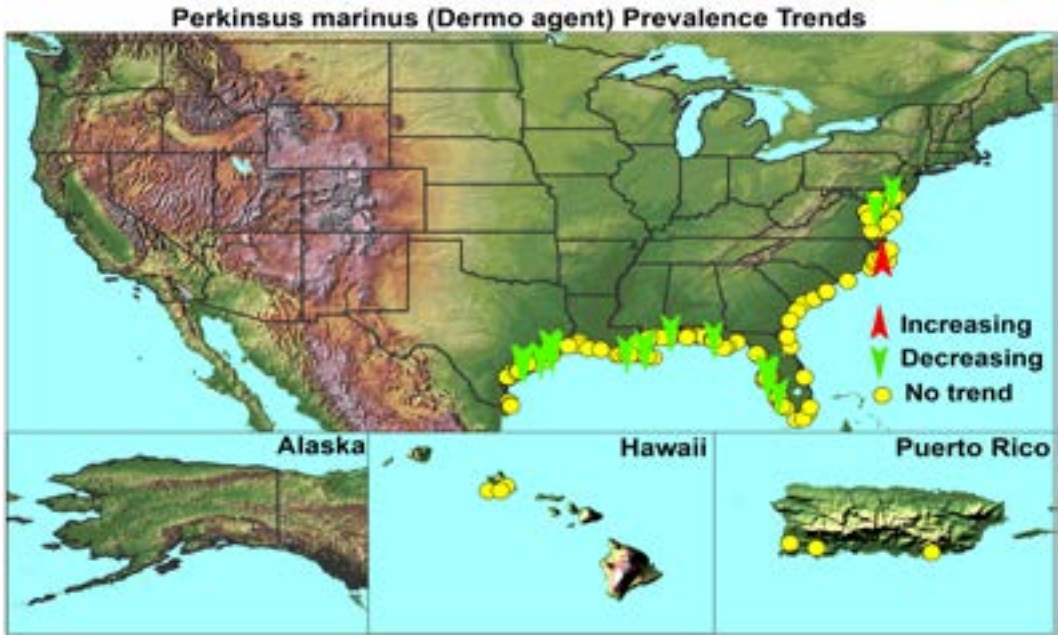


Figure 17. Site-specific long-term temporal variation of *P. marinus* prevalence in oysters.

Ciliates (Single cell Protozoans)

Overview

Ciliates are single-celled eukaryotes with hair-like organelles called cilia which are used for movement and food collection (Figure 18). Ciliates can be found in every aquatic system, such as ponds, lakes, rivers, and oceans. They reproduce primarily by cell division. However, in some instances two cells can fuse together to form new organisms. Parasitic ciliates are transmitted from host to host through water and can cause serious tissue damage or stress in the host organisms (Meyers and Burton, 2009). Ciliates are found in in the gills, gut, and digestive tract of oysters and blue mussels, but not in zebra mussels (Kim et al., 2006). Ciliate infection does not appear to elicit any obvious pathological conditions or host responses in mussels and oysters, however, mature and multiplying maturing ciliates can cause cell distended condition, called a xenoma, in the host tissue (Brandão et al., 2013). Xenoma findings are presented later in this report.

Current Status and Temporal Trends

The vast majority of both mussel and oyster sites are low ciliate infection (Figures 19 and 20). However hot-spots for ciliate infection prevalence were more frequent at blue mussel (Figure 19) relative to oyster sites (Figure 20). Medium infection intensity for ciliate were mostly observed at oyster sites, including locations in Chesapeake Bay, VA; Charleston Harbor, SC; Apalachicola Bay, FL; and Calcasieu Lake, LA where (Figure 20).

Ciliate infections are on the rise in the Gulf of Mexico and at a few locations along the East coast (Figure 21). Some decreasing trends were also observed at locations in Massachusetts Bay, including Buzzards Bay and Boston Harbor, as well as a location in Santee River, SC (Figure 21).

Correlations

Ciliate infection prevalence was positively correlated with manganese in blue mussels from the West coast (Appendix A).

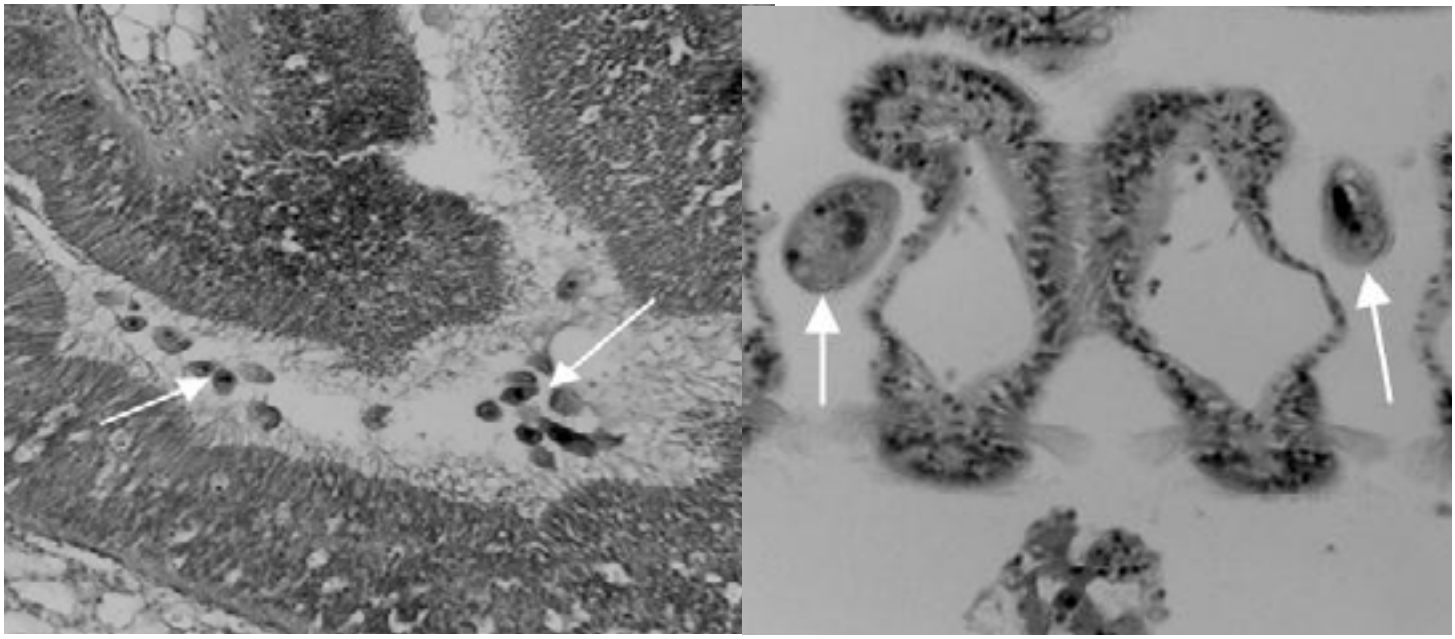


Figure 18. Ciliates in the intestine of an oyster and between gill filaments of a blue mussel (Kim et al., 2006).

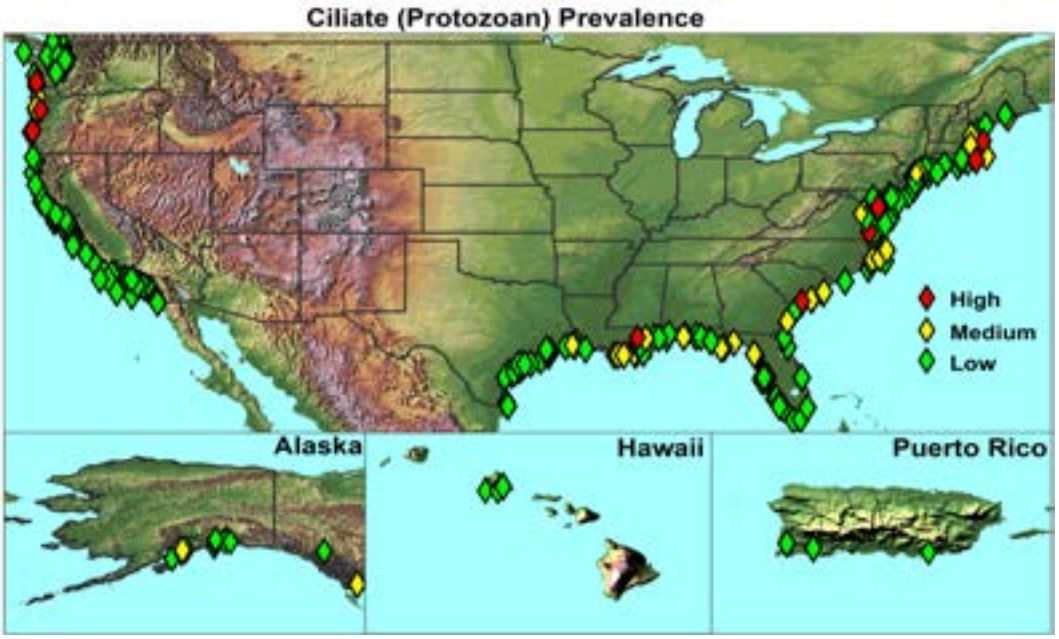


Figure 19. Spatial distribution of ciliate prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

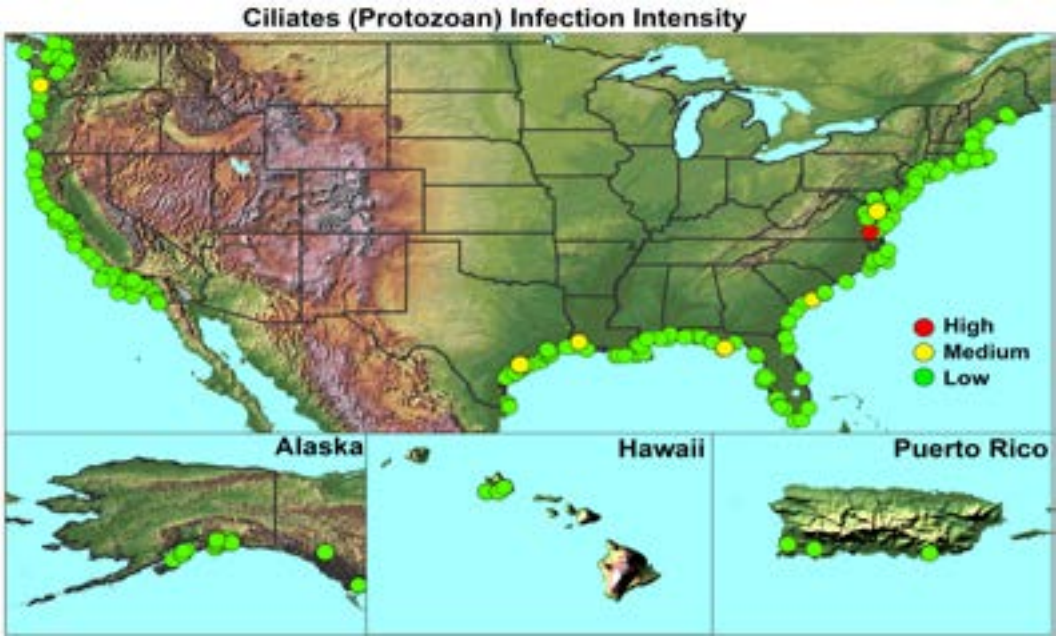


Figure 20. Spatial distribution of ciliates infection intensity in oysters and mussels, based on 2008-2009 Mussel Watch data.

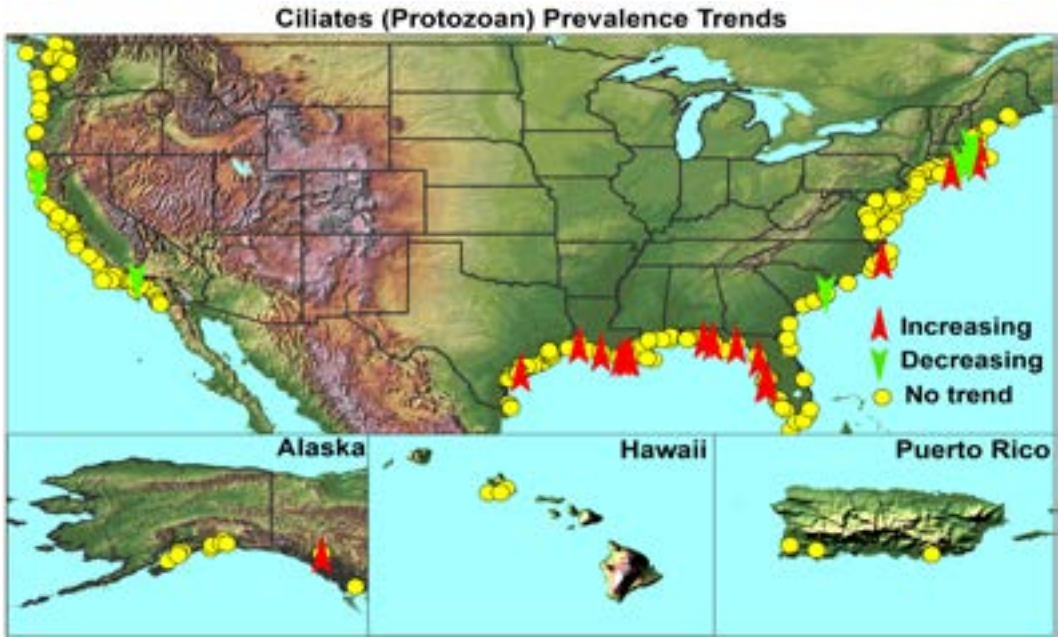


Figure 21. Site-specific long-term temporal trends of ciliate prevalence in oysters and mussels.

Cestodes (Tapeworms)

Overview

Cestodes are a class of parasitic flatworms commonly called tapeworms because of their extended length in adult stage. Cestodes are hermaphrodites, but self-fertilization is rare and cross-fertilization is the main reproduction practice, thus additional organisms are essential for the continuation of the species within the host. The life cycle of tapeworms requires one intermediate host (e.g. bivalves) which harbor eggs and larvae (encysted sporocysts and metacercariea larvae), and one definitive host (vertebrates), which harbor the adult worms (Roberts et al., 2005). There are over a thousand species of tapeworms that parasitize various animals, including bivalves as juveniles, while their adults often live in the digestive tract of vertebrates like humans. In humans, light infection usually does not show any remarkable symptoms. However, abdominal discomfort, gastric pain followed by vomiting and diarrhea can be observed in heavier infection (Bogitsh and Carter, 2005). In bivalves, cellular reaction to the infection is characterized by encapsulation of larval cestodes in connective tissue (Figure 22). Thus, the infection does not seem to significantly cause harm to the host bivalves (Cheng, 1966).

Current Status and Temporal Trends

The spatial distribution based on prevalence values (Figure 23) and infection intensity (Figure 24) indicated that cestodes, which were only found in oysters, were generally low. However, the prevalence values indicated some regional differences. Prevalence was relatively high in the coastal waters along the Gulf of Mexico (Apalachicola Bay, Lower Laguna Madre and Tampa Bay), while medium prevalence ranges were mostly observed along the southeast cost. Cestode infection intensities were not much more severe in oysters from the coastal waters of the Gulf of Mexico than oysters from the East coast (Figure 24).

The vast majority of monitoring sites showed no temporal trends in cestode prevalence (Figure 25). Honolulu Harbor, HI, and Beaufort Inlet, NC showed increasing trends, while cestode prevalence decreased in Charlotte Harbor and Rookery Bay located along the southern Florida Gulf coast (Figure 25).

Correlations

Cestode infections in American oysters were significantly, but weakly correlated ($p > 0.0001$) with trace metals body burdens (Appendix A), with a positive association with arsenic tissue concentration and negative correlations with cadmium and nickel body burden.

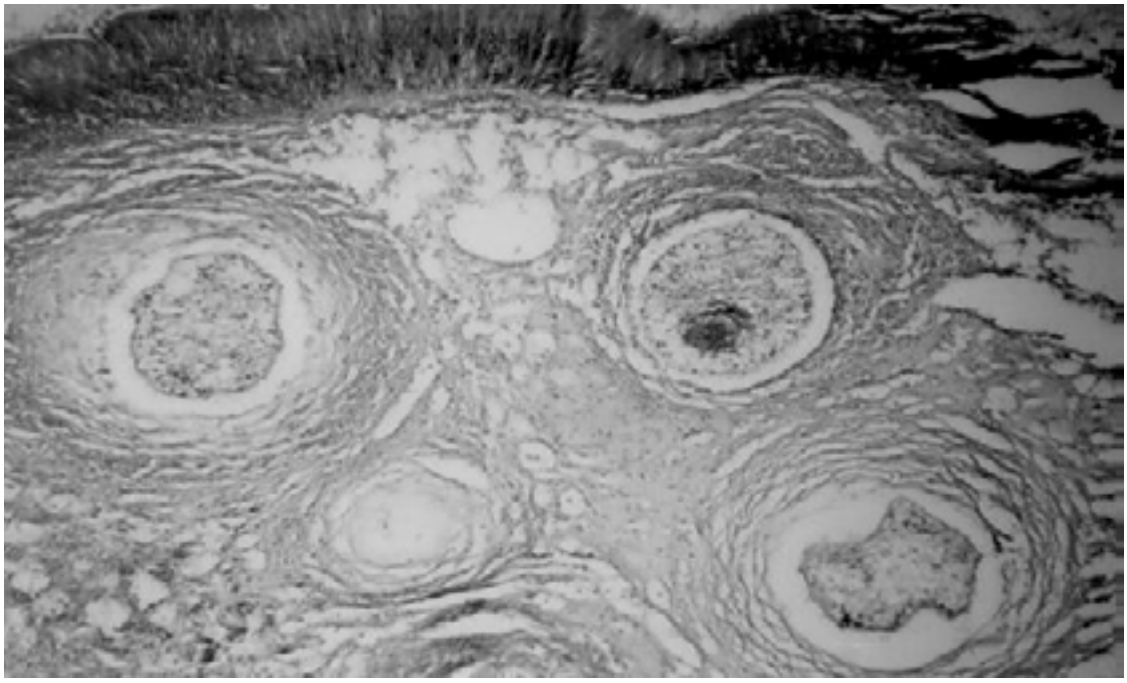


Figure 22. Encapsulated cestode larvae surrounding the stomach of an American oyster (Kim et al., 2006).

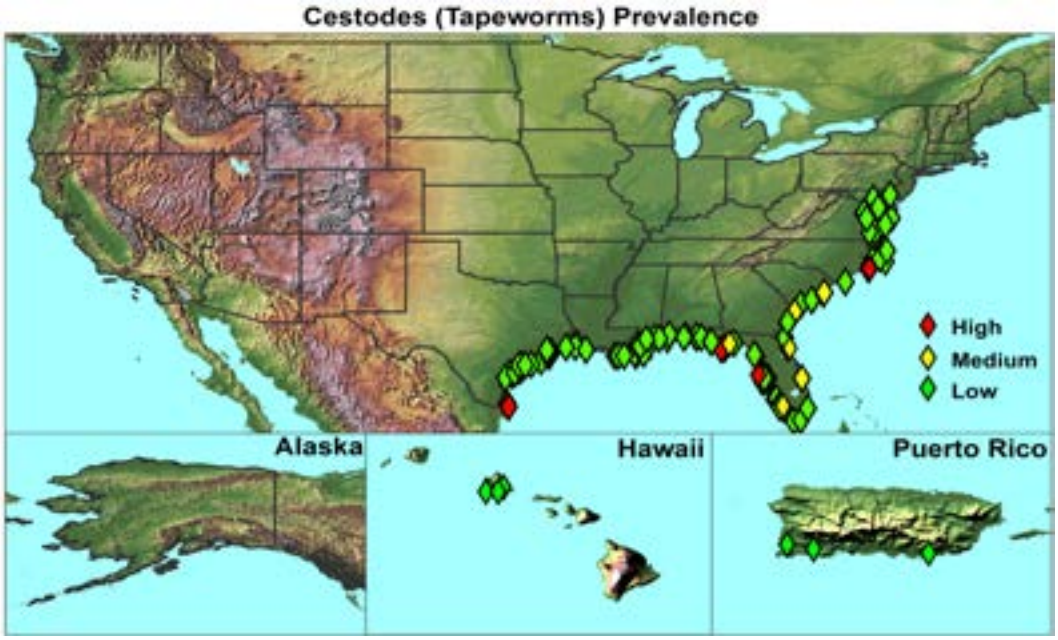


Figure 23. Spatial distribution of cestode prevalence in oysters, based on 2008-2009 Mussel Watch data.

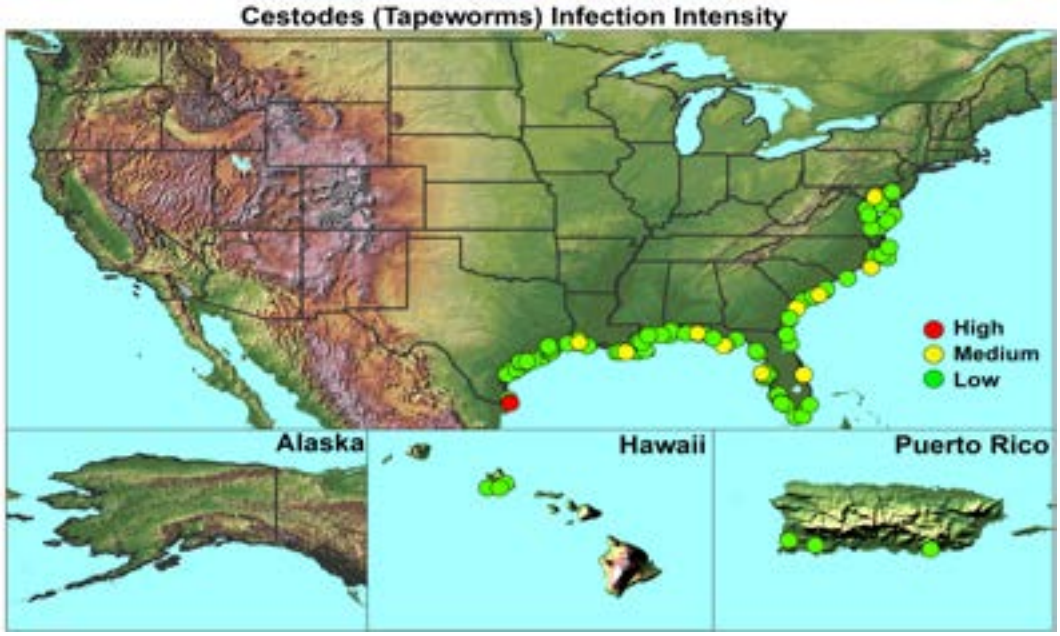


Figure 24. Spatial distribution of cestode infection intensity in oysters, based on 2008-2009 Mussel Watch data.

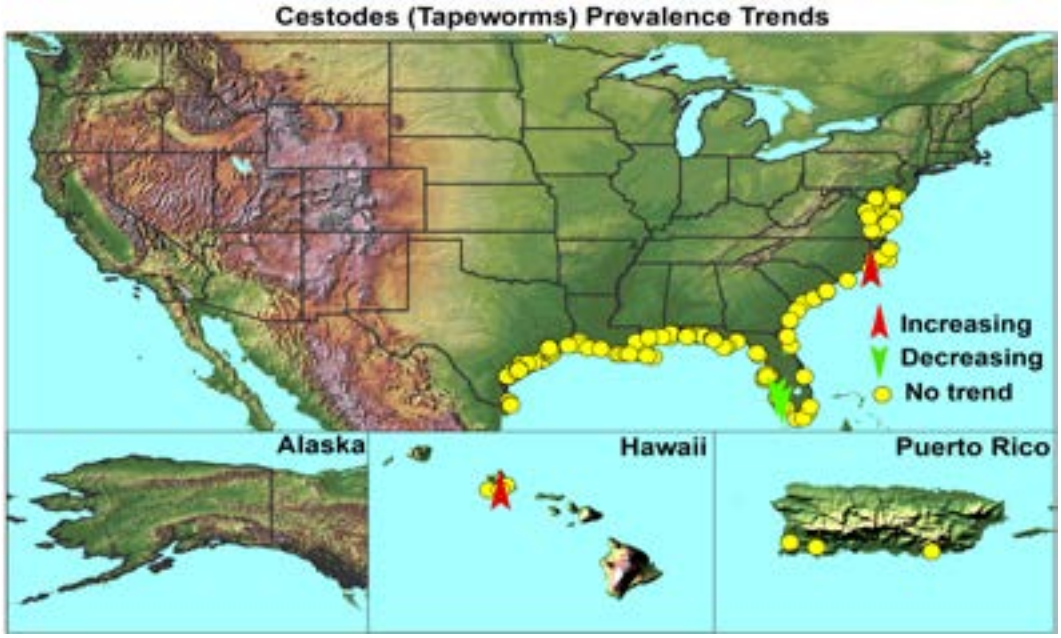


Figure 25. Site-specific long-term temporal trends of cestode prevalence in oysters.

Trematodes (Flatworms, Flukes)

Overview

Trematodes are a group of parasitic flatworms within the phylum Platyhelminthes that have a distinctive external feature of having two suckers, one near the mouth and the other on the organism's ventral surface. Figure 26 depicts a Bucephalus sp. trematode (Meyers and Burton, 2009). Nearly all trematodes are parasites of freshwater and marine mollusks and vertebrates, and require two host species to complete their life cycle. As larvae, they live in the digestive and reproductive tissues of bivalves, often causing sterilization (Cheng, 1967; Sindermann, 1970). If the parasite is present in large numbers, it can cause tissue destruction, and death (Meyers and Burton, 2009). Meyers and Burton (2009) also observed that, in mussels, trematodes can lower byssal thread production, cause infertility, and affect pearl formation. If ingested by humans, trematodes can cause severe intestinal illnesses (Meyers and Burton, 2009).

Current Status and Temporal Trends

Low to medium trematode infections were found in most coastal regions. The northeast Atlantic was the only area with high prevalence and infection intensity of trematodes (Figures 27 and 28).

Three sites in the Hudson River and Boston Harbor showed increases in trematodes, while Jones Inlet in Long Island Sound was the only site with a decreasing trend (Figure 29).

Correlations

No significant correlations were found between contaminant body burdens of the sentinel bivalves and prevalence of the trematode infection.

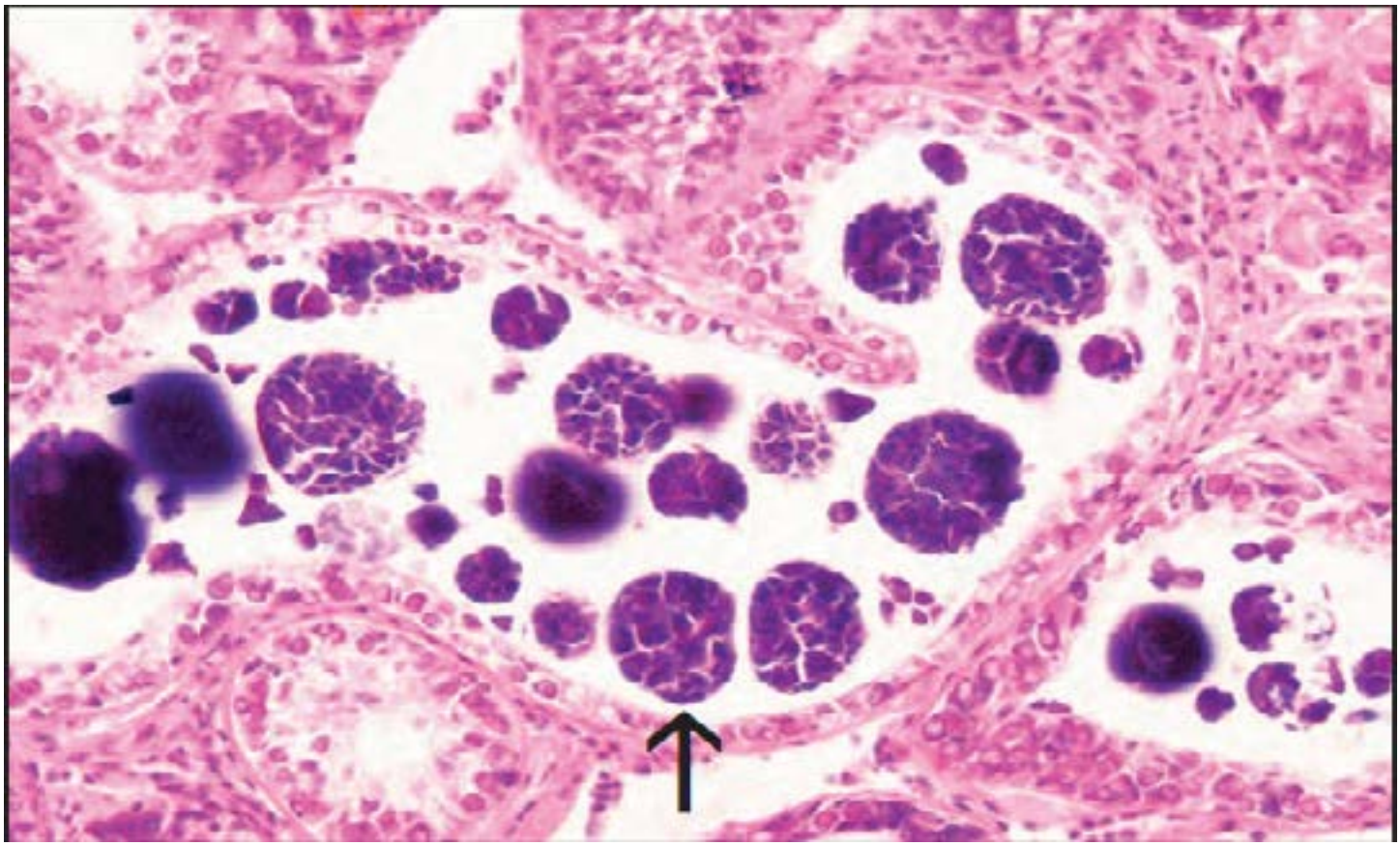


Figure 26. Trematode sporocyst (Bucephalus sp.) infetion in blue mussels (Meyers and Burton, 2009).

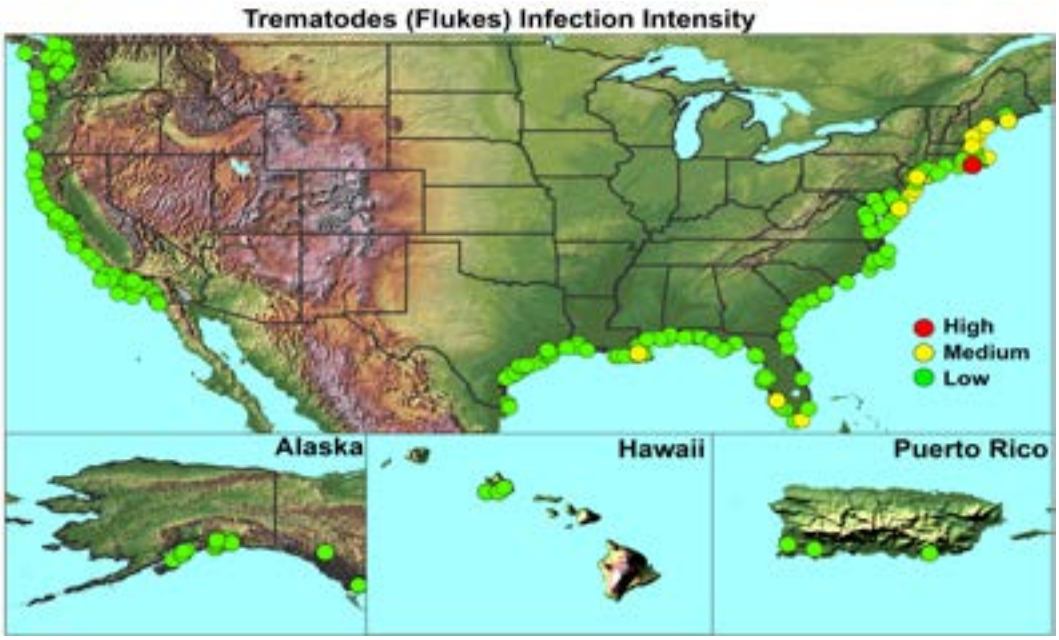


Figure 27. Spatial distribution of trematode prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

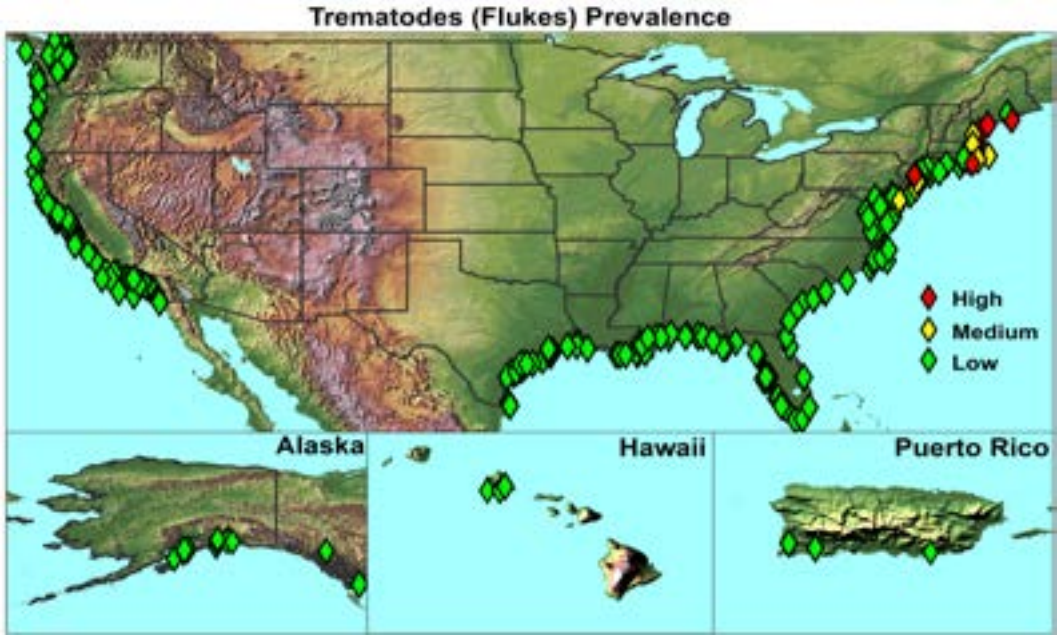


Figure 28. Spatial distribution of trematode infection intensity in oysters and mussels, based on 2008-2009 Mussel Watch data

Figure 29. Site-specific long-term temporal variation of trematode prevalence in oysters and mussels.

Nematodes (Roundworms)

Overview

Nematodes (roundworms) are the second most diverse group of animals, second only to arthropods. Roundworms (parasitic cylindrical worms) have a tubular digestive system with openings at both ends of the cylindrical body. Over half of the 28,000 described species are pathogenic (Hugot et al., 2001). They have multiple development stages; according to Cheng (1978), nematodes that infect mollusks, such as oysters and mussels, are mainly found at larval stages (Figure 30), while adults can be found in the predators of the mollusks, such as the oyster drill. Cheng (1966) suggested that larval nematodes invade oysters via the digestive tract and migrate through tissues by way of blood vessels. Infections in mollusks can cause granulomas in infected cells as well as the destruction of adjacent host tissues (Meyers and Burton, 2009). In some cases, cellular responses in the host include infiltration of hemocytes around the area where the worm is located (Kim et al., 2006).

Current Status and Temporal Trends

Most of the medium and high prevalence and infection intensities were found in the coastal waters of the Gulf of Mexico (Figures 31 and 32), while the Atlantic seaboard showed mostly low levels of nematodes.

Increasing temporal trends of nematode infection were observed at several locations in the Gulf of Mexico (Figure 33). The flatworm's infections were increasing at sites in Barataria Bay and Mississippi River in LA, Chactawhatchee Bay and Pensacola Bay in FL, and Matagorda Bay and Galveston Bay in TX (where the highest infection intensity also occurred). Decreasing trends were found at Flamingo in Florida Bay, Ben Davis Point in Delaware Bay, and Breton Sound, LA.

Correlations

A positive ($p < 0.001$), but weak ($\rho = 0.3$) correlation was observed between nematode infection prevalence and cadmium body burden in American oysters (Appendix A).

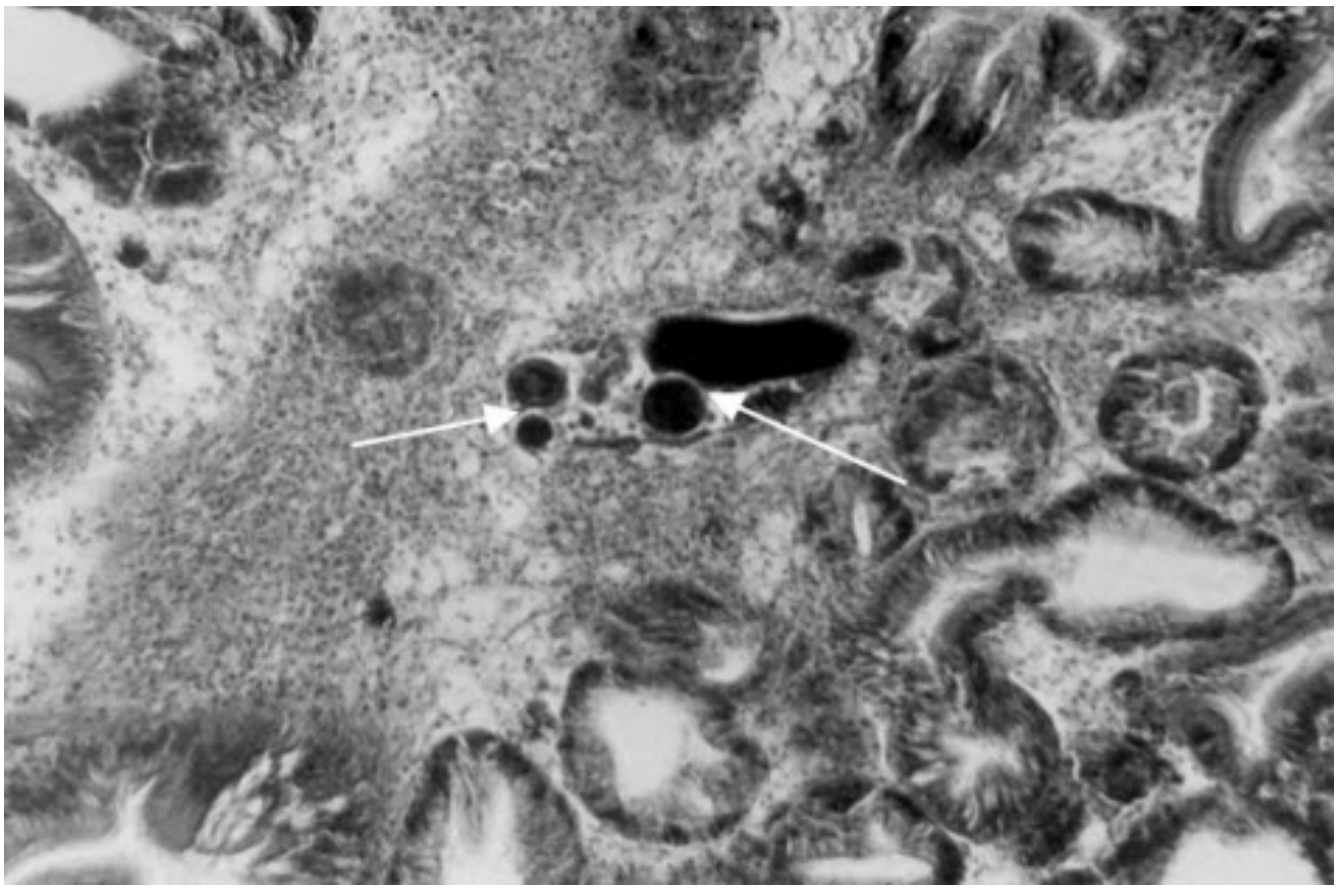


Figure 30. Sections of unidentified nematode larvae in the digestive gland connective tissue of an American oyster (Kim et al., 2006).

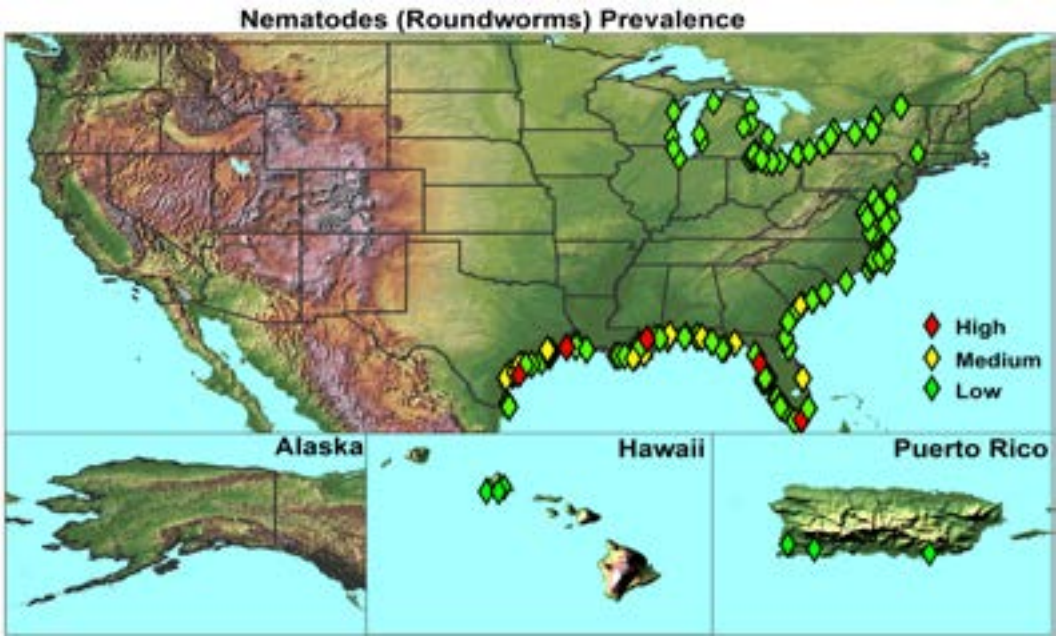


Figure 31. Spatial distribution of nematode prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

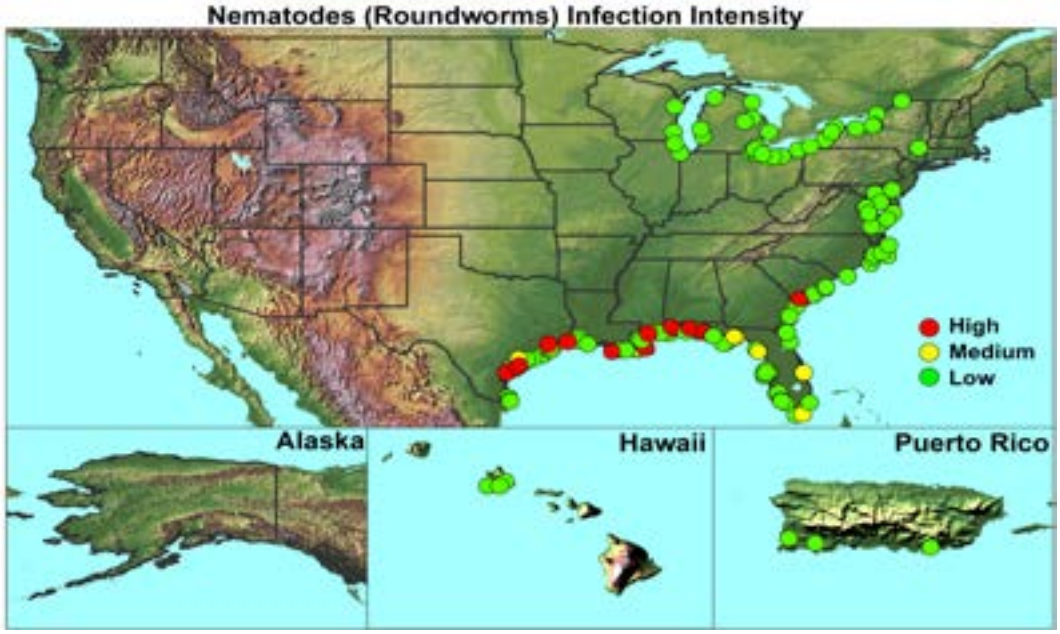


Figure 32. Spatial distribution of nematode infection intensity in oysters and mussels, based on 2008-2009 Mussel Watch data.

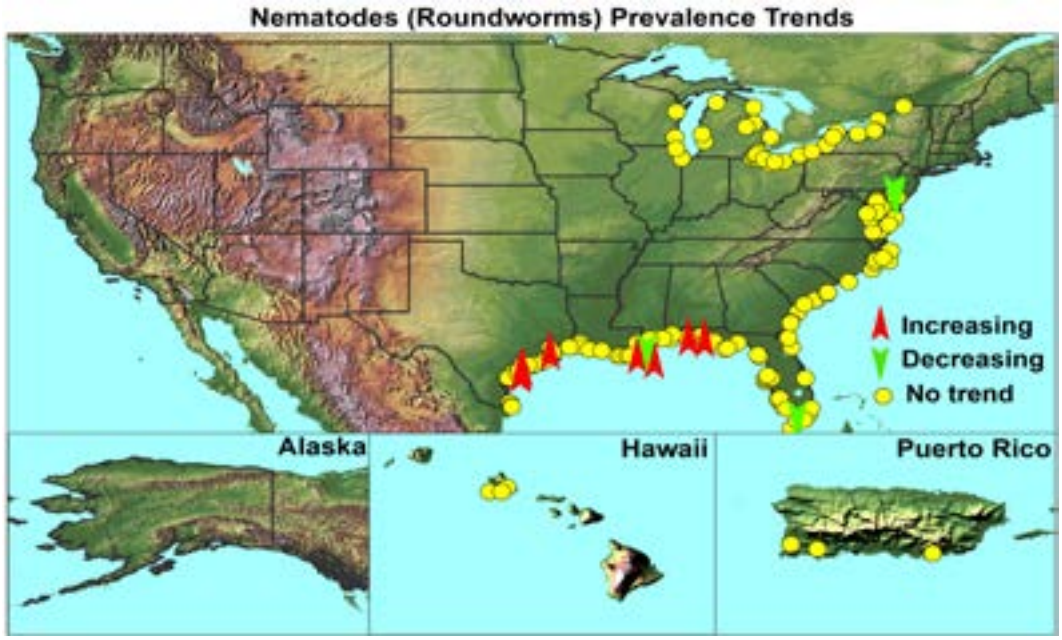


Figure 33. Site-specific long-term temporal variation of nematode prevalence in oysters and mussels.

Copepods

Overview

Copepods are small (1-2 mm) multi-cellular animals of the phylum Arthopoda, subphylum Crustacea. They are aquatic animals with exoskeletons. Parasitic copepods lay their eggs in the water and they are subsequently ingested by bivalves during filter feeding. (Heegaard, 1962; Darwin and Stefanich, 1966). They are mainly found in gills (Figure 34), but can also be found in the digestive tract. Appendages of the copepods (e.g. antennae) can cause erosion and metaplasia of the intestinal epithelium. It is estimated that nearly half of the 13,000 species of copepods are parasitic (Heegaard, 1962). Parasitic copepods infecting bivalves may be either obligate endoparasites which affect the alimentary tract of bivalves, or ectoparasites, which affect the mantle and gills.

Current Status and Temporal Trends

The highest copepod infections were observed in blue mussels from Santa Monica Bay and Tijuana River estuary CA, Possession Point, WA, and Jamaica Bay, NY. Oysters from Lake Barre in LA were also found to harbor elevated concentration of the parasite (Figure 35). Medium range copepod infections were observed along the East, West and Gulf coasts, but also in Alaska where the blue mussels from the Homer Spit site (Figure 35). Parasitic copepod infection intensity mainly ranged from low to medium (Figure 36). The infection intensity values were, however, relatively severe at three blue mussels site (Boston Harbor in MA, Pennobscot Bay in ME, and Marina Del Rey in CA) and one American oyster site (Ace basin in SC) (Figure 36). Only three sites showed any temporal trends; they were all blue mussel sites in the Northeast (Figure 37).

Correlation

No significant correlations were found between contaminant body burdens of the sentinel bivalves and prevalence of the parasitic copepod infection.

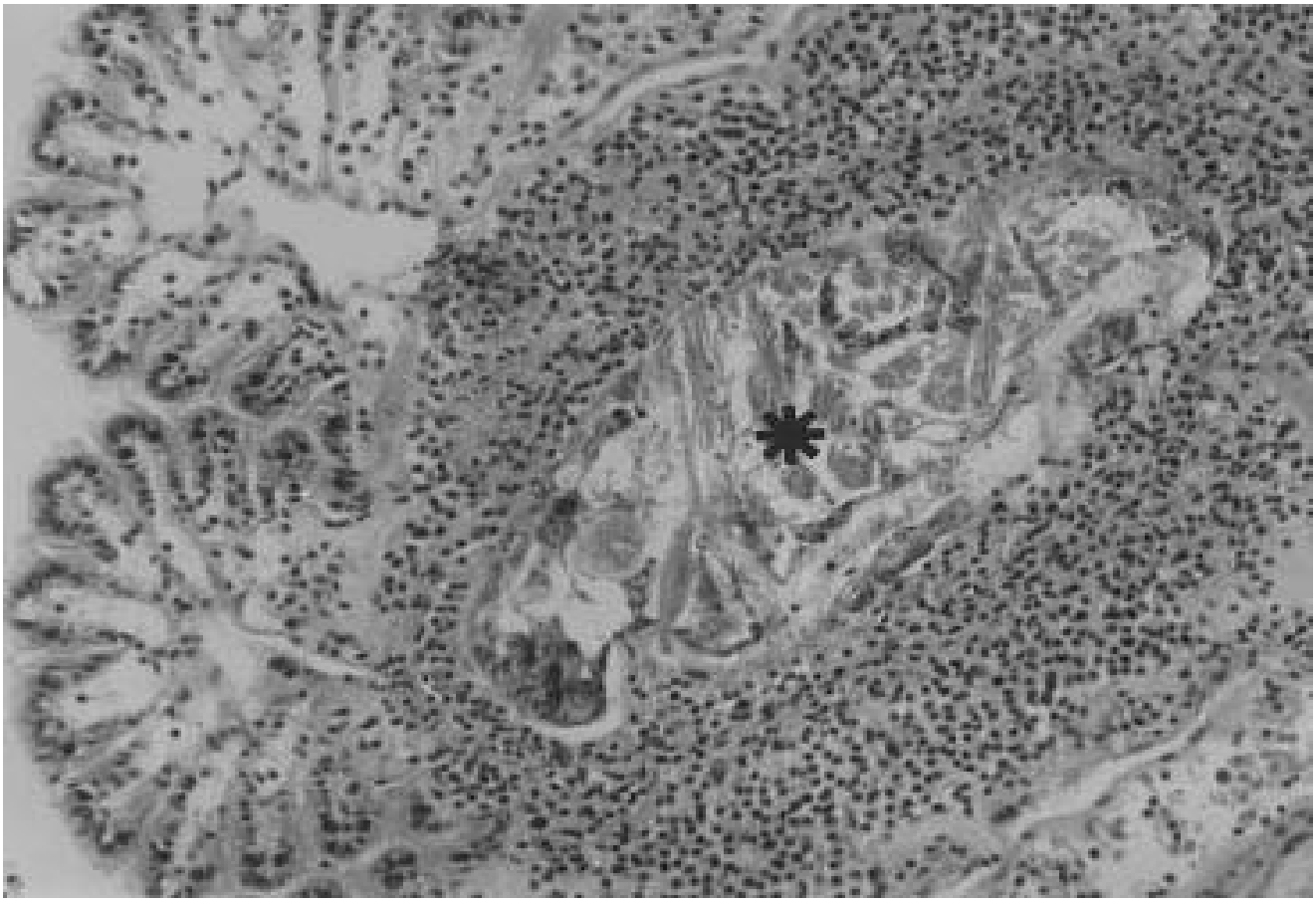


Figure 34. Copepod (*) in gill isolated by an intense hemocyte reaction (Carballal et al., 2001).

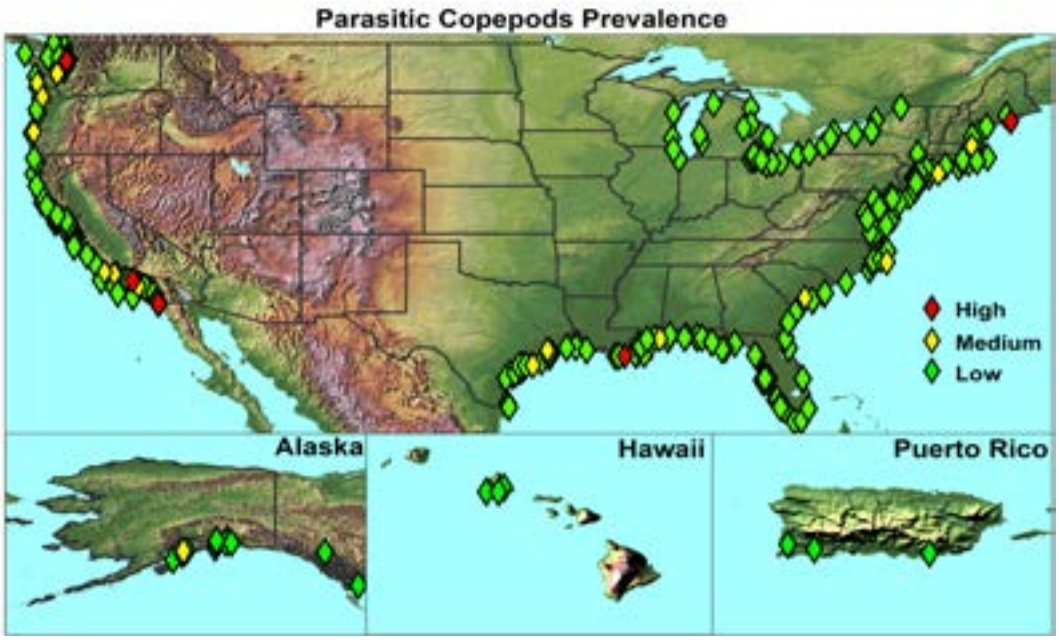


Figure 35. Spatial distribution of copepod prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

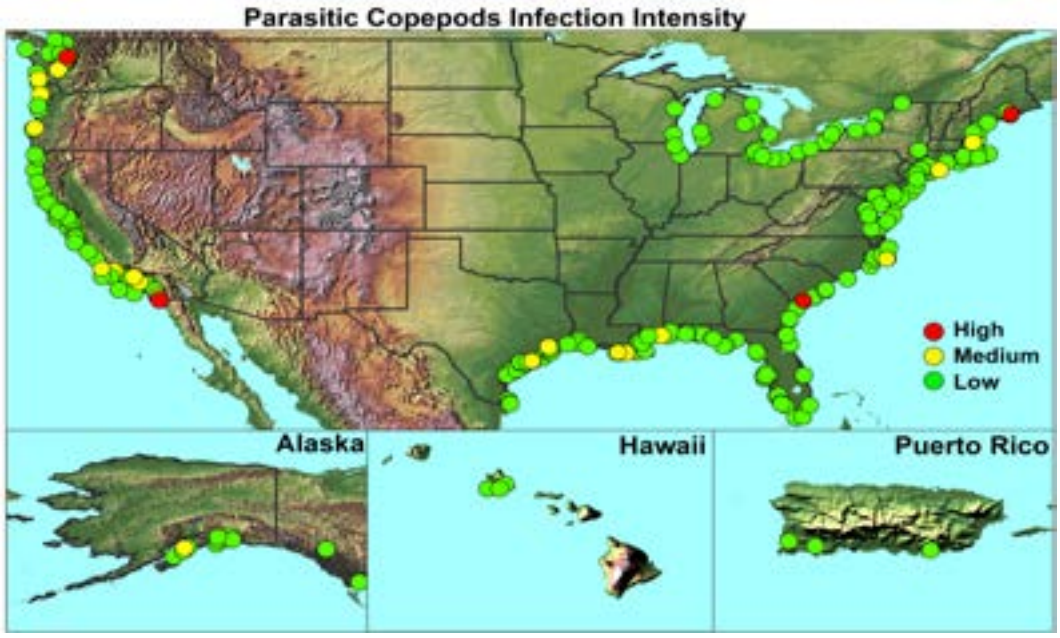


Figure 36. Spatial distribution of copepod infection intensity in oysters and mussels, based on 2008-2009 Mussel Watch data.

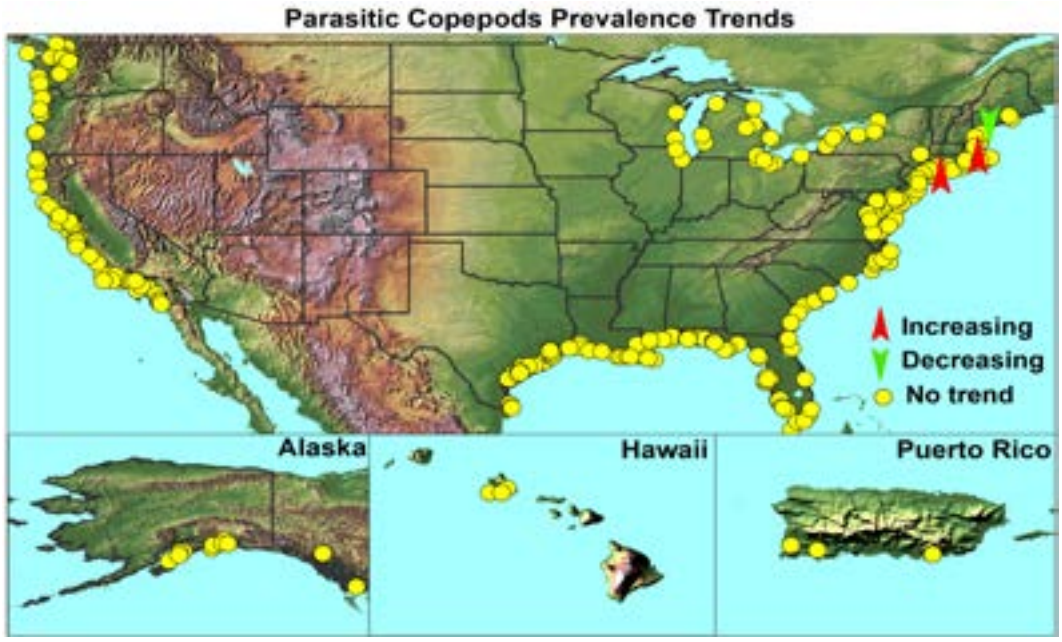


Figure 37. Site-specific long-term temporal variation of copepod prevalence in oysters and mussels.

Pinnotherid Crabs (Pea crabs)

Overview

Pea crabs are small crustaceans in the Pinnotheridae family, which exclusively parasitizes bivalves (Figure 38). Pea crabs are occasionally found in the mantle cavity of oysters and blue mussels (Kim et al., 2006). When very small they can also be found in the gills or in the water-conducting channels in the back of the gills (Haven et al., 1978). Pea crabs can have detrimental impacts on the host by removing food particles captured in the bivalve's gill, which over time can lead to food depravation (Kennedy et al., 1996). Also, direct injury can occur when the crab is connected to the host's gill by causing erosion, and the walking legs damage the gill tissue as it seeks food in the mucous strings (Stauber, 1945; Meyers and Burton, 2009). Bivalves with pea crabs have been found to contain less tissue mass per shell cavity volume (Haven, 1958).

Current Status and Temporal Trends

Pea crab infections were found at low prevalence and intensity at virtually all blue mussels and oyster sites nationwide, with the exception of some locations in the Mid-Atlantic and Gulf coasts, where medium to high infections were observed (Figures 39 and 40). The pea crab infections were the most severe at the Dandy Point in Chesapeake Bay, VA; Altamaha River, GA; and Port Isable and South bay in Lower Laguna Madre, TX (Figure 40).

Pea crab infections in blue mussels and oysters were fairly static. The only trend was at a blue mussel site located near the Housatonic River in Long Island Sound where a significant decreasing trend was recorded (Figure 41).

Correlation

No significant correlations were found between contaminant body burdens of the sentinel bivalves and prevalence of the pea crab infection.

Figure 38. A pea crab (Pinnotheridae family) living inside an American oyster (Howard et al., 2004).

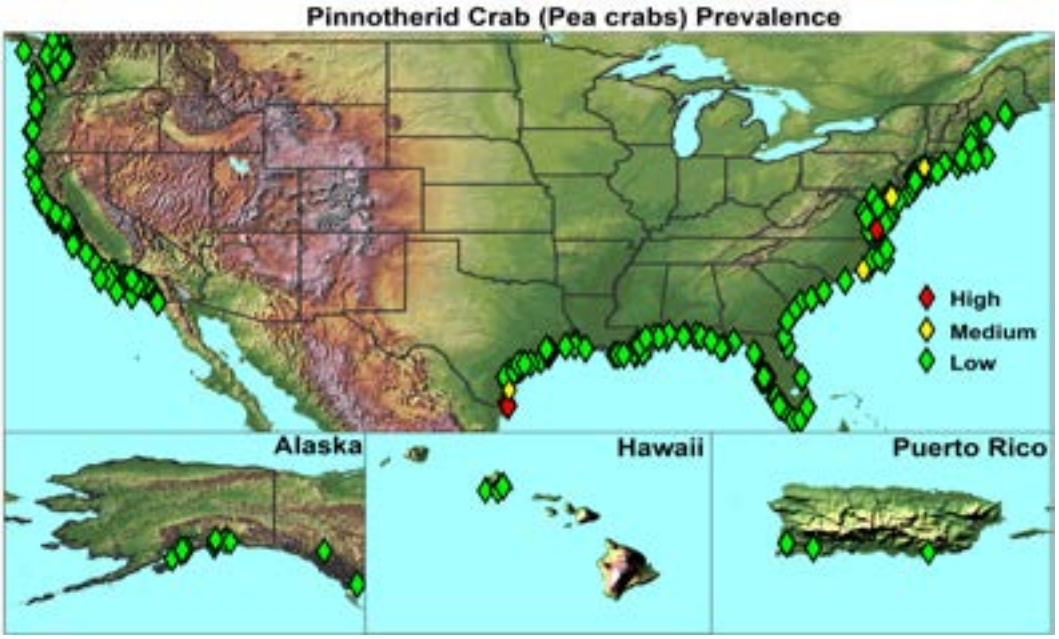


Figure 39. Spatial distribution of pinnotherid crab prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

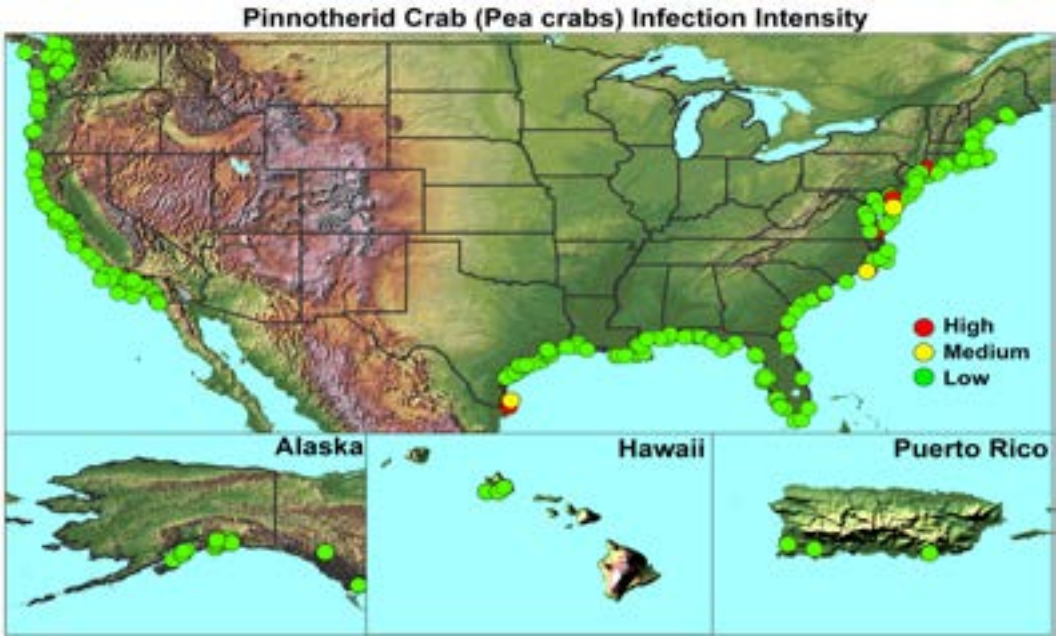


Figure 40. Spatial distribution of pinnotherid crab infection intensity in oysters and mussels, based on 2008-2009 Mussel Watch data.

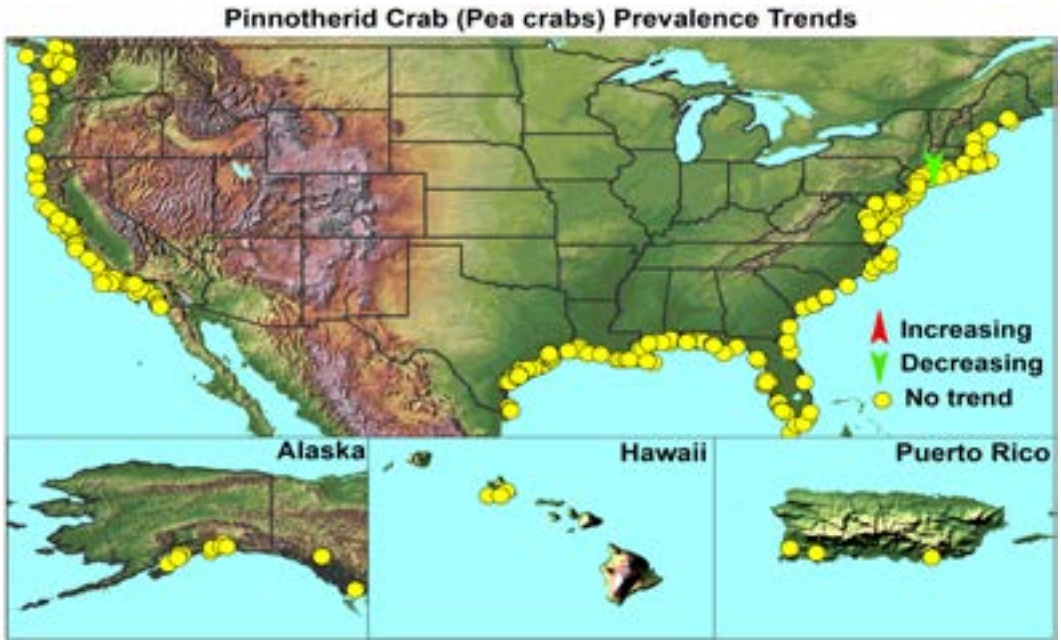


Figure 41. Site-specific long-term temporal variation of pinnotherid crab prevalence in oysters and mussels.

DISEASES

Tissue Hemocytic Infiltration (Tissue Inflammation)

Overview

Hemocytic infiltration (Figure 42) is the concentration of immune system cells (called hemocytes) that cause tissue inflammation. Hemocytes are the phagocytic cells of invertebrates that engulf particles or bacteria at infected sites and remove them from the organism. Their purpose is to neutralize or isolate potentially damaging substances (Kennedy et al., 1996). The presence of these cells indicates that the bivalve is responding to a pathogen. There are two types of infiltration of hemocytes that occur in bivalves known as focal and diffuse. Diffuse is distinguished from focal when hemocytes are distributed broadly over a large section of tissue without a clear center or focal point of highest hemocyte concentration (Kim et al., 2006).

Current Status and Temporal Trends

Hemocytic infiltration or tissue inflammation occurred at variable levels in all the coastal waters (Figures 43 and 44), though the Great Lakes were consistently low. Coastal waters of the northeast had the highest number of sites, with high prevalence. Medium to high incidence of tissue inflammation prevalence and occurrence intensity was also observed in Alaska, Hawaii and Puerto Rico (Figures 43 and 44).

The condition of tissue hemocytic infiltration appeared to be increasing throughout U.S. coastal regions with the exception of the Great Lakes, Puerto Rico, and Alaska (Figure 45). The Gulf coast recorded more locations with increasing temporal trends of the condition.

Correlations

No significant correlations were found between contaminant body burdens of the sentinel bivalves and prevalence of the hemocytic infiltration condition.

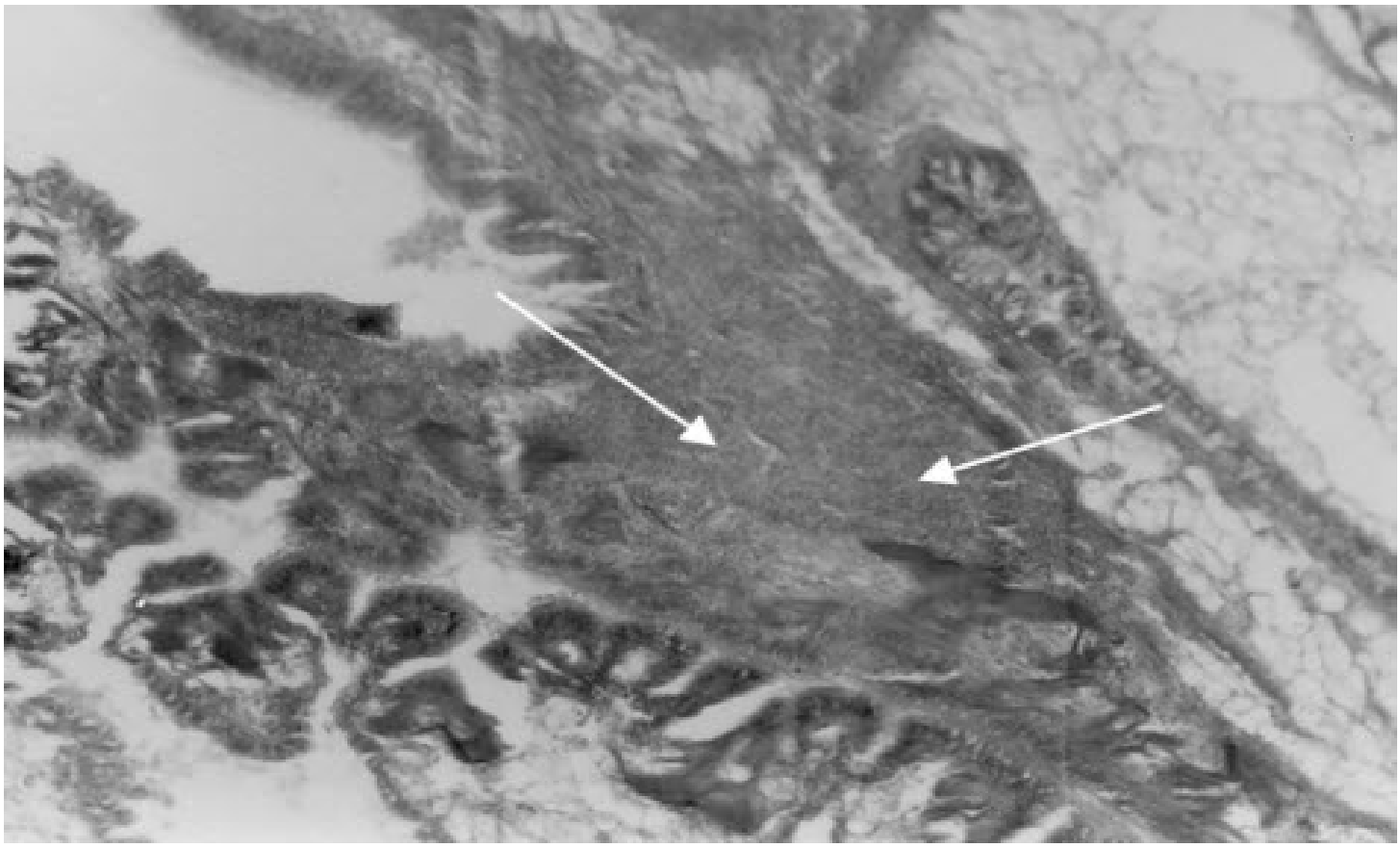


Figure 42. Hemocytic infiltration near the gill base of an American oyster. Arrows indicate examples (Kim et al., 2006).

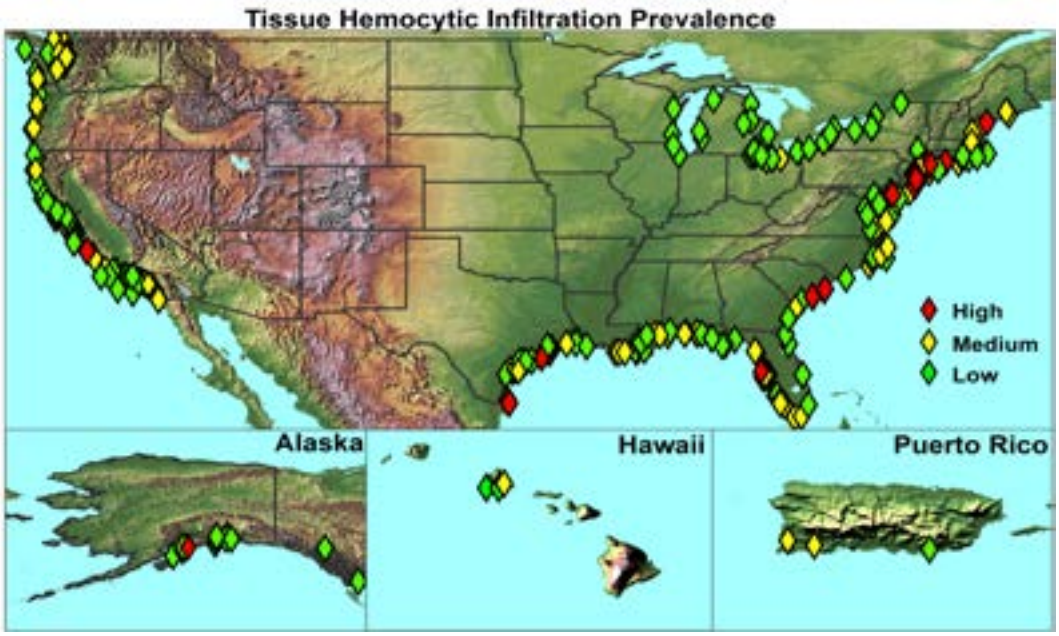


Figure 43. Spatial distribution of hemocytic infiltration prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

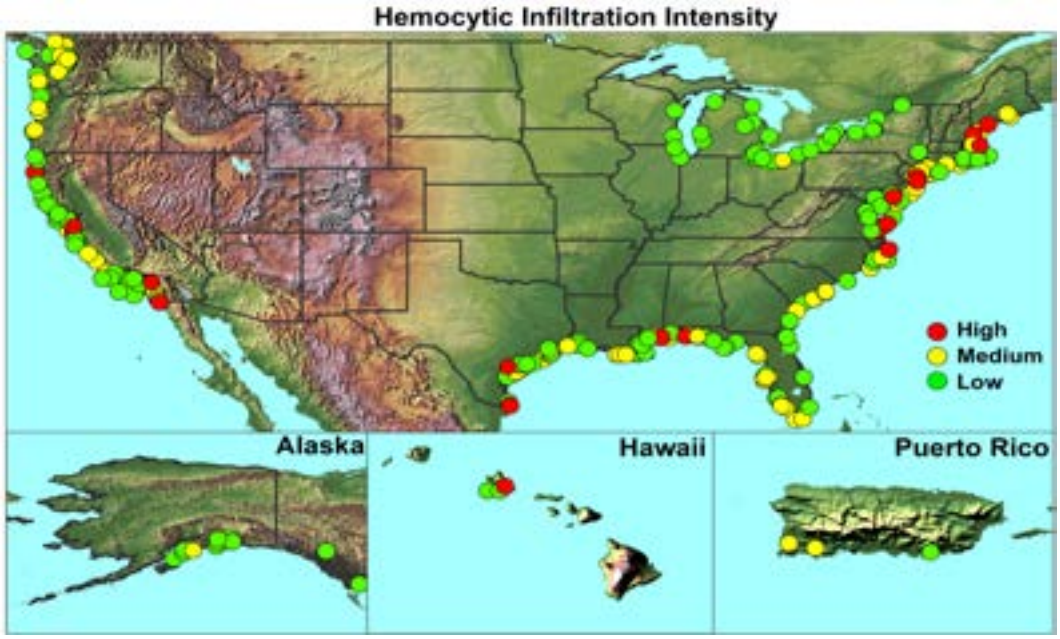


Figure 44. Spatial distribution of hemocytic infiltration occurrence intensity in oysters and mussels, based on 2008-2009 Mussel Watch data.

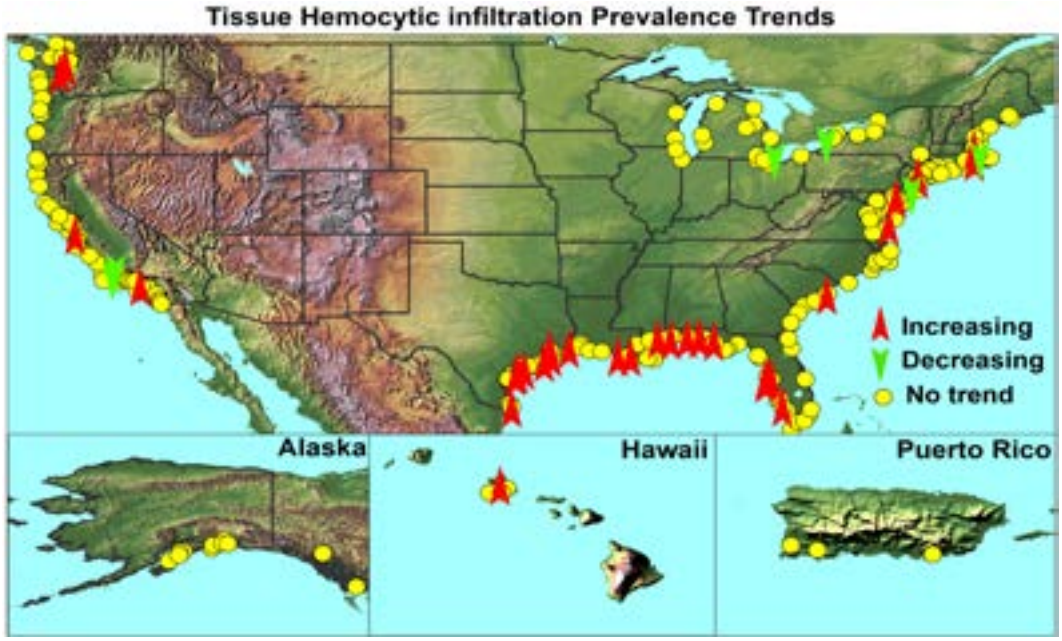


Figure 45. Site-specific long-term temporal variation of tissue hemocytic infiltration prevalence in oysters and mussels.

Ceroid Bodies

Overview

Ceroid bodies are a form of lipofuscinosis, a metabolic cellular disease (Figure 46). It is caused by a lack of enzymes which allows a waste product (ceroid body lipfuscin) to accumulate in body cells (Zaroogian and Yevich, 1993). Proteins are present with lipids resulting in a color, brownish-yellow, which demonstrates that ceroid bodies are aggregates linked to metabolite accumulation and detoxification (Zaroogian and Yevich, 1993). Ceroids are found in the organs of many animals, interfere with normal cell functioning, and also cause aging. Ceroids occur in greater abundance in oysters than other bivalve types (Kim et al., 2006).

Current Status and Temporal Trends

With the notable exception of the Pacific Northwest and the Great Lakes, all other coastal areas of the U.S. exhibited monitoring sites with high occurrence values (Figure 47). Ceroid condition were more frequent in oysters, with 81% of the high prevalence cases found in American oyster (Figure 47); this is consistent with previously published results (Kim et al., 2006). Even though ceriod bodies were highly prevalent, they occurred at low intensity (Figure 48). The highest intensity values of ceroid condition were found in Charlotte Harbor, St. Johns River and Cedar Key in FL, Swan Point, MD, and Mesquite Bay and Nueces Bay in TX.

The high occurrence in the Gulf has been relatively stable over time (Figure 49). Most decreasing trends were in mussels in the Great Lakes. There are only three sites noted with an increasing trend of ceroid bodies, which includes Bud Inlet in Puget Sound, WA; in Bodega Bay, CA; and in Cape Charles, Chesapeake Bay, VA (Figure 49).

Correlations

The presence of ceroid bodies gave positive correlations with arsenic body burdens in blue mussels from the West coast (Appendix A). Presence of ceroid bobies may be linked to cell aging, but in regard to chemical contaminants, positive correlations may suggest cellular response to exposure considering that ceroid bodies are an indication of cell stress.

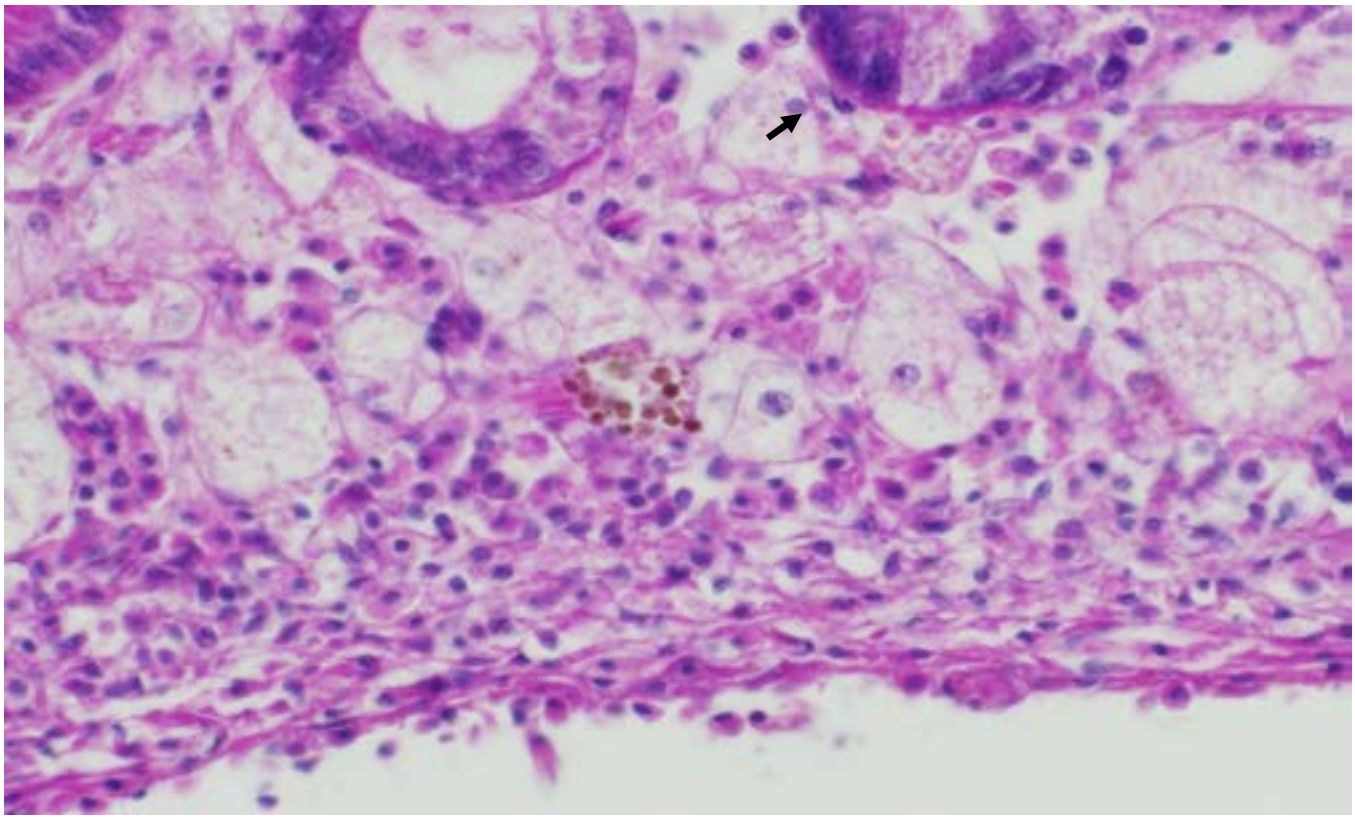


Figure 46. Ceroid bodies in gonad tissue of an American oyster (40x mag, MHE stained). Photo by Joe Marcino, Maryland Department of Natural Resources.

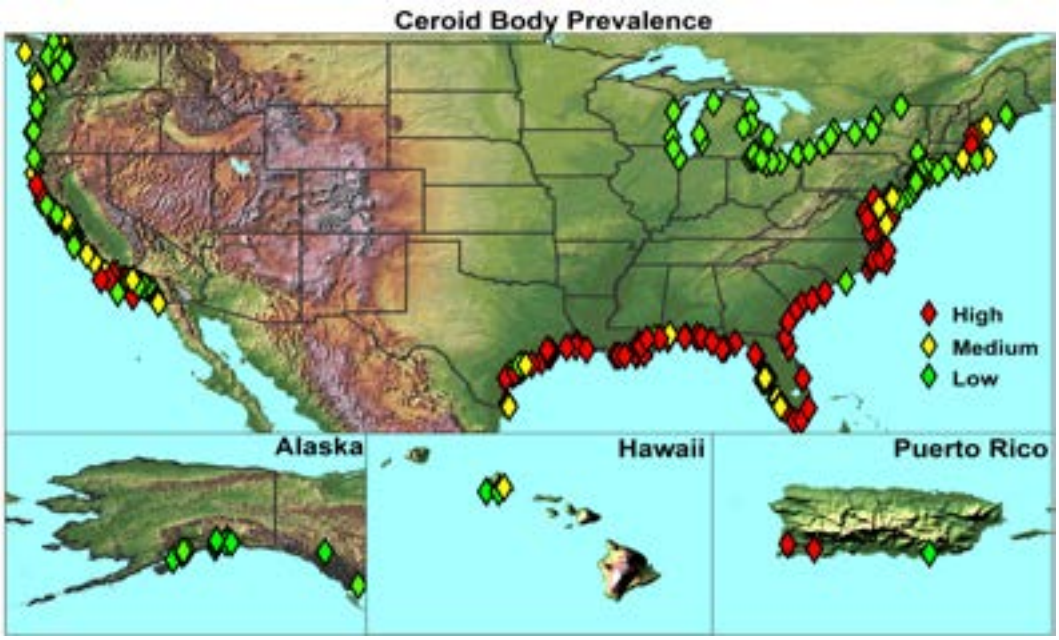


Figure 47. Spatial distribution of ceroid bodies prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data

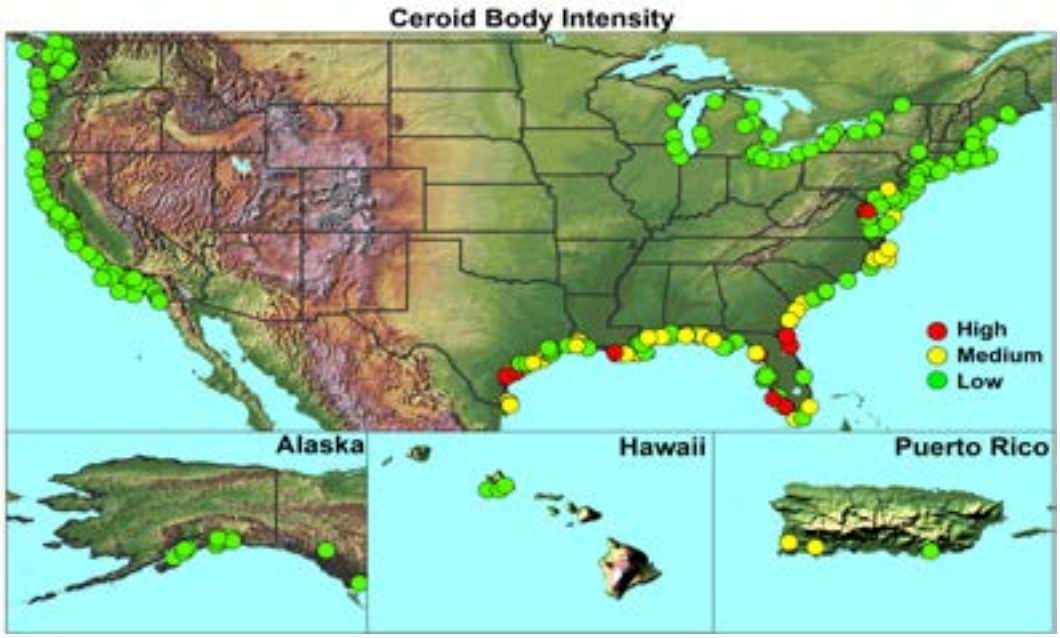


Figure 48. Spatial distribution of ceroid bodies occurrence intensity in oysters and mussels, based on 2008-2009 Mussel Watch data.

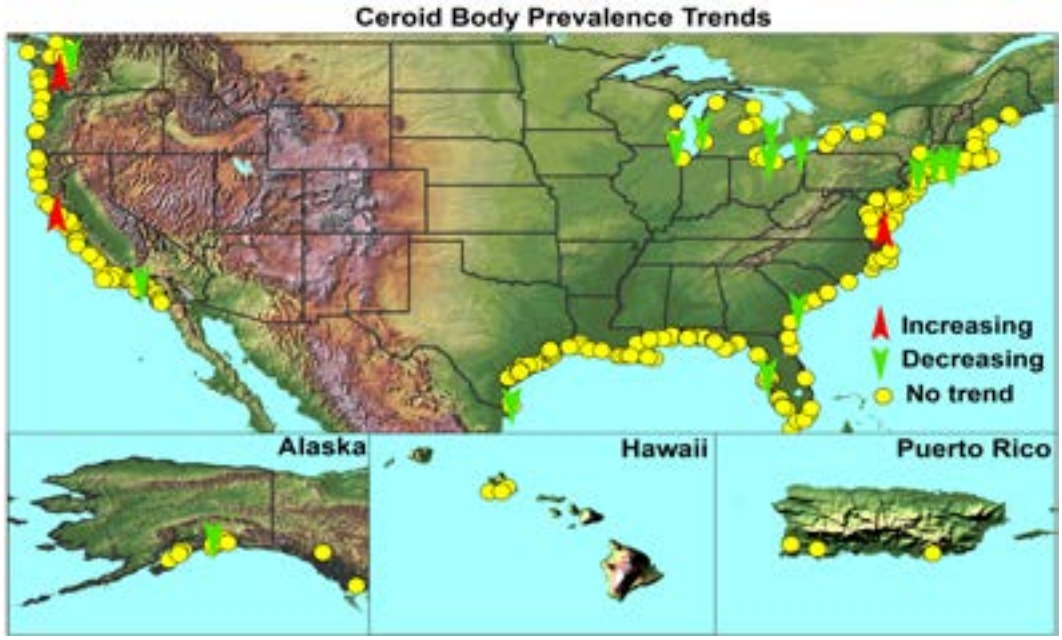


Figure 49. Site-specific long-term temporal variation of ceroid body prevalence in oysters and mussels.

Digestive Gland Atrophy

Overview

Atrophy of the digestive gland (Figure 50) is characterized by the thinning of the digestive tubule walls (Winstead, 1995). The condition is an abnormal organ structure that has serious impacts on bivalve growth. It is linked to stressors, such as exposure to contaminants and poor nutrition (Winstead, 1995). Thin digestive tubule walls may hinder food intake and processing (Winstead, 1995), the high level of digestive gland atrophy incidence in the bivalves could lead to poor nutrition and eventually to low reproduction. Atrophy of the digestive gland has been used along with biomarkers to evaluate the environment's recovery after an oil spill (Garmendia et al., 2011). Kim and Powell (2009) have also made linkages of trends of digestive tubule atrophy to interannual variation in climate such as El Niño.

Current Status and Temporal Trends

Of all the histopathology conditions measured, atrophy of the digestive gland was the most common and widespread in the sentinel bivalves (Figures 51 and 52). High prevalence levels were found in all species measured and all geographies, except the western Great Lakes (Figure 51). Most of the monitoring sites also recorded medium to high range for the intensity of this pathology condition (Figure 52).

Most areas have remained static in the occurrence of digestive gland atrophy (Figure 53). Only Galveston Bay, TX and Pensacola Bay, FL have shown increasing occurrences of digestive gland atrophy. Decreasing trends in digestive gland atrophy occurred at five monitoring sites, including Barber's Point Harbor, HI; Stony Point and Peach Orchard Point in Lake Erie, OH; Lake Borgne and Breton Sound, LA; and Charlotte Harbor, FL.

Correlations

No significant correlations were found between contaminant body burdens and prevalence values of digestive gland atrophy condition in the sentinel bivalves.

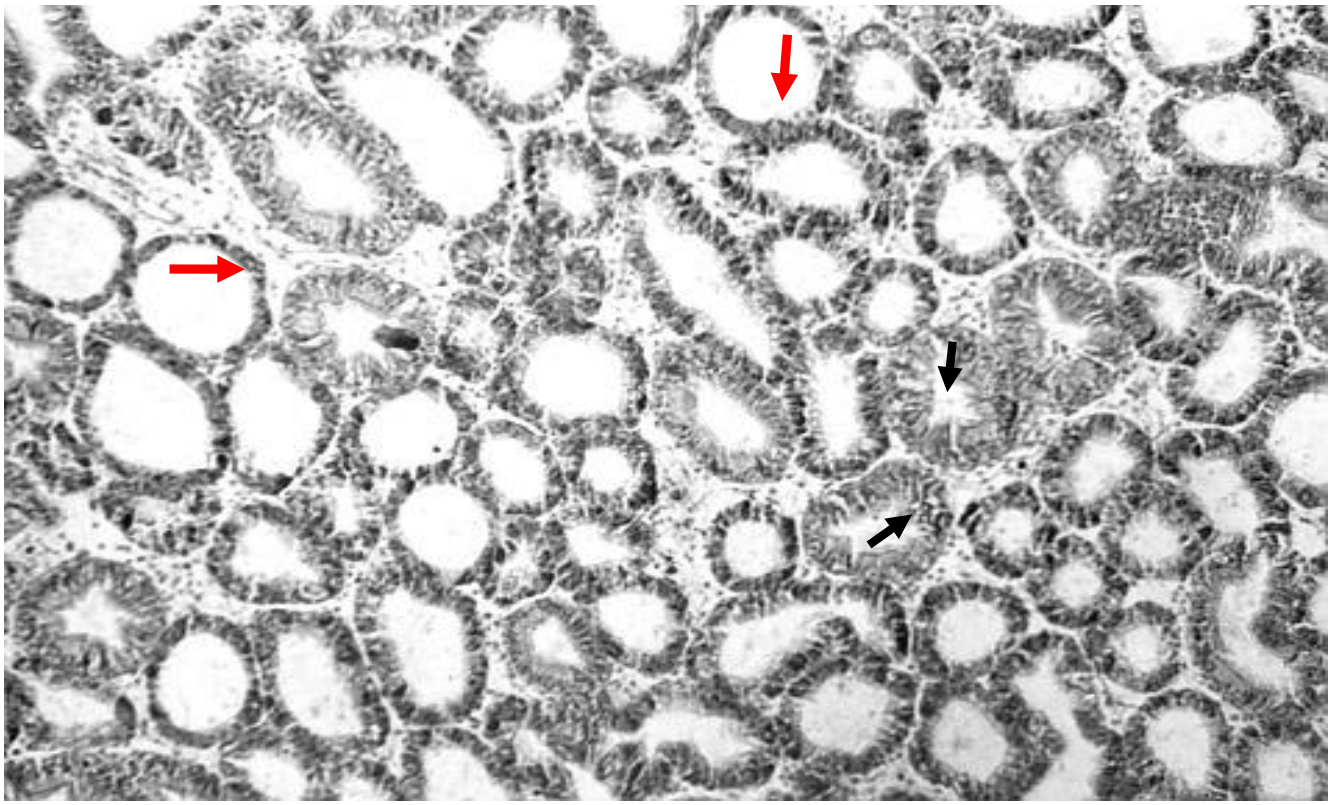


Figure 50. Digestive gland atrophy in American oyster (red arrow) characterized by atrophic epithelia resulting in rounded and enlarged lumina compared to quadriradiate lumina (black arrow) formed by normal thick epithelium (Kim et al., 2006).

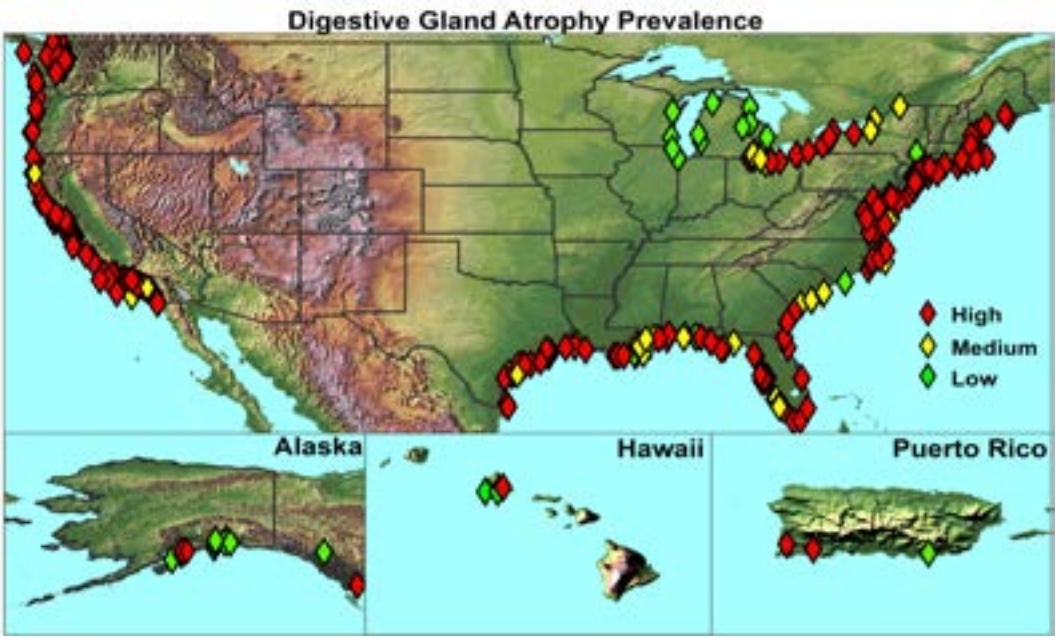


Figure 51. Spatial distribution of digestive gland atrophy prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

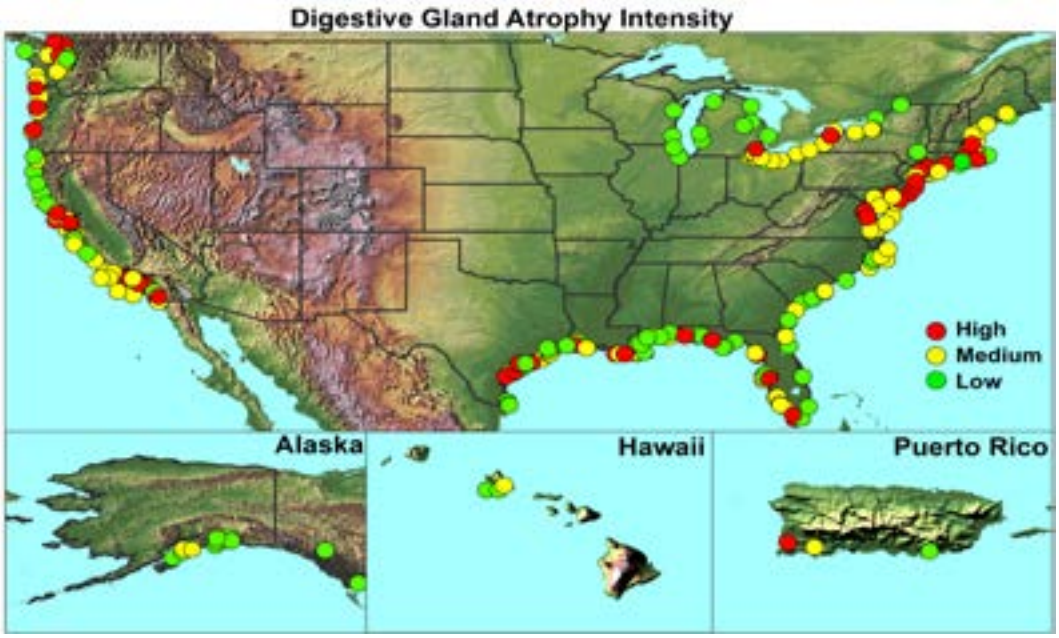


Figure 52. Spatial distribution of digestive gland atrophy occurrence intensity in oysters and mussels, based on 2008-2009 Mussel Watch data.

Figure 53. Site-specific long-term temporal variation of digestive gland atrophy prevalence in oysters and mussels.

Tissue Necrosis (Tissue Death)

Overview

Necrosis (Figure 54) is an advanced stage of tissue breakdown that is distinguished from other forms of tissue death by drastic changes visible to the naked eye (Majno and Joris, 1995). It is generally caused by factors external to the living cell or tissue. In bivalves, necrosis is often caused by pathogenic infection (Meyers and Burton, 2009). Necrosis does not elicit the immune system by sending chemical signals, so dead cells due to necrosis are not removed by phagocytes and these dead cells build up within the bivalve tissue. Bacillary necrosis disease in larval and juvenile bivalves often results in such extensive cellular destruction that it causes high mortality (Tubiash et al., 1965).

Current Status and Temporal Trends

Values of prevalence and intensity of occurrence for tissue necrosis in the sentinel bivalves were low for most of the country (Figures 55 and 56). Hot-spot for tissue necrosis were observed at blue mussel monitoring sites in Duxbury Bay, MA; Long Island Sound, NY; and Marina Del Rey, San Diego Bay and San Pedro Harbor, CA. (Figure 56).

Temporal trends of necrosis condition in the bivalves were static (Figure 57). The only trends were in Massachusetts, where necrosis increased in Duxbury Bay but decreased in Buzzards Bay.

Correlation

No significant correlations were found between contaminant body burdens and prevalence values of tissue necrosis condition in the sentinel bivalves.

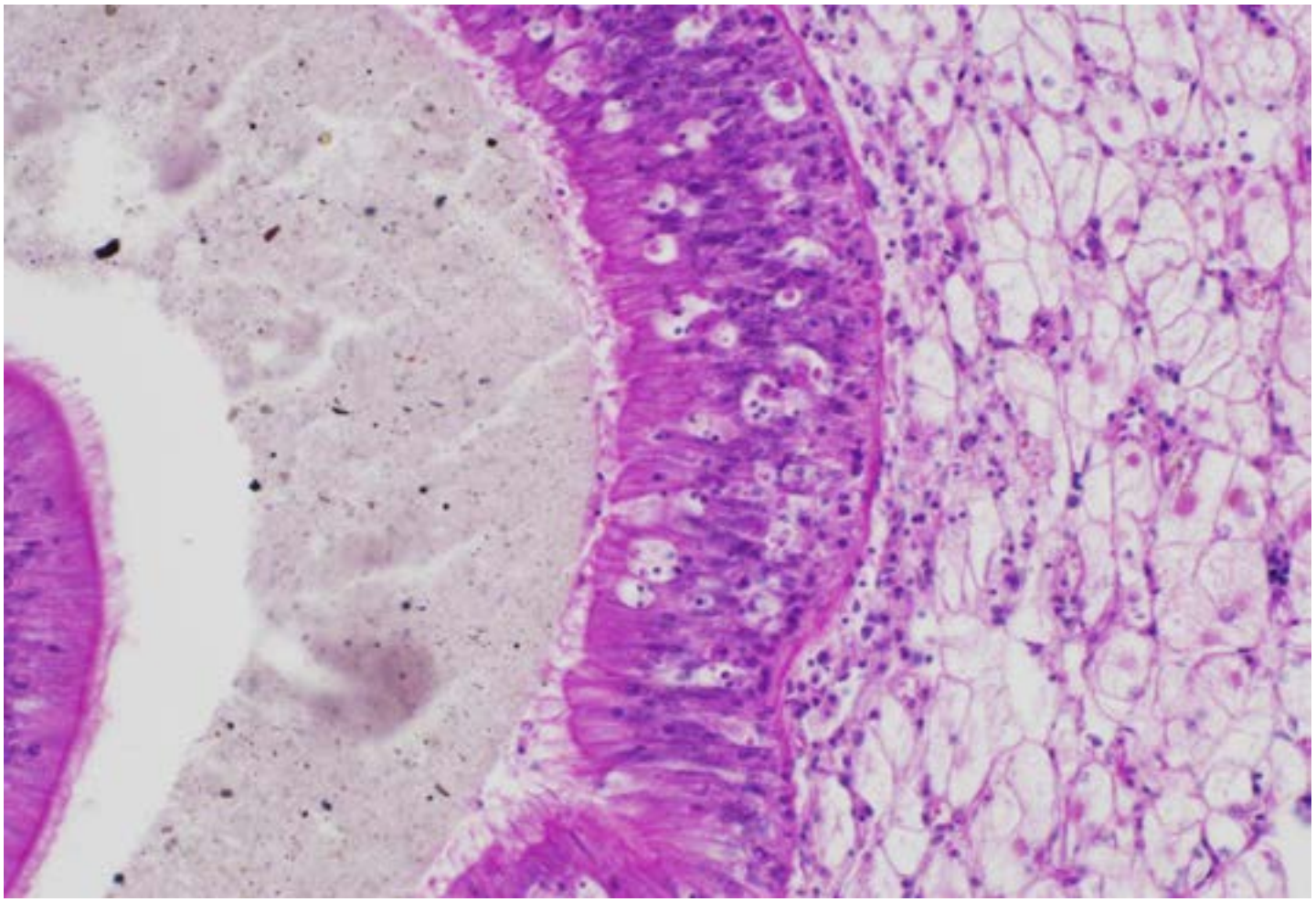


Figure 54. Photo of necrosis in the intestine of an American oyster (20x mag, MHE stain). Photo by Joe Marcino, Maryland Department of Natural Resources.

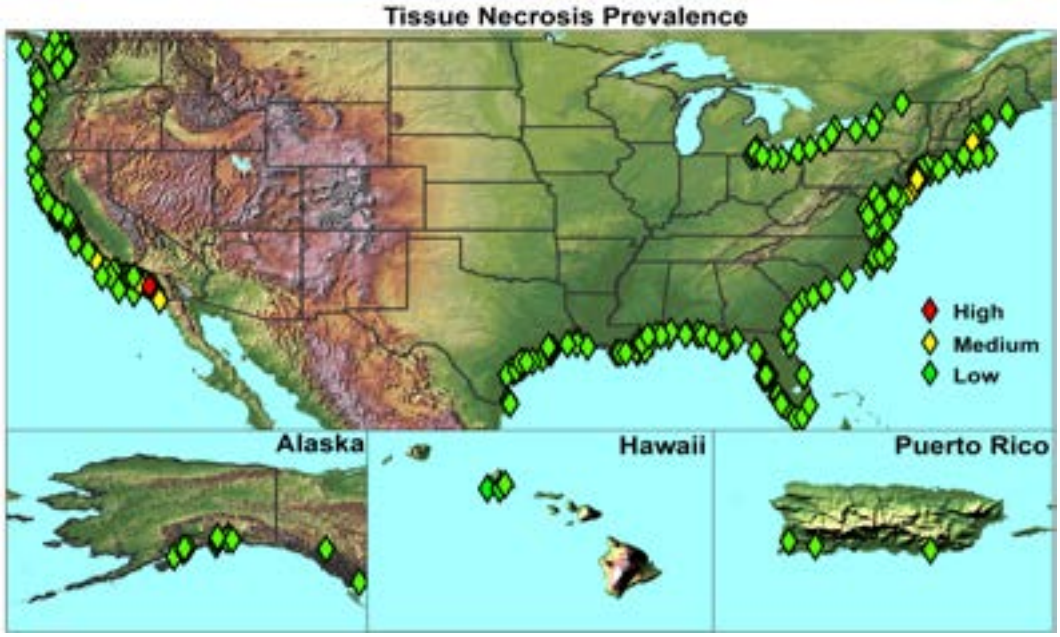


Figure 55. Spatial distribution of tissue necrosis prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

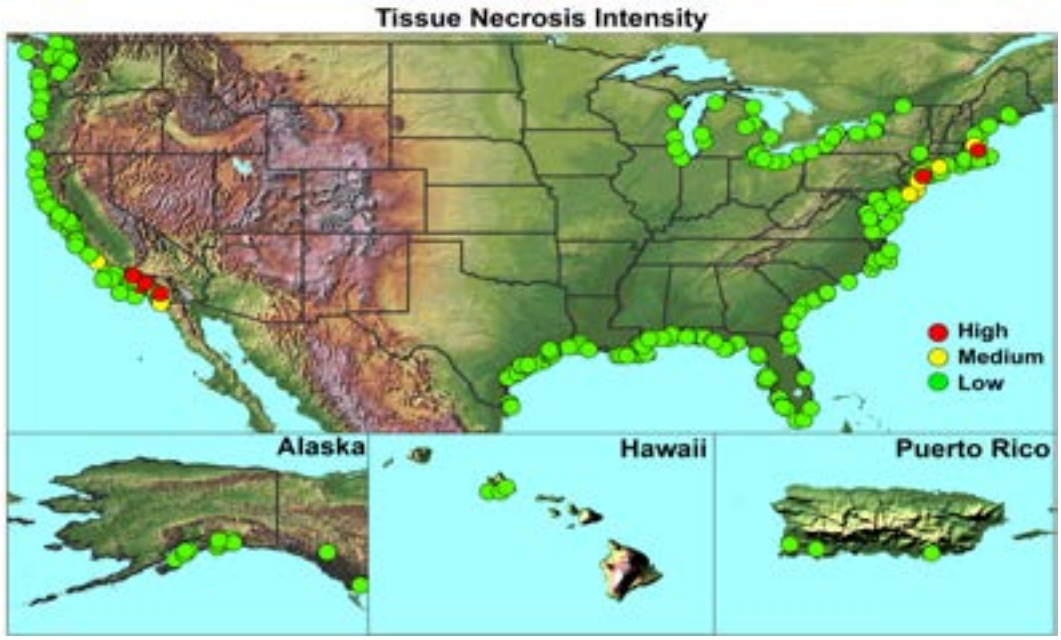


Figure 56. Spatial distribution of tissue necrosis occurrence intensity in oysters and mussels, based on 2008-2009 Mussel Watch data.

Figure 57. Site-specific long-term temporal variation of tissue necrosis prevalence in oysters and mussels.

Xenomas

Overview

Xenoma (Figure 58) is defined as an enlargement of tissue infected by parasites (Kim et al. 2006). When intracellular parasites accumulate within host cells, it causes hypertrophy (xenoma) of the infected cell and its nucleus (Boehs et al., 2009). Multiplying ciliates are known to cause xenoma in oysters and blue mussels. Xenoma causes local epithelial erosion and most likely impedes internal water flow (Scarpa et al., 2006).

Current Status and Temporal Trends

Overall, the prevalence of xenoma in the sentinel bivalves was low (Figure 59), as well as the intensity of occurrence (Figure 60). Among blue mussels there was only a single high prevalence site, Eliot Bay in Puget Sound. Among oysters, Lake Charles in Calcasieu Land, LA and Mullet Key Bayou in Tampa Bay, FL displayed high prevalence values. Most of the medium prevalence and intensity of xenoma condition occurred in the Gulf coast (Figures 59 and 60), while oysters in the southeast US only had low levels.

Increasing temporal trends of the xenoma condition were observed at only three coastal locations around the US (Figure 61): American oysters at locations in Matagorda Bay, TX and Apalachee Bay, FL, and a blue mussel location in Puget Sound, WA.

Correlation

No significant correlations were found between contaminant body burdens and prevalence values of xenoma condition in the sentinel bivalves.

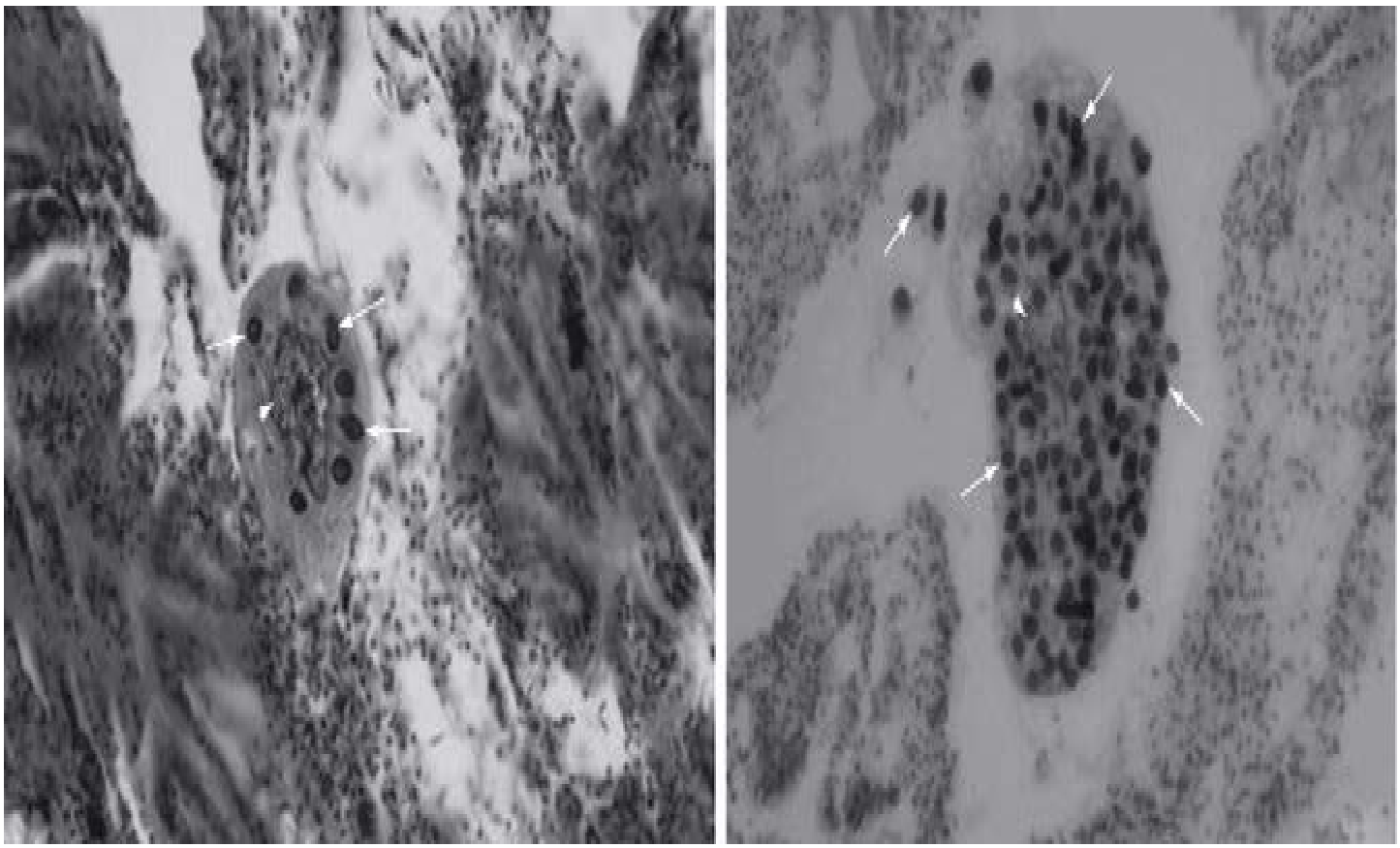


Figure 58. Xenomas in the gills of the mangrove oyster in earlier and more advanced stage. Arrows = ciliates; arrowheads = nucleus of the host cell (Boehs et al., 2009).

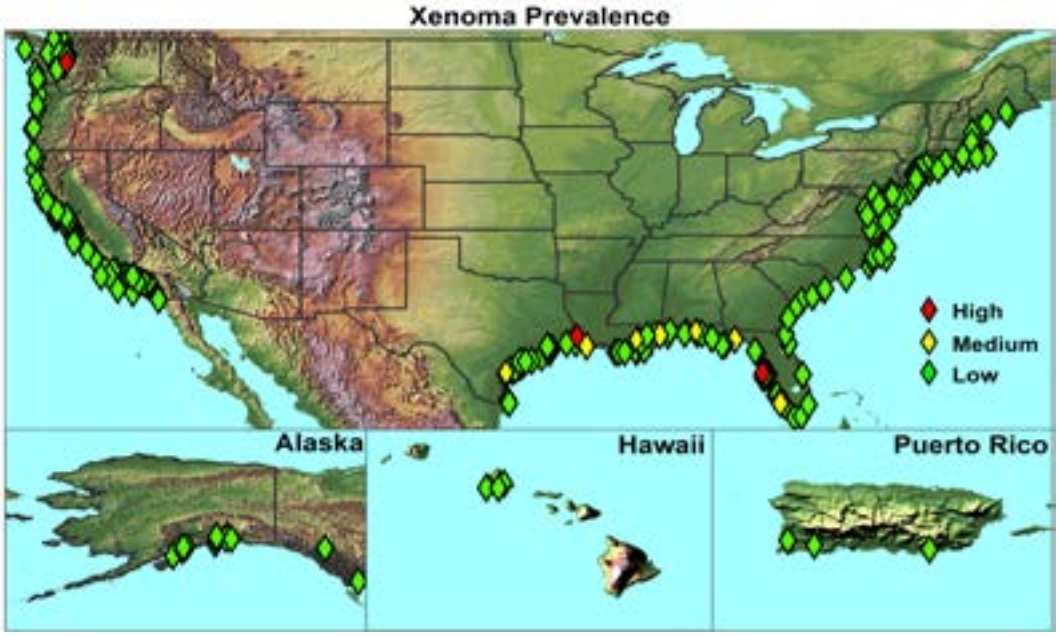


Figure 59. Spatial distribution of xenoma prevalence in oysters and mussels, based on 2008-2009 Mussel Watch data.

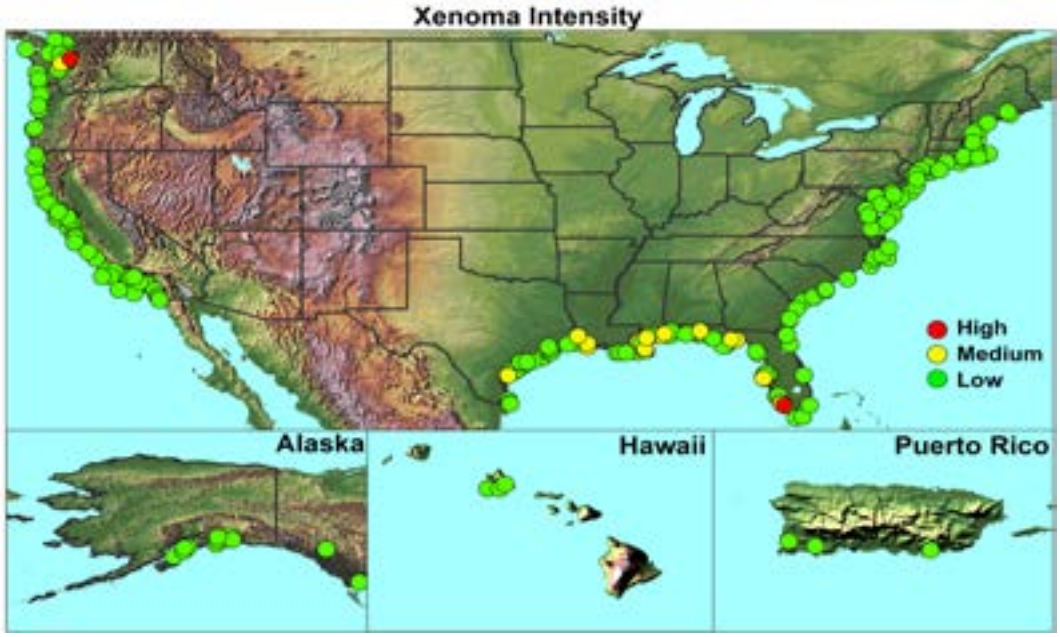


Figure 60. Spatial distribution of xenoma occurrence intensity in oysters and mussels, based on 2008-2009 Mussel Watch data.

Figure 61. Site-specific long-term temporal variation of xenoma prevalence in oysters and mussels.

SYNTHESIS

Average prevalence and intensity values for each histopathology parameter are summarized by bivalve species in Tables 5 and 6. Additionally, for parasites and diseases, the proportion of bivalves that were infected were compared among the bivalve types (Table 7) and between regions (Table 8). The assessment of current status of parasite infection and disease occurrences coupled with results in Tables 5 and 7 indicated a broad distribution of the pathology parameters in coastal waters at national scale. Where occurrences were extremely high or extremely low, no significant ($p > 0.05$) differences were observed among the different bivalve types (Table 7). Those were the cases of infection intensity of ciliates, xenoma and pinnotherid crabs, and also the severity of tissue conditions, such as unusual digestive tubule ($p > 0.05$). Prevalence and intensity of infection of all other parasite taxa and tissue conditions were found to be significantly different ($p < 0.05$) among the different bivalve types (Table 6). It is noteworthy to highlight the fact that cases of significantly higher intensities of parasite infections and tissue conditions were observed more frequently ($p < 0.05$) in the oysters. However, the East coast blue mussels had significantly higher numbers ($p < 0.05$) of some adverse conditions, including tissue necrosis, unusual digestive tubules, and trematodes. While zebra mussels were found to harbor few parasites and significantly lower cases of tissue conditions, the blue mussels had medium to high range of parasitic infection. Differences between the incidence of disease and parasitism of zebra mussels (Great Lakes) and the other species studied may be a result of the fact that zebra mussels are found in freshwater and so have a different biology than the rest of the mollusk species studied. Therefore the lower pathology of zebra mussels should

Table 5. Average prevalence of parasites and diseases by region and by bivalve type.

Parameter	American oyster (east)	Blue mussel (east)	American oyster (Gulf)	Blue mussel (west)	Zebra mussels
Ceroid body	0.89	0.49	0.93	0.29	0.14
Cestodes	0.17	nd	0.11	nd	nd
Prokaryotes	0.09	0.08	0.03	0.01	nd
Ciliates	0.29	0.37	0.12	0.27	nd
Copepods	0.01	0.02	0.01	0.10	0.00
Digestive gland atrophy	0.97	1.00	0.87	1.00	0.96
Hemocytic infiltration	0.27	0.39	0.12	0.16	0.04
H. nelsoni	0.06	nd	0.00	nd	nd
Necrosis	0.00	0.06	0.00	0.01	0.00
Nematodes	0.02	nd	0.06	nd	0.00
Gregarines	1.35	0.00	1.16	0.33	nd
Pea Crab	0.04	0.01	0.00	0.00	nd
Trematodes	0.00	0.52	0.01	0.01	nd
Xenoma	0.01	0.00	0.02	0.01	nd

Table 6. Average intensity of parasite infections and diseases by region and by bivalve type. (nd indicates conditions that do not occur in a given bivalve species).

Parameter	American oyster (east)	Blue mussel (east)	American oyster (Gulf)	Blue mussel (west)	Zebra mussels
Ceroid body	74.52	15.35	62.03	9.35	8.22
Cestodes	0.72	nd	0.56	nd	nd
Prokaryotes	2.36	0.60	1.09	0.39	nd
Ciliates	8.76	3.08	1.87	2.23	nd
Copepods	0.04	0.12	0.06	0.41	0.00
P. marinus	1.34	nd	1.59	nd	nd
Digestive gland atrophy	2.38	2.54	2.10	2.63	1.86
Hemocytic infiltration	1.00	1.73	0.45	0.69	0.19
H. nelsoni	2.18	nd	1.00	nd	nd
Necrosis	0.00	0.41	0.00	0.07	0.02
Nematodes	1.84		2.12		1.00
Gregarines	20.12	0.08	38.20	4.65	
Pea crab	1.00	0.93	1.00		nd
Trematodes	0.06	2.88	0.15	0.07	
Xenoma	2.79	2.00	2.16	2.77	nd

not be construed to imply that the Great Lakes actually have lower contaminant levels than the marine coastal and estuarine areas of the country. Overall, occurrence and intensity of parasite infection and tissue condition in the sentinel bivalves by coastal region follow the trend, from highest to lowest: Gulf coast oysters > East coast oysters > East coast blue mussels > West coast blue mussels > zebra mussels (Tables 6 and 7).

The susceptibility of bivalves to harbor more or less parasites was assessed using parasite taxa richness. Parasite taxa richness was calculated as the total number of the parasites found in a bivalve species and geographic location. Parasite taxa richness for 2008-2009 differed broadly among host bivalves and between geographic locations (Figure 62). Annual parasite taxa richness in oysters varied from 2.5 in Hawaii to 17.9 in the Gulf of Mexico (Appendix B). With an annual average of 12.2, the West coast blue mussels were significantly higher ($p < 0.05$) in parasite taxa richness than the East coast mussels, which had annual average of about 9.8 (Appendix B). The American oysters harbored significantly more parasite taxa than the other bivalve types ($p < 0.0001$). Gulf coast oysters had significantly more diverse parasite types than East coast oysters, which in turn harbored more parasite types than oysters from Hawaii and Puerto Rico ($p < 0.0001$). Among all sentinel bivalve types, zebra mussels had the least diverse parasite types with an annual average of 1.1 ($p < 0.0001$).

Over the monitoring period (1995 – 2009), annual average parasite taxa richness in Gulf coast oysters was fairly constant except for the years between 2003 and 2006, where the values fluctuated (Figure 63a). The most variations in parasite taxa richness values were recorded in the blue mussels (Figure 63b). Annual averages of parasite taxa richness in Northeast blue mussels varied between 6 and 13, while those in West coast blue mussels fluctuated between 10 and 16. Except for a few instances (1998, 2007 and 2008) where there were no overlaps, parasite taxa richness appeared to fluctuate similarly in blue mussels from both coasts.

The differences in parasite taxa richness among the bivalve species may be attributable to an amalgam of factors, including the host bivalves' biological susceptibility as well as water quality and water temperature. The NS&T program uses different mollusks with dissimilar habitats, feeding rate, and physiological processes all of which could contribute to their predisposition to disease and parasite infection. Thus, the susceptibility of the sentinel bivalves to harbor different parasites taxa could be emphasized by their biology. Most shellfish species harbor naturally an array of parasites (Murchelano and MacLean, 1990). However, if the quality of the water in which they dwell is affected by stressors such as pollutants, increased surface temperature or low dissolved oxygen, their physiological processes can be impacted increasing their susceptibility to fight infection (MacKenzie et al., 1995). In this study, although some hot-spots of parasites infection and disease occurrence were observed at various levels across the NS&T monitoring sites, correlations between parasites and diseases were rare. (Appendix A). However, elsewhere, exposure to contaminants was known to impact aquatic bivalve immune system and facilitate parasitism and occurrence of diseases (Weis et al., 1995; Johnson et al., 1992; MacKenzie et al., 1995). Rocher et al. (2006) showed that contaminants including metals activate stress response in zebra mussels and blue mussels and stress could increase the susceptibility to opportunistic parasite infection. Several reports based on the NS&T monitoring data (Apeti et al., 2009 and 2012; Kimbrough et al., 2008) indicated that heavy metals and organic contaminants body burden in the sentinel bivalves are often very low (relative to US Food and Drug Administration guidelines), which may explain the lack of strong correlations with the pathology parameters.

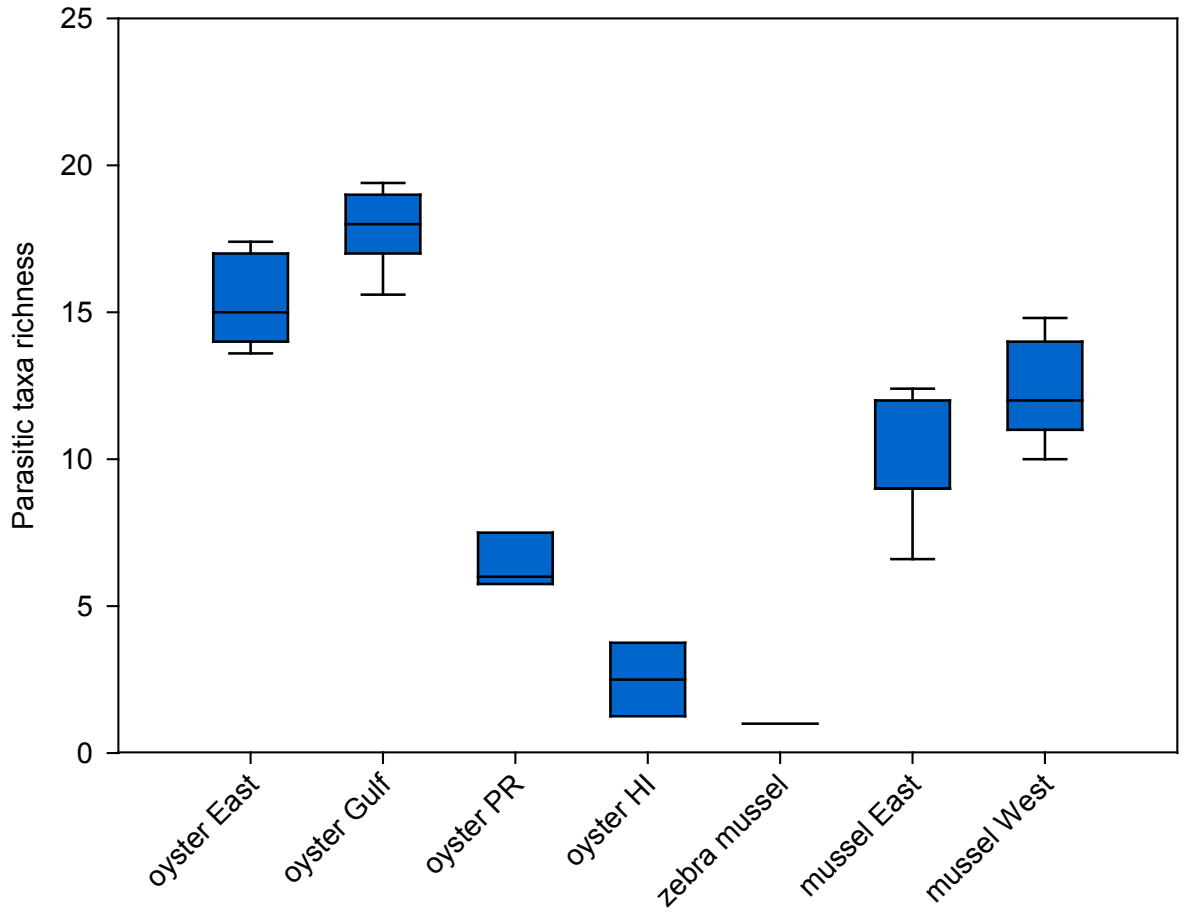


Figure 62. Boxplot illustrating parasite taxa richness in different host bivalve types by region. The upper and bottom limits of the boxplots represent the 25th and 75th percentiles, the whiskers represent the 5th and 95th percentiles, and the line in the middle of the box is the median.

Table 7. Interspecies comparison using analysis of variance (ANOVA) applied to the 2008-2009 prevalence results. Species not connected with the same letter are statistically different at $p < 0.05$. (nd indicates conditions that do not occur in a given bivalve species).

Parameter	American oyster	Blue mussels (west)	Blue mussels (east)	Zebra mussels	
Parasite					
Cestodes	A	nd	nd	nd	
Ciliates	B	C	A	nd	
Copepods	A	A	A	A	
Pea crab	A	B	AB	nd	
P. marinus	A	nd	nd	nd	
H. nelsoni	A	nd	nd	nd	
Nematodes	A	nd	nd	B	
Gregarines	A	B	C	nd	
Prokaryotes	A	B	A	nd	
Trematodes	B	B	A	nd	
Disease					
Ceroid body	A	B	BC	C	
Digestive gland atrophy	B	A	A	B	
Hemocytic infiltration	B	BC	A	C	
Necrosis	B	AB	A	B	
Xenoma	A	AB	B	nd	

Aside from contaminants, other water quality parameters including coastal flow regime, water residence time, runoff, and types of land use in adjacent watersheds and more importantly increased surface temperatures, were demonstrated to promote increased parasite infection in bivalves (Ward and Lafferty, 2004). Higher temperatures above thermal tolerance was shown to significantly stress organisms, increasing their susceptibility to some diseases, especially in aquatic ecosystems (Lafferty et al, 2004; Robinson et al, 2005). Environmental factors such as climate change along with the rising trend of sea surface temperature constitute potential stressors impacting the health of marine and coastal resources (Cook et al., 1978). Recently, a number of biochemical alterations and emergence of disease found in marine and coastal environments have been linked to climate change that is shifting the disease landscape globally (Harvell et al., 2002).

Outbreaks of bivalve diseases and parasites could have potential human health and economic consequences. Bivalves such as American oyster are important commercial fisheries; outbreaks of pathogen such as P. marinus and H. nelsoni, which are detrimental to oysters can cause reduced stock as result of massive die-off (Ewart and Ford, 1993). Other parasites such as PIB (Chlamydia and Rickettsia), cestodes (tapeworms), nematodes (roundworms) and trematodes (flukes) only parasitize mollusks as juveniles, while their adult forms often live in the digestive tract of vertebrates like humans where they can cause serious health effects (Bogitsh and Carter, 2005).

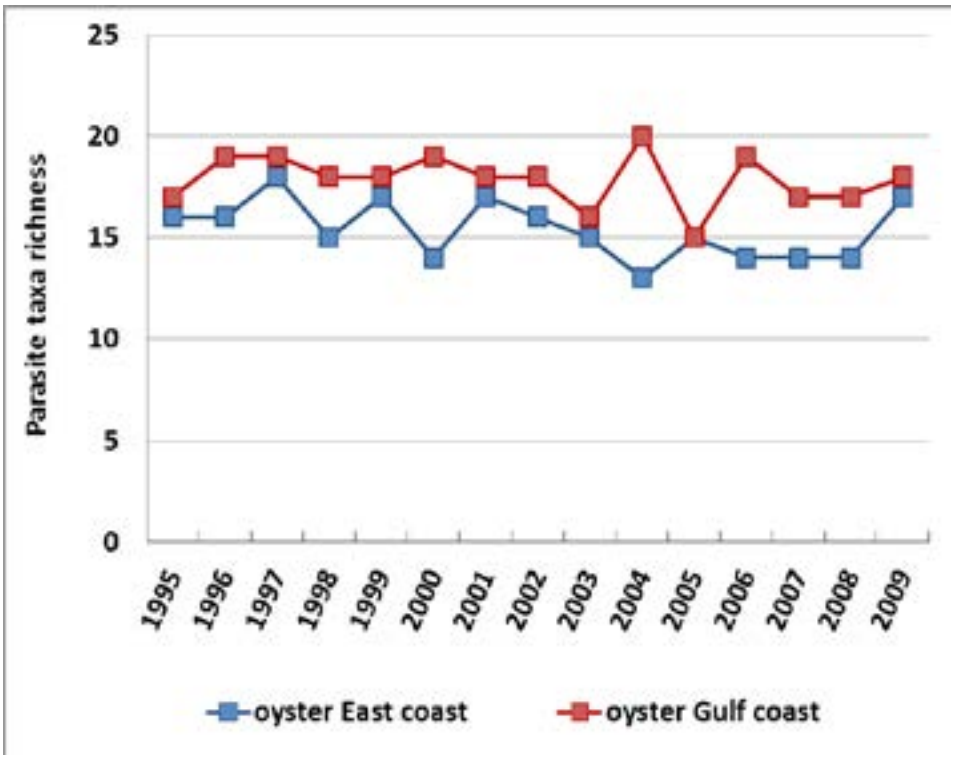


Figure 63a. Temporal variation of parasite taxa richness in oysters from the East coast and the Gulf of Mexico.

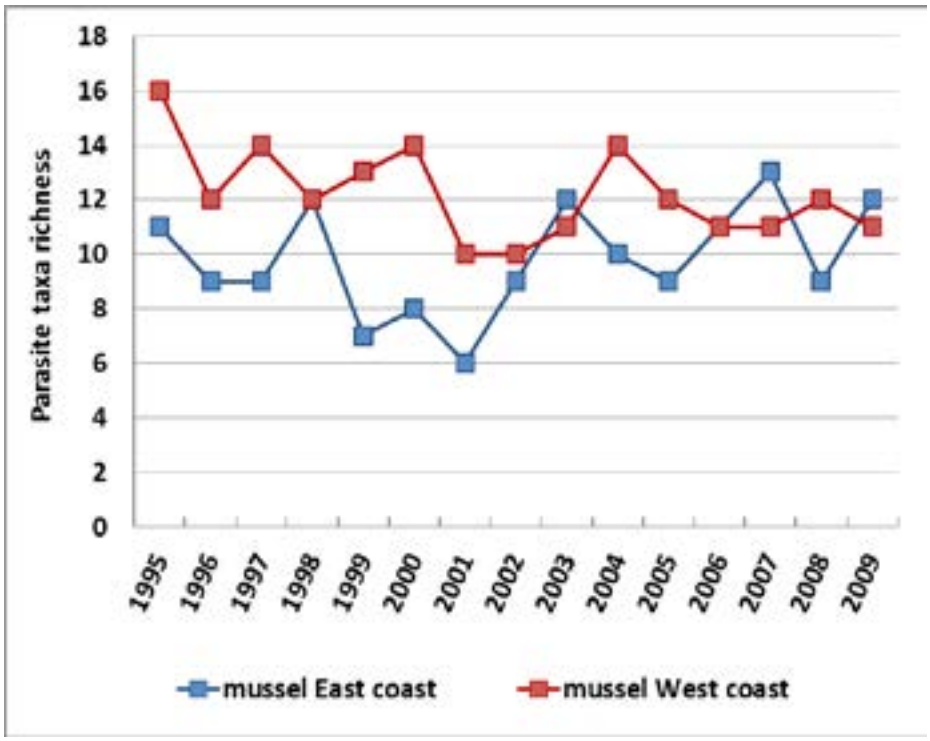


Figure 63b. Temporal variation of parasite taxa richness in blue mussels from the East and West coasts of the U.S.

Table 8. Regional contrast using analysis of variance (ANOVA) applied to the 2008-2009 prevalence results. Coastal regions not connected with the same letter are statistically different at $p < 0.05$. (nd indicates conditions that do not occur in a given bivalve species).

Parameter	Southeast	GOM	Northeast	Mid-Atlantic	West coast	Great Lakes
Parasite						
Cestodes	A	B	nd	B	nd	nd
Ciliates	AB	BC	A	B	BC	nd
Copepods	A	A	A	A	A	A
Pea crab	A	A	A	A	A	A
P. marinus	A	A	nd	B	nd	nd
H. nelsoni	A	B	nd	A	nd	nd
Nematodes	B	A	nd	B	nd	
Gregarines	A	A	C	BC	B	nd
Prokaryotes	A	BC	BC	AB	C	nd
Trematodes	C	C	A	B	C	nd
Disease						
Ceroid body	A	A	B	B	B	C
Digestive gland atrophy	BC	C	AB	A	A	C
Hemocytic infiltration	ABC	AB	A	A	BC	C
Necrosis	AB	B	AB	A	AB	AB
Xenoma	B	A	B	B	B	nd

CONCLUSION

Diverse groups of parasite taxa and types of diseases are measured in sentinel bivalve organisms as part of the NOAA/NCCOS NS&T Mussel Watch coastal monitoring program. The prevalence and intensity of parasites infection and occurrence of diseases and tissue conditions in bivalves varied broadly and their status and trends assessments suggested patterns of not only site-specific, but also regional differences.

For the parasites and diseases, high prevalence and intensity were observed more frequently in oysters than in the other bivalves. Blue mussels from the West and East coasts have medium to high range of parasitic infection, although some of the highest occurrences of disease were found in blue mussels from the West and East coasts, including tissue necrosis and digestive gland atrophy. Other histology conditions, such as ceroid bodies, hemocytic infiltration and digestive gland atrophy were common among all bivalves. Overall, occurrence and intensity of parasite infection and tissue condition in the sentinel bivalves by coastal region follow the trend, from highest to lowest: Gulf coast oysters > East coast oysters > East coast blue mussels > West coast blue mussels > zebra mussels.

The occurrence of parasites and disease at more significant magnitudes ($p > 0.05$) in the Gulf of Mexico and southeast coast over the the rest of the coastal regions did raise the question of regional scope ecosystem health issues. However, the lack of strong correlation between the histopathology parameters and contaminant body burdens suggested that the causes for regional differences may lie elsewhere.

Some hot-spots of parasites infection and disease occurrence were observed at various levels across the NS&T monitoring sites. Currently our data are insufficient to make a strong case for water quality inference relative to occurrence and distribution of the bivalve parasites and diseases in our coastal waters. However, by using multiple species belonging to various coastal zones, the NOAA NS&T data is unique in providing long-term bivalve histopathology monitoring measurements that could be used as baseline information at local, regional and national scales.

Additionally, the parasites and diseases monitored were in most cases fairly static at over the monitoring time period assessed in this study. In a few instances, however, some site-specific decreasing and increasing trends were observed for some of the histopathology parameters. These temporal trends were not extensive enough to infer a rational conclusion on water quality problems and parasite and disease prevalance.

Environmental stressors, including anthropogenic events, climate change and the resulting impacts of sea level rise and increased surface temperature, will continuously influence the distribution of disease and pathogen infections in our coastal waters. In case of unforeseen events, such as shellfish diseases or pathogen outbreaks, the MWP would be unique in providing baseline histopathology data to coastal resource managers. Additionally, for coastal resource management perspective, the MWP's histopathology monitoring data offer a very valuable baseline information to potentially understand changes in bivalve condition. Thus, for management purposes, sustained monitoring of bivalves' health condition are warranted because time series baseline data, such as the NS&T monitoring data, are important for early problem detection, which is a key for cost-effective mitigation and protection of our coastal resources.

LITERATURE CITED

Andrews, J.D. 1966. Oyster mortality studies in Virginia. V. Epizootiology of MSX, a protistan parasite of oysters. Ecology 47:19-31.

Andrews, J.D. 1979. Oyster diseases in Chesapeake Bay. 1979. Marine Fisheries Review. 1(2): 54-53.

Apeti, D.A., Johnson, W.E., Kimbrough, K.L. and Lauenstein, G.G. 2011. National Status and Trends Mussel Watch Program: Field Methods 2011 Update. NOAA National Centers for Coastal Ocean Science, Center for Coastal Monitoring and Assessment. NOAA NCCOS Technical Memorandum 134. Silver Spring, MD. 46 pp.

Apeti, D.A., Lauenstein, G.G., and Riedel, G.F. 2009. Cadmium distribution in coastal sediments and mollusks of the US. Mar. Pollut. Bull.58(7):1016-1024.

Apeti, D.A., Lauenstein, G.G., and, Evans, D.W. 2012. Recent status of total mercury and methyl mercury in the coastal waters of the northern Gulf of Mexico using oysters and sediments from NOAA's mussel watch program Marine Pollution Bulletin, 64(11):2399-2408.

Berner, L.H., McGowan, J., Martin, J.H. and Teal, J. 1976. Sampling marine organisms. In: Strategies for Marine Pollution Monitoring, E. D. Goldberg, (ed.). John Wiley & Sons, NY. pp. 269-273.

Boehs, G., Lenz, T.M. and Villalba, A. 2009. Xenomas in Crassostrea rhizophorae (Ostreidae) from Camamu Bay, Bahia, Brazil. Braz. J. Biol. 69:457-458.

Bogitsh, B.J., Carter, C.E. 2005. Human Parasitology, 3rd Edition. Academic Press, pp. 273-277. ISBN 0-12-088468-2

Brandão, R.P., Boehs, G. and da Silva, P.M. 2013. Health assessment of the oyster Crassostrea rhizophorae on the southern coast of Bahia, northeastern Brazil. Rev. Bras. Parasitol. Vet. 22:84-91.

Carballal, M.J. Iglesias D., Santamarina J., Ferro-Soto, B. and Villalba, A. 2001. Parasites and Pathologic onditions of the Cockle Cerastoderma edule Populations of the Coast of Galicia (NW Spain). Journal of Invertebrate Pathology, 78: 87–97

Cheng, T.C. 1965. Histochemical observations on changes in the lipid composition of the Eastern oyster, Eastern oyster (Gmelin), parasitized by the Trematodes Bucephalus sp. J. Invert. Pathol. 7:398-407.

Cheng, T.C. 1966. Perivascular leukocytosis and other types of cellular reactions in the oyster Eastern oyster experimentally infected with the nematode Angiostongylus cantonensis. J. Invert. Pathol. 8:52-58.

Cheng, T.C. 1967. Marine molluscs as hosts for symbioses with a review of known parasites of commercially important species. Adv. Mar. Biol. 5:1-424.

Cheng, T.C. 1978. Larval nematodes parasitic in shellfish. Marine Fisheries Review 40:39-42.

Cook, T., Folli, M., Klinck, J., Ford, S. and Miller, J. 1978. The relationship between increasing sea-surface temperature and the northward spread of Perkinsus marinus (Dermo) disease epizootic in oysters. Estuarine, Coastal and Shelf Science, 46:587-597.

Couch, J.A., and Rosenfield, A. 1968. Epizootiology of Minchinia nelson in oysters introduced to Chincoteague Bay, Virginia. Proc. Natl. Shellfish. Assoc. 58:52-59

Craig, M.A., Powell, E.N., Fay, R.R and Brooks, J.M. 1989. Distribution of Perkinsus marinus in Gulf coast oyster populations. Estuaries, 12: 82-91.

Darwin, E.J. and Stefanich, F.A. 1966. Some Common Parasites of the Fishes of Alaska. Alaska Department of Fish and Game, Juneau, AK. http://www.adfg.alaska.gov/fedaidpdfs/afrbIL.089.pdf

Elston, R.A. 1990. Mollusc diseases: Guide for the shellfish Farmer. Washington Sea Grant Publication. University of Washington Press, Seattle. 73 pp.

Ewart, W.J. and Ford, E.S. 1993. History and impact of MSX and Dermo diseases on oyster stocks in the Northeast region. Northeastern Regional Aquaculture Center, University of Massachusetts Dartmouth. Factsheet No. 200 – 1993.

Farrington, J.W. 1983. Bivalves as sentinels of coastal chemical pollution: the Mussel (and oyster) Watch. Oceanus 26:18-29.

Farrington, J.W., Albaiges, J., Burns, K.A., Dunn, B.P., Eaton, P., Laseter, J.L., Parker, P.L. and Wise, S. 1980. Fossil fuels. In: The International Mussel Watch. National Research Council. National Academy of Sciences - Office of Publications, Washington, D.C. pp. 7-77.

Ford, S. 2011. Dermo disease of oysters caused by Perkinsus marinus. Revised and updated by Susan E. Ford. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. International Council for the Exploration of the Sea, Leaflet No. 30. 5 pp.

Ford, S.E. and Figueras, A.J. 1988. Effects of subleth-

al infection by the parasite Haplosporidium nelsoni (MSX) on gametogenesis, spawning, and sex ratios of oysters in Delaware Bay, USA. Dis. Aquat. Org., 4:121-33.

Garmendia , L., Soto ,M., Vicario, U., Kim , Y., Cajaraville, M.P. and Marigómez, I. 2011, Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: Tissue-level biomarkers and histopathology. J. Envion. Monit. 13: 915-932.

Harper, D. and Benton, M. 2009. Introduction to Paleobiology and the Fossil Record. Wiley-Blackwell. pp. 207.

Harvell, C.D., Mitchell, E.C., Ward, R.J., Altizer, S., Dobson, P.A., Ostfeld, S.R. and Samuel, D.M. 2002. Climate warming and disease risks for terrestrial and marine biota. Science, 296:2158-2162.Haskin, H.H., Canzonier, W.J. and Myhre, J.L. 1965. The history of MSX on Delaware Bay oyster grounds, 1957-65 (Abstract). Amer. Malacol. Union Bull. 32:20-21.

Haven, D.S. 1958. Effects of pea crabs Pinnotheres ostreum on oysters Crassostrea virginica. Proc. Natl. Shellfish. Assoc. 49:77-86.

Haven, D.S., Hargis Jr., W.J. and Kendall, P.C. 1978. The oyster industry of Virginia: Its status, problems, and promise. Virginia Institute of Marine Science, Spec. Papers in Mar. Sci. 4. 1024 pp.

Hebert, P.D.N., Muncaster, B.W. and Mackie, G.L. 1989. Ecological and genetic studies on Dreissena polymorpha (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. 46: 1587-1591.

Heegaard, P. 1962. Parasitic Copepoda from Australian waters. Records of the Australian Museum 25:149-233.

Hilbish, T.J., Mullinax, A., Dolven, S.I., Meyer, A., Koehn, R.K., and Rawson, P.D. 2000. Origin of the antitropical distribution pattern in marine mussels (Mytilus spp.): routes and timing of transequatorial migration. Mar. Biol. (Berl.) 136:69-77.

Howard, D.W., Lewis, E.J., Keller, B.J. and Smith, C.S. 2004. Histological techniques for marine bivalve mollusks and crustaceans. NOAA Tech. Memo. NOS NCCOS 5, 218 pp.

Hugot J.-P., Baujard, P. and Morand, S. 2001. Biodiversity in helminths and nematodes as a field of study: an overview. Nematology 3:199–208.

ICES (International Council for the Exploration of the Sea). 2012. Report of the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO), 31 January – 04 February 2012, Lisbon, Portugal. ICES CM 2012/SSGHIE: 03. 63pp.

Johnson, L.L., Stehr, C.M., Olson, O.P., Myers, M.S., Pierce, S.M., McCain, B.B. and Varanasi, U. 1992. National Benthic Surveillance Project: Northeast Coast. Fish histopathology and relationships between lesions and chemical contaminants (1987-89). NOAA Tech. Memo. NMFS-NWFSC-4. NOAA/NMFS, Seattle, WA. 95 pp.

Jovanovich, M.C. and Marion, K.R. 1987. Seasonal variation in uptake and depuration of anthracene by the brackish water clam Rangia cuneata. Mar. Biol. (Berl.) 95: 395–403.

Kemp,W.M., Boynton,W.R., Adolf, J.E., Boesch, D.F., Boicourt,W.C., Brush, G., Cornwell, J.C., Fisher, T.R., Glibert, P.M., Hagy, J.D., Harding, L.W., Houde, E.D., Kimmel, D.G., Miller, W.D., Newell, R.I.E., Roman, M.R., Smith, E.M., Stevenson, J.C. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Mar. Ecol. Prog. Ser. 303, 1-29.

Kennedy, V.S., Newell, R.I.E. and Eble, A.F.. 1996. The Eastern Oyster, Crassostrea virginica: . College Park, MD: Maryland Sea Grant College.

Kim, Y. and Powell, E.N. "Distribution of parasites and pathologies in sentinel bivalves: NOAA Status and Trends “Mussel Watch” Program." Journal of Shellfish Research 26.4 (2007):1115-1151.

Kim, Y. and Powell, E.N. 2009. Effects of climate variability on interannual variation in parasites, pathologies, and physiological attributes of bivalves from the U.S. East, Gulf, and West Coasts. Environmental Bioindicators 4:67-96.

Kim, Y., Ashton-Alcox, K.A. and Powell. E.N. 2006. Histological Techniques for Marine Bivalve Molluscs: Update. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS, 27. 76 pp.

Kim, Y., Ashton-Alcox, K.A., and Powell, E.N. 2006. Histological Techniques for Marine Bivalve Molluscs: Update. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 27. pp. 53-64.

Kimbrough, K.L., and Lauenstein, G.G. 2006. Trace Metal Analytical Methods of the National Status and Trends Program: 2000-2006. US Dept. Commerce., NOAA Technical Memorandum 29, NOS NCCOS, Silver Spring, Maryland.

Kimbrough, K.L., Johnson, W.E., Lauenstein, G.G., Christensen, J.D. and Apeti, D.A. 2008. An Assessment of Two Decades of Contaminant Monitoring in the Nation's Coastal Zone. Silver Spring, MD. NOAA Technical Memorandum. NOS NCCOS 74. 105 pp.

Kimbrough, K.L., Lauenstein, G.G. and Johnson, W.E. 2007. Organic Contaminant Analytical Methods of the National Status and Trends Program: Update 2000-2006. Silver Spring, MD. NOAA Technical Memoranda NOS NCCOS 30.

Lafferty, K.D., Porter, J.W. and Ford, S.E. 2004. Are diseases increasing in the ocean? Annu. Rev. Ecol. Syst., 35, 31-54.

Lauenstein, G.G. and Cantillo, A.Y., 1998. Sampling and Analytical Methods of the National Status and Trends Program Mussel Watch Project: 1993-1996 Update. NOAA Tech. Memo. NOS NCCOS 130. Silver Spring, Maryland.

MacKenzie, K., Williams, H.H., Williams, B., McVicar, A.H and Siddall, R. 1995. Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. Adv. Parasitol., 35:85-114.

Mackin, J.G. 1962. Oyster diseases caused by Dermocystidium marinum and other microorganisms in Louisiana. Publ. Inst. Mar. Sci. Univ. Tex. 7:132-229.

Majno, G. and Joris, I. 1995. Apoptosis, oncosis, and necrosis. An overview of cell death. America Journal of Pathology 146:3-15.

May, B. and Marsden, J.E. 1992. Genetic identification and implications of another invasive species of dreissenid mussel in the Great Lakes. Can. J. Fish. Aquat. Sci. 49: 1501-1506

Menzel, R.W. and Hopkins, S.H. 1955. Growth of oysters parasitized by the fungus Dermocystidium marinum and by the Trematodes Bucephalus cuculus. J. Invert. Pathol. 41:333-342.

Meyers, T.R. and Burton, T. 2009. Diseases of wild and cultured shellfish in Alaska. Anchorage, AK. Alaska Dept. of Fish and Game, Fish Pathology Laboratories.

Murchelano, R.A. and MacLean S.A. 1990. Histopathology Atlas of the Registry of Marine Pathology. NOAA/NOS/National Ocean Pollution Program Office, Oxford, Maryland. 77 pp.

Padovan, I.P., Tavares, L.A., Corral, L., Padovan, P.A., and Azevedo, C. 2003. Fine structure of the oocyst of Nematopsis mytella (apicomplexa, porosporidae), a parasite of the mussel Mytella falcata and of the oyster Crassostrea rhizophorae (Mollusca, bivalvia) from the northeastern Atlantic coast of Brazil, 20(351), 141–145.

Powell, E.N. and Ellis, M.S. 1998. Perkinsus marinus assay. Pages 228–233 in G.G. Lauenstein and A.Y. Cantillo, eds. Sampling and analytical methods of the National Status and Trends Program Mussel Watch Projects: 1993–1996 update. NOAA Tech. Memo. NOS/ORCA 130, Silver Spring.

Roberts, L., Janovy Jr., J. and Schmidt, G.D. 2005. Foundations of Parasitology (8th edn). McGraw-Hill Companies, Inc., New York. ISBN 0-07-128458-3.

Robinson, A.R., Learmonth, A.J., Huston, M.A., Macleod, D.C., Sparks, H.T., Leech, I.D., Pierce J.G., Rehfisch, M.M. and Crick, Q.P.H. 2005. Climate Change and Migratory Species. British Trust for Ornithology. BTO Research Report 414. The Nunnery, Thetford, Norfolk

Rocher, B., Le Goff, J., Peluhet, L., Briand, M., Manduzio, H., Gallois, J., Devier, M., Geffard, O., Gricourt, L., Augagneur, S., Budzinski, H., Pottier, D., Andre, V., Lebailly, P. and Cachot, J. 2006. Genotoxicant accumulation and cellular defence activation in bivalves chronically exposed to waterborne contaminants from the Seine River. Aquat. Toxicol. 79:65-77.

Scarpa, E., Ford, S., Smith, B. and Bushek, D. 2006. An investigation of Ciliates xenoma in Cassostrea virginica. J. Shellfish Res. 25:772-773.

Sindermann, C.J. 1970. Principle Diseases of Marine Fish and Shellfish. Academic Press, New York. 369 pp.

Sindermann, C.J. 1990. Principal diseases of Marine Fish and Shellfish, 2nd Edition, Volume 2, Diseases of Marine Shellfish. Academic Press, San Diego. 516 pp.

Sindermann, C.J., and Lightner, D.V. Disease Diagnosis and Control in North American Marine Aquaculture. Amsterdam: Elsevier, 1988. Print.

Snieszko, S.F.A. 1970. Symposium on Diseases of Fishes and Shellfishes. Washington: American Fisheries Society, 1970. Print.

Stauber, L.A. 1945. Pinnotheres ostreum, parasitic on the Eastern oyster, Ostrea (Gryphaea) virginica. Biol. Bull. 88:269-291.

Tripp, B.W. and Farrington, J.W. 1984. Using sentinel organisms to monitor chemical changes in the coastal zone: progress or paralysis. Submitted to the Coastal

Society, 9th Annual Conference, October 1984, Atlantic City, NJ. Woods Hole Oceanographic Institution Contribution No. 5830.

Tubiash, S.H., Chanley, P. and Leifson, E. 1965. Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. Journal of Bacteriology, American Society for Microbiology, 90(4):1036-1044.

Ward, R.J. and Lafferty, D.K. 2004. The elusive baseline of marine disease:are disease in ocean ecosystems increasing? Public Library Of Science, PLOS Biology, 2(4):542 - 547.

Weis, P., Weis, J.S. Couch, J. Daniels, C. and Chen , T. 1995. Pathological and genotoxicological observations in oysters (Eastern oyster) living on chromated copper arsenate (CCA)-treated wood. Mar. Environ. Res. 39:275-278.

Winstead, J.T. 1995. Digestive tubule atrophy in Eastern oysters, Crassostrea virginica (Gmelin, 1791), exposed to salinity and starvation stress. J. Shellfish Res., 14:105–111.

Zaroogian, G. and Yevich, P. 1993. Cytology and biochemistry of brown cells in Crassostrea virginica collected at clean and contaminated stations. Environ. Pollut. 79:191-197.

Appendix A
Correlation between parasitic taxa and contaminants measured by the NS&T Mussel Watch Program. Results represent pair of parameters which displayed statically significant relationships ($\rho > 0.3$; $p < 0.05$).

Species	Histopathology parameter	Contaminant	Spearman ρ	P value
American oyster	Cestodes	Arsenic	0.492	< 0.001
American oyster	Cestodes	Cadmium	-0.422	< 0.001
American oyster	Cestodes	Nickel	-0.377	< 0.001
American oyster	Nematodes	Cadmium	0.300	< 0.001
Blue mussels (west)	Gregarines	Arsenic	0.329	< 0.001
Blue mussels (west)	Gregarines	Lead	0.315	< 0.001
Blue mussels (west)	Gregarines	Manganese	-0.343	< 0.001
Blue mussels (west)	Ciliates	Manganese	0.349	< 0.001
Blue mussels (west)	Ceroid body	Arsenic	0.369	< 0.001
American oyster	Gregarines	Cestodes	0.306	< 0.001
American oyster	P. marinus	Gregarines	0.307	< 0.001
Blue mussels (east)	Digestive gland atrophy	Necrosis	0.338	< 0.001

Appendix B
Annual parasite taxon richness by bivalve type and geographic location.

	Oysters				Zebra Mussels	Blue Mussels		
Year	East	Gulf	Puerto Rico	Hawaii	Great Lakes	East	West	Alaska
1995	16	17			1	11	16	7
1996	16	19	6			9	12	
1997	18	19			2	9	14	3
1998	15	18	9	1	1	12	12	
1999	17	18				7	13	3
2000	14	19	7		1	8	14	
2001	17	18			1	6	10	3
2002	16	18	5	4		9	10	
2003	15	16				12	11	4
2004	13	20		3	1	10	14	
2005	15	15			1	9	12	5
2006	14	19	6	2		11	11	
2007	14	17				13	11	4
2008	14	17	6			9	12	3
2009	17	18			1	12	11	3
Average	15.4	17.9	6.5	2.5	1.1	9.8	12.2	3.9

U.S. Department of Commerce
Rebecca Blank, *Deputy Secretary*

National Oceanic and Atmospheric Administration
Kathryn Sullivan, *Acting Under Secretary for Oceans and Atmosphere*

The mission of the National Centers for Coastal Ocean Science is to provide managers with scientific information and tools needed to balance society's environmental, social and economic goals. For more information, visit: http://www.coastalscience.noaa.gov/.