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TEMPORAL AND SPATIAL VARIABILITY OF NATIVE GEODUCK (*PANOPEA GENEROSA*) ENDOSYMBIONTS IN THE PACIFIC NORTHWEST

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ABSTRACT Lucrative commercial cultivation of Pacific geoduck (*Panopea generosa*) has developed in the United States within the past 20 y, making it one of the most economically important commercial shellfish species harvested for export. Aquaculture of the species exists in close proximity to native populations, but very little is known about the health of native populations. Baseline information on endosymbiont identification, prevalence, intensity, and geographic distribution are necessary to facilitate management and/or mitigation of potential disease interactions between cultured and natural shellfish stocks. A survey of Pacific geoduck (*P. generosa*) parasites from three natural populations in Washington state (Totten Inlet, Thorndyke Bay, Freshwater Bay) was conducted in 2008 to 2010. Histopathology of 634 animals was used to explore trends of parasite presence and to identify potential environmental factors (site distribution, collection depth, and season) that influence parasite assemblages. Endosymbionts observed on histological examination included *Rickettsia*-like organisms (RLOs) in the ctenidia ($n = 246$); an unidentified metazoan parasite in the siphon epithelium ($n = 220$); and microsporidia-like species in the intestine ($n = 103$), siphon muscle ($n = 28$), and ova (a *Steinhausia*-like parasite; $n = 99$). This study reveals the presence of three microsporidia-like organisms (including *Steinhausia*-like parasites) not previously described in geoducks. Assemblages of most parasites showed strong seasonal variations and site-specific distributions throughout the year. The presence of *Rickettsia*-like organisms may be driven by seasonal elevated temperatures, and was extremely common at Freshwater Bay. Metazoans and microsporidia were common in South Puget Sound and exhibited high infection intensity year-round. Spawning season drove *Steinhausia*-like parasite presence with no spatial driver. Baseline information on natural parasite levels, distribution, and infection loads complements ongoing monitoring of natural geoduck population dynamics, and provides crucial information to evaluate future disease events should they occur.

KEY WORDS: geoduck, disease, parasite, shellfish, Washington state, *Panopea generosa*

INTRODUCTION

Baseline information on the health status and prevalence of parasites and diseases in wild populations is necessary to understand potential interactions between wild and farmed shellfish, such as spillover (e.g., farmed to wild) and spillback effects (e.g., wild to farmed) (Daszak et al. 2000). Parasites and diseases present at low densities in wild populations may elevate to epidemic status as a result of the increases in population density or shifts in environmental conditions within culture settings (May et al. 1981). Shellfish transport has been long thought to spread disease potentially within wild and cultured populations. Strict shellfish transportation regulations exist as important management tools to help control disease interactions and to prevent further transmission. Movements of shellfish stock or seed may pose a significant threat to native populations, especially if animals are not monitored properly for disease or parasite presence. Unmonitored stock transport by growers or scientists and ballast discharge are suspected modes of transmission for some of the major shellfish diseases, including bonamiasis of the Asian oyster (*Crassostrea ariakensis*) (Carnegie et al. 2008), Denman Island disease of the European oyster (*Ostrea edulis*) (Gagné 2008), and two diseases, *Haplosporidium nelsoni* (or multinucleated sphere unknown, or MSX) and *Perkinsus marinus*, in the eastern oyster (*Crassostrea virginica*) (Burreson et al. 2000, Burreson & Ford 2004, Ford & Smolowitz 2007).

The Pacific geoduck (*Panopea generosa* Gould, 1850) is a large, burrowing hiatellid clam found in low intertidal and subtidal sediments throughout the Northeast Pacific coast, including the United States (Alaska, Washington state, California), Canada (British Columbia), and Mexico (North Baja Pacific Coast). Geoducks are one of the most economically important commercial shellfish species harvested for export (Hoffmann et al. 2000, Bower & Blackbourn 2003). A commercial Washington state geoduck fishery initiated in 1970 became highly lucrative during the 1990s through live exports to Asia; subsequent commercial cultivation of the species was developed in response to additional market demands. Washington state is at the forefront of geoduck aquaculture, which currently occurs in close proximity to wild geoduck aggregations targeted in the commercial fishery.

Few studies have been conducted regarding parasite load, natural distribution patterns, and epizootics specific to geoducks. However, this clam is known to experience several morphological abnormalities, including warts, pustules, discoloration of the periostracum, and infectious agents such as protozoas and *Rickettsia*-like prokaryotes (Kent et al. 1987, Bower & Blackbourn 2003). The ongoing evolution of the geoduck aquaculture industry presents a unique opportunity to evaluate and, potentially, mitigate negative effects of cultured–wild interactions in geoducks. To enhance our understanding of disease ecology within native geoduck populations, a comprehensive histopathological survey of three sites in Washington state was initiated in southern Puget Sound, Hood Canal, and the Strait of Juan de Fuca. These areas represent locations of natural geoduck aggregations where native populations reside

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within close proximity to cultured geoduck stocks. The goal of this study was (1) to explore trends of parasite presence within wild geoduck populations and (2) to identify geographic patterns (site and collection depth) and seasonal trends in the diversity of parasite assemblages. Information on parasite distribution (spatial and temporal) and abundance, coupled with the host response to infection, will provide needed baseline data for future species management and will assist in future research regarding the impact of these diseases on northwest populations of Pacific geoducks.

MATERIALS AND METHODS

Sample Collection and Histology

A target of 60 Pacific geoducks that ranged in size from 80–225 mm (mean ± SD, 141 ± 31.13 mm) were collected randomly by Washington Department of Fish and Wildlife divers at two depth strata from three natural populations in Washington state over multiple seasons during a 2-y period. Sites included Totten Inlet (latitude, 47.1697; longitude, –122.9617; $n = 224$), Thorndyke Bay (latitude, 47.8042; longitude, –122.7344; $n = 173$), and Freshwater Bay (latitude, 48.1439; longitude, –123.5848; $n = 237$; Fig. 1). To capture the presence of parasites more prevalent in warmer or colder seasons, animals were collected during the following months: October 2007 and July 2008 to represent warmer periods, and May 2007, February 2009, and April 2009 to represent cooler periods. Water depth was determined using mean lower low water (MLLW), or the average value of lower low-water height each tidal day observed over the National Tidal Datum Epoch by the National Oceanic and Atmospheric Administration. Collection depths were either shallow (10–30 ft MLLW) or deep (30–70 ft MLLW). Freshwater Bay geoducks were aggregated only in shallow depths at the time of sampling and therefore were not collected in deep water.

Animals were dissected within 24 h of harvesting. Length, width, and depth of shells were measured. Three 2–3-mm cross-sections were excised from each animal to obtain tissues from the following organs: siphon, ctenidia, labial palps, mantle, heart, digestive organs, and gonad. Any gross lesions were recorded, and sections were removed for histological processing

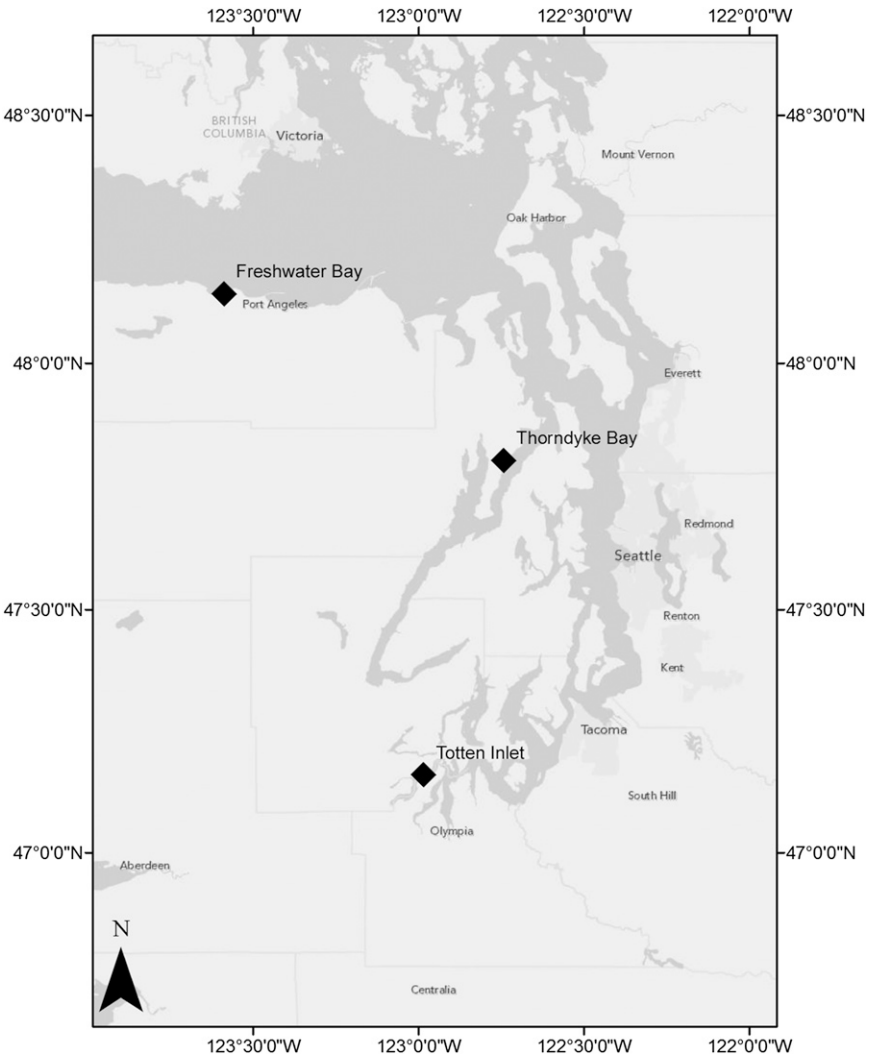

Figure 1. Geoduck sampling sites in Washington state.

and future molecular characterization. All tissue samples were preserved in Davidson's solution for 24 h and stored in 70% ethanol until processed for routine paraffin histology (Shaw & Battle 1957, Luna 1968). Deparaffinized tissue sections were stained with hematoxylin–eosin and examined for parasite presence by light microscopy. If warranted, specific stains for bacteria or fungi detection such as Gram stain or periodic acid Schiff stain (PAS) were prepared (Luna 1968).

Observed pathogens were grouped into broad taxonomic categories: *Rickettsia*-like organisms (RLOs), microsporidia-like organisms (MLO), and metazoan parasites. For each category, tissue sections were assigned a semiquantitative score of 0–4 per field of view: 0, no parasites; 1, few parasites (<10); 2, small numbers of parasites (11–20); 3, moderate numbers of parasites (21–30); and 4, large numbers of parasites (>30). The parasite data set consisted of 634 geoducks and five tissue sections (ctenidia, siphon muscle, siphon surface epithelium, intestine, and ova) containing five parasite categories: (1) RLO (ctenidia), (2) metazoa (siphon external epithelium), and MLO in the (3) siphon muscle, (4) intestine, and (5) ova. A parasite abundance matrix was organized into unique animal identification numbers described by parasite taxa and environmental variables: harvest depth (shallow, deep), season collected (winter, December to February; spring, March to May; summer, June to August; fall, September to November), and site (Thorndyke Bay, Totten Inlet, Freshwater Bay).

Statistical Analysis

Generalized linear models (GLMs) were created with the binomial family distribution and the logit link function and were used to test significance of terms (site, collection depth, season) associated with geoduck parasite presence or absence. Residual scaled deviance values were used to measure goodness of fit of the final GLM models. Tukey's honest significant difference tests were used for pairwise comparisons of parasite frequency according to the model of best fit. Kruskal–Wallis one-way analysis of variance (ANOVA) was used to compare ranked parasite intensities among sites and seasons. The chi-square test was used to test for differences in parasite prevalence between depth strata. Post hoc pairwise comparisons of Kruskal–Wallis ANOVAs were performed using Dunn's method. Generalized linear models, ANOVAs, and chi square and Tukey's honest significant difference tests were performed using R software v. 2.11.1 (R Development Core Team 2012). Post hoc analyses were performed with SigmaPlot software v. 11.0 (Systat Software, Inc.).

RESULTS

Parasite Morphology and Characterization

The most common geoduck parasites observed on histological examination included an RLO in the ctenidia (39%; Fig. 2A), an unidentified metazoan in the siphon external epithelium (35%; Fig. 2B), a *Steinhausia*-like organism (SLO) in the ovum (16%; Fig. 2C), and MLO in the intestine (16%; Fig. 2D) and siphon muscle (4%; Fig. 2E, F, Table 1). *Rickettsia*-like organisms were characterized by the presence of basophilic inclusions that stained violet with hematoxylin–eosin within the ctenidia epithelium (Fig. 2A) and were Gram negative. Inclusions were spherical and measured 13.22 ± 0.85 μm (mean ± SD) in maximum dimension ($n = 5$); individual RLOs were too small to measure. No host response was observed in association with RLO infections. Metazoa within the siphon epithelium were characterized as multicellular organisms surrounded by an eosinophilic keratin-like cuticle, some of which contained ova, and measured 128.81 ± 49.48 μm in length and 74.04 ± 36.57 μm in width ($n = 15$; Fig. 2B). In addition, *Steinhausia*-like microsporidians were observed within oocytes and were characterized by the presence of spherical eosinophilic inclusion bodies and sporocysts that contained numerous 1–2-μm basophilic spores (Fig. 2C). No host response was observed in association with the *Steinhausia*-like infections. Two spherical stages of MLO were observed in inflammatory lesions within the intestinal submucosa. The larger merogonic stage measured 4.89 ± 1.16 μm ($n = 15$) and the smaller, spore-like stages measured 0.85 ± 0.28 μm ($n = 15$) and were found in intracytoplasmic sporocysts of hemocytes (Fig. 2D). Multifocal inflammatory lesions that contained several sporocysts of an MLO were observed in the siphon musculature of some geoducks. Sporocysts measured a mean of 13.43 ± 3.5 μm ($n = 20$) and contained 4–15 spores (mean, 6.8 ± 2.8 spores per sporocyst; $n = 20$), which measured a mean of 2.91 ± 0.47 μm ($n = 15$; Fig. 2E). The spores stained PAS positive and were not acid fast.

Overall Parasite Prevalence and Intensity

Parasite intensity was measured using a semiquantitative score of 1–4, as described earlier (Fig. 3). Parasite prevalence varied among seasons for all parasites except for the SLO (chi square = 0.44, $df = 1$, $P > 0.05$). Prevalence for RLOs was greater in geoducks collected in the shallow depths (chi square = 4.8, $df = 1$, $P < 0.05$). Siphon MLO were observed only in shallow collection depths. Both the intestinal MLO and metazoan parasites were more prevalent at the deeper collection depths (chi square = 26.99, $df = 1$, $P < 0.001$; chi square = 58.28, $df = 1$, $P < 0.001$, respectively). Overall infection intensities differed by season (Kruskal–Wallis H statistic = 60.385, $df = 3$, $P < 0.001$).

Rickettsia-*like Organisms*

The most commonly encountered parasite was an RLO within ctenidial epithelia, which was observed in 39% of the sampled geoducks (Fig. 2A, Table 1). Prevalence of RLOs was greatest in Freshwater Bay (62%) relative to both Thorndyke Bay (35%) and Totten Inlet (19%; Fig. 4D, Table 2). Although overall seasonal trends in RLO prevalence were not determined because of significant interactions between season and site (Table 1), seasonal trends in RLO infection intensity varied within Freshwater and Thorndyke bays (Freshwater Bay: H = 41.23, $df = 2$, $P < 0.001$; Thorndyke Bay: H = 15.08, $df = 2$, $P < 0.001$; Totten Inlet: H = 2.70, $df = 2$, $P > 0.05$; Fig. 3D, Table 2). Over all sites, RLO intensities varied among seasons, with the highest intensities observed in summer (parasite intensity score, 2.13 ± 0.14) and winter (parasite intensity score, 1.75 ± 0.75; Table 1). No significant difference in RLO infection intensity was detected among sites (H = 3.09, $df = 2$, $P > 0.05$; Fig. 3D, Table 2).

Metazoan Parasites

Metazoan parasites were observed in the siphon epithelium of 35% of the geoducks sampled in this study (Fig. 2B, Table 1).

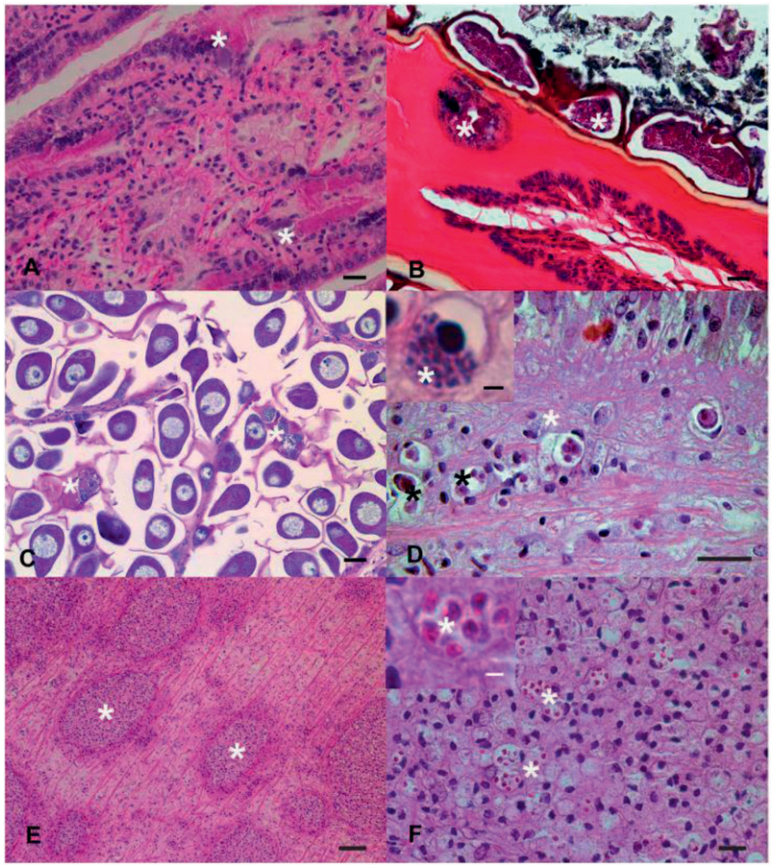

Figure 2. Commonly observed parasites in wild geoducks in Washington state. White asterisks indicate parasite presence. (A) Noted are *Rickettsia*-like inclusion bodies in geoduck ctenidia tissue (bar = 13 μm). (B) Metazoan parasites (bar = 25 μm). (C). Seen are *Steinhausia*-like microsporidians with oocytes (bar = 25 μm). (D) Microsporidia-like organism (MLO) parasites within intestinal submucosa illustrating meronts (black asterisks) and spores (white asterisks and inset image; bar = 20 μm, inset bar = 2 μm). (E) Low magnification illustrating the multifocal nature of the MLO within the siphon musculature (bar = 50 μm). (F) High magnification of siphonal MLO (bar = 8 μm, inset bar = 2 μm). Stained with hematoxylin–eosin.

Overall seasonal trends in metazoan prevalence were not determined because of significant interactions between season and site (Table 1). Prevalence of siphon metazoa varied among sites, with the highest levels observed in geoducks from Totten Inlet (57%) and Thorndyke Bay (46%) relative to only 9% of Freshwater Bay (overall: $H = 53.65$, $df = 2$, $P \leq 0.001$; Fig. 4). Similar seasonal trends in metazoan prevalence were observed in geoducks from Freshwater and Thorndyke bays, where summer prevalence exceeded those of all other seasons (Table 2). Animals from both sites exhibited similar prevalence patterns of metazoan parasites; no seasonal trend was observed in Totten Inlet animals (Fig. 4A, Table 2). Across all sites, metazoan infection intensity was significantly lower in the spring compared with winter and summer (winter: Dunn's multiple-comparison Q statistic = 2.83, $P < 0.05$; summer: $Q = 2.72$, $P < 0.05$; Fig. 3A, Table 1). Totten Inlet geoducks had higher intensity metazoan infections (parasite intensity score, 3.26 ± 0.11) relative to those in animals from both Freshwater (parasite intensity score, 1.60 ± 0.26) and Thorndyke (parasite intensity score, 2.03 ± 0.14; $P < 0.05$) bays, which were similar to one another ($Q = 1.16$, $P > 0.05$).

Steinhausia-*like Organisms*

Steinhausia-like organism parasites were observed in oocytes of 16% of total geoducks sampled in this study (Fig. 2C, Table 1). Mean prevalence (28%33%) and intensity (parasite intensity score, 1.08 ± 0.06–1.26 ± 0.08) of SLO infection were similar among sites (intensity: $H = 2.12$, $df = 2$, $P > 0.05$; Table 2). Site was not a significant term in the final GLM for SLO presence ($F = 1.12$, $df = 2$, $P > 0.05$). Across all sites, SLO prevalence was greatest in the winter (70.7%) and spring (58.0%) relative to summer (14.3%) and fall (1.9%; $P < 0.05$; Fig. 4E, Table 1). Differences in SLO parasite infection intensity by season were not detected ($H = 2.06$, $df = 2$, $P > 0.05$; Fig. 3E).

Intestinal Microsporidia-like Organisms

Intestinal MLO were observed in 16% of all geoducks sampled in this study (Fig. 2D, Table 1); no overall seasonal trends in prevalence were observed ($F = 0.94$, $df = 3$, $P > 0.05$; Fig. 4B, Table 1). Prevalence varied among locale, with the most infections observed in Totten Inlet animals (34%; $P < 0.05$) relative to those from Thorndyke Bay (17%) and Freshwater Bay (4%; Fig. 4B), which were similar to one another ($P = 0.16$; Fig. 4B, Table 2). Mean infection intensity was similar among sites ($H = 4.94$, $df = 2$, $P > 0.05$; Fig. 3B, Table 2). Infection intensities varied with season across all sites ($H = 14.34$, $df = 2$, $P < 0.05$; Fig. 3B, Table 1). Fall intensity (parasite intensity score, 2.46 ± 0.20) was greater than spring (parasite intensity

TABLE 1.

Overall mean parasite prevalence and intensity in natural populations of Washington state *Panopea generosa*.

Parasite	Tissue	Prevalence					Intensity†				
		Overall prevalence (%)	Winter ($n = 89$)	Spring ($n = 204$)	Summer ($n = 161$)	Fall ($n = 180$)	Overall mean intensity ± SE	Winter ($n = 94$)	Spring ($n = 210$)	Summer ($n = 99$)	Fall ($n = 193$)
Rickettsia-like organism	Gill	39	4.7*	36.1*	44.5*	57.8*	2.01 ± 0.14	1.75 ± 0.75^{A}	1.18 ± 0.05^{AD}	2.13 ± 0.14^{AB}	1.60 ± 0.08^{AC}
Metazoan	Siphon epithelium	35	50.0*	24.6*	52.0*	32.7*	2.70 ± 0.09	3.05 ± 0.2^{A}	2.19 ± 0.19^{B}	2.94 ± 0.19^{A}	2.54 ± 0.19^{AB}
Steinhausia-like organism	Oocytes	16	70.7[a]	58.0[a]	14.3[b]	1.9[b]	1.20 ± 0.02	1.14 ± 0.06^{A}	1.24 ± 0.08^{A}	1.10 ± 0.10^{A}	1.50 ± 0.71^{A}
Microsporidia-like organism, intestine	Intestinal submucosa	16	22.6[a]	16.2[a]	16.8[a]	15.1[a]	1.87 ± 0.09	1.47 ± 0.19^{A}	1.75 ± 0.16^{B}	1.73 ± 0.15^{B}	2.46 ± 0.20^{AB}
Microsporidia-like organism, siphon	Siphon musculature	4	0.0[a]	2.2[a]	9.9[a]	5.2[a]	2.79 ± 019	0^{A}	2.25 ± 0.48^{A}	3.13 ± 2.26^{A}	2.44 ± 0.34^{A}

* Significant differences in prevalence not determined. † Parasite intensity is based on a semiquantitative score of 0–4: 0, no parasites; 1, few parasites (<10); 2, small numbers of parasites (11–20); 3, moderate numbers of parasites (21–30); 4, large numbers of parasites (>30). Significant interactions between season and site detected. Different letters indicate significant differences in prevalence (lowercase) or intensity (uppercase); alphabetical order reflects values ordered higher to lower.

score, 1.75 ± 0.16) and summer (parasite intensity score, 1.73 ± 0.15), but significantly exceeded that observed in winter, when the lowest mean infection intensity (parasite intensity score, 1.47 ± 0.19) was observed ($Q = 3.33$, $P < 0.05$).

Siphon Microsporidia-like Organisms

Siphon MLO were observed the least frequently (4%) of all characterized parasites encountered in geoducks sampled in this study (Fig. 2E, F; Table 1); no overall seasonal trends in prevalence or intensity were observed ($P > 0.05$; Figs. 3C and 4C, Table 1). Overall prevalence was similar among seasons and ranged from 0% in winter to 9.9% in summer (Table 1). Prevalence of the siphonal MLO varied among sites. Nine percent of Totten Inlet animals and 6% of those from Thorndyke Bay were infected, whereas no MLO were observed in the siphon of Freshwater Bay geoducks (Fig. 4C, Table 2). Mean overall infection intensity was high (parasite intensity score, 2.79 ± 0.19) and was similar among seasons ($H = 4.7$, $df = 2$, $P > 0.05$; Fig. 3C, Table 1). Siphon muscle MLO were observed in the highest infection intensities at Totten Inlet (parasite intensity score, 2.67 ± 0.26) and Thorndyke Bay (parasite intensity score, 3.00 ± 0.30), and intensity differences were nonsignificant between the two sites (Mann-Whitney *U*-test, 75; $P > 0.05$; Table 2).

DISCUSSION

This study revealed five morphologically distinct endosymbionts of natural Pacific geoduck populations in the Pacific Northwest: an RLO in the ctenidia, an unidentified metazoan in the siphon epithelium, *Steinhausia*-like spp. in oocytes, and two other MLO within siphon muscle and intestinal submucosa. To our knowledge, this is the first report of microsporidia-like parasites, including *Steinhausia*-like parasites, in geoducks. This study provides an initial characterization of endoparasites in wild Puget Sound geoduck populations, and suggests that seasonal and geographic differences in distribution and infection intensity should be taken into account when moving animals among locales.

Putative Identification and Seasonal Distribution of Geoduck Parasites

Intracytoplasmic *Rickettsia*-like colonies (inclusion bodies) are commonly observed in a variety of molluscan species worldwide, such as oysters, abalone, and clams, including the geoduck (Elston 1986, Fries & Grant 1991, Friedman et al. 2000, Bower & Blackbourn 2003). The most common geoduck parasite (39%) observed in this study were RLOs. Microscopic examination revealed that RLO prevalence peaked in warmer months (fall sampling), with the greatest infection intensity observed during summer months. This finding suggests that elevated temperature may be an important driver of RLO presence in geoducks, and it complements experimental trials of other *Rickettsia* investigations in invertebrate species (e.g., Moore et al. 2000, Friedman et al. 2002, Braid et al. 2005, Vilchis et al. 2005). Transmission experiments of one RLO, "*Candidatus* Xenohaliotis californiensis," in abalone (*Haliotis spp*.) indicate that elevated seawater temperature significantly enhanced parasite transmission and accelerated progression of the disease (Moore et al. 2000, Friedman et al. 2002, Braid et al. 2005, Vilchis et al. 2005). In geoduck populations, RLO

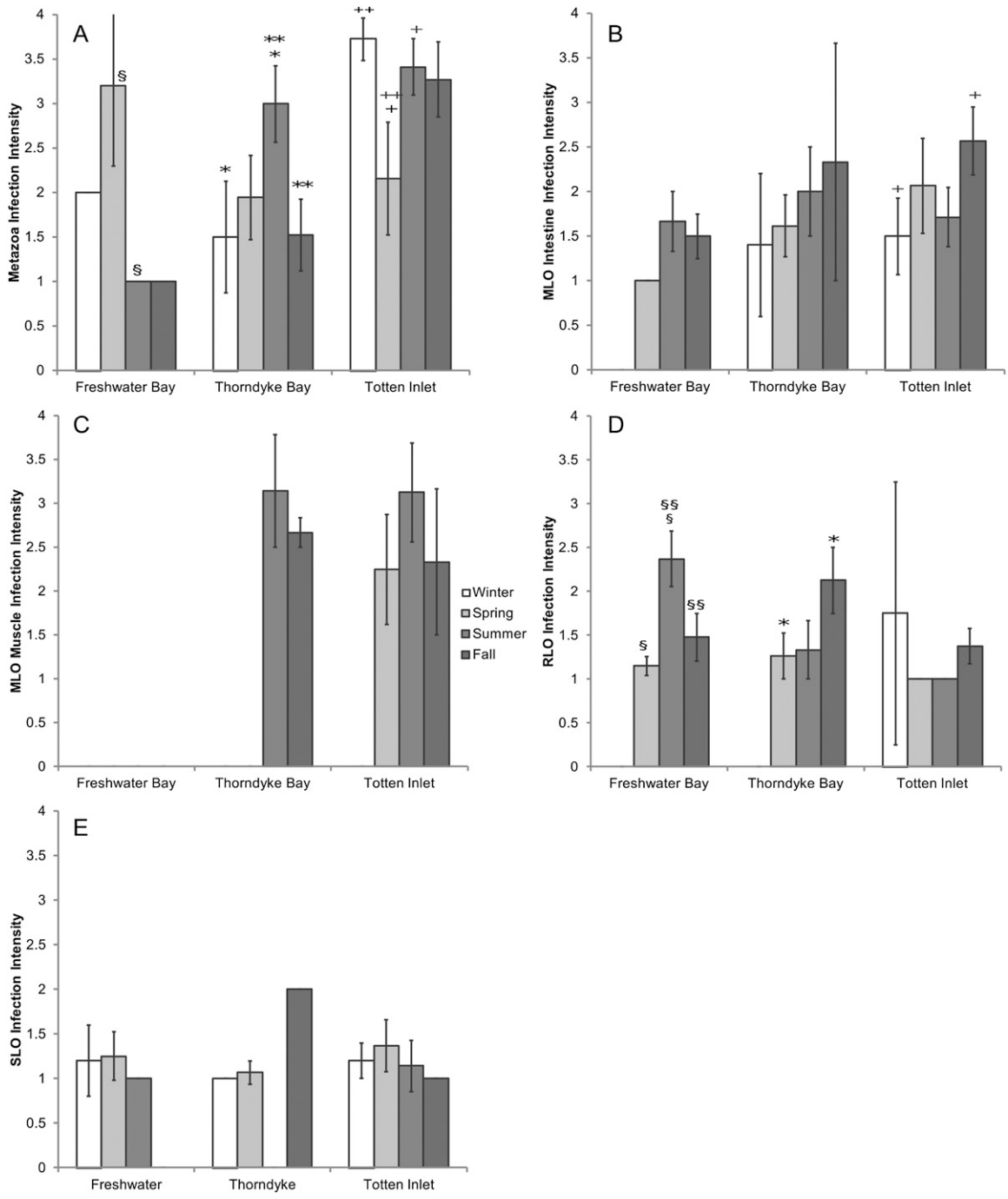

Figure 3. (A–E) Infection intensity in *Panopea generosa* by site and season. Parasite groups metazoa (A), intestinal microsporidia (microsporidia-like [MLO] intestine) (B), siphon muscle microsporidia (MLO muscle) (C), *Rickettsia*-like organism (RLO) (D), and *Steinhausia*-like organism (SLO) (E) observed from histology in geoducks collected from Freshwater Bay, Thorndyke Bay, and Totten Inlet. Error bars represent 95% confidence intervals. § or §§Freshwater Bay pairwise comparisons indicating significant difference between seasons. * or **Thorndyke Bay pairwise comparisons indicating significant difference between seasons. + or ++Totten Inlet pairwise comparisons indicating significant difference between seasons.

reproduction may also increase with elevated temperature and may lead to the trends observed.

In the current study, metazoan infections in geoducks were present year-round in high intensity at all sites and seasons other than those from Freshwater Bay, where both prevalence and intensity were low. The relatively high occurrence and elevated infection intensities observed may be the result of an accumulation of these parasites over time (Rohde 1984); age data from future studies are necessary to confirm this prediction. Geoducks are known to be one of the longest living bivalve molluscs, and in fact, Bureau et al. (2002) used growth rings, verified as annual by the bomb radiocarbon signal (Vadopalas et al. 2011), to estimate the age of one geoduck at 168 y. Animals collected in this study were

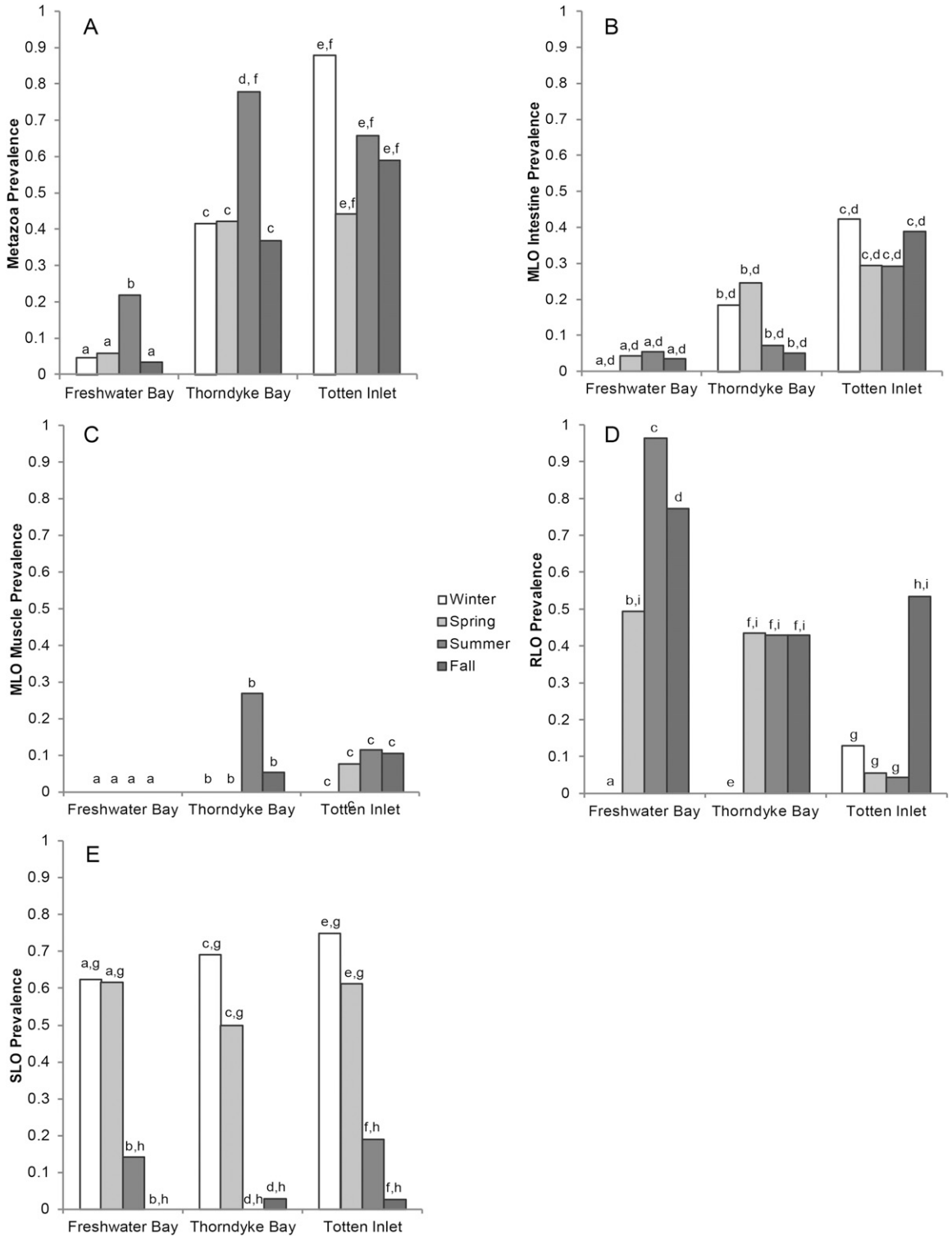

Figure 4. Proportions of parasite groups metazoa (A), intestinal microsporidia (microsporidia-like [MLO] intestine) (B), siphon muscle microsporidia (MLO muscle) (C), *Rickettsia*-like organism (RLO) (D), and *Steinhausia*-like organism (SLO) (E) observed from histology in geoducks collected from Freshwater Bay, Thorndyke Bay, and Totten Inlet. Bars with the same letters are statistically similar, while differing letters represent significant differences in the measured response.

recruits and assumed to be collected at random with respect to age. Although shell length was collected for all specimens, shell length correlates poorly with age after asymptotic length is attained at age 5–15 y (Goodwin & Pease 1991, Hagen & Jaenicke 1997, Hoffmann et al. 2000, Campbell et al. 2004).

Microsporidian infections have not been identified previously in geoducks. Currently, microsporidia have been reported

TABLE 2.

Parasite prevalence and intensity among sites and seasons.

Parasite	Freshwater Bay ($n = 237$)			Thorndyke Bay ($n = 173$)			Totten Inlet ($n = 224$)		
	Prevalence (%)	Intensity (mean ± SE)	Seasonal prevalence trends	Prevalence (%)	Intensity (mean ± SE)	Seasonal prevalence trends	Prevalence (%)	Intensity (mean ± SE)	Seasonal prevalence trends
Rickettsia-like organism	62^{a}	1.70 ± 0.09^{A}	Su > F > Sp	35^{b}	1.60 ± 0.11^{A}	W < Sp = S = F	19^{b}	1.36 ± 0.10^{A}	F > W = Sp = Su
Metazoan	9^{b}	1.60 ± 0.26^{B}	Su > W = Sp = F	46^{a}	2.03 ± 0.14^{B}	Su > W = Sp = F	57^{a}	3.26 ± 0.11^{A}	No trend
Steinhausia-like organism	32^{a}	1.23 ± 0.10^{A}	W = Sp > F = Su	28^{a}	1.08 ± 0.06^{A}	W = Sp > F = Su	33^{a}	1.26 ± 0.08^{A}	W = Sp > F = Su
Microsporidia-like organism, intestine	4^{b}	1.33 ± 0.17^{A}	No trend	17^{b}	1.70 ± 0.18^{A}	No trend	34^{a}	2.00 ± 0.12^{A}	No trend
Microsporidia-like organism, siphon	0^{a}	NA	No trend	6^{b}	3.00 ± 0.30^{A}	No trend	9^{c}	2.67 ± 0.26^{A}	No trend

F, fall; NA, not applicable; Sp, spring; Su, summer; W, winter. Different letters indicate significant differences in prevalence (lowercase) or intensity (uppercase) among sites ($P < 0.05$).

only in oysters, mussels, and cockles from Europe, Australia, California, and the eastern United States (Figueras et al. 1991, Comtet et al. 2003, Graczyk et al. 2006). Of the three MLO observed in geoducks in the current study, only those observed within oocytes (SLOs) were consistent morphologically with a known microsporidian genus observed previously in oocytes of some bivalve species. This parasite was morphologically similar to members of the genus *Steinhausia*, such as *Steinhausia mytiloyum*, which parasitizes oocytes of mussels (*Mytilus galloprovincialis*) (Figueras et al. 1991, Graczyk et al. 2006).

The other microsporidia-like parasites identified in geoduck intestine and siphon muscle do not have all the classic characteristics of microsporidia (Garcia 2002). Microsporidia are obligate intracellular protists that form spores (Garcia 2002). Like several other taxa, the life cycle of microsporidia includes an asexual reproduction (merogony) and sexual reproduction via the production of spores, with the infectious stage responsible for host-to-host transmission (Garcia 2002). Both of these stages were observed in geoducks. However, the two life stages were not always observed within the same individual. Of all geoducks examined with either intestinal or siphon muscle MLO parasites, nine were observed with both MLO life stages (7%). The intestinal MLO parasites in geoducks had a plasmodium-like morphology, which may represent meronts, whereas the siphon muscle MLO contained spore-like stages. Although the spores stained PAS positive, typical of microsporidia, they were not acid fast, one of the characteristics of the microsporidia taxon (Garcia 2002), suggesting that these parasites may belong to another taxon or are distantly related to known microsporidia. Both MLO parasites elicited a host inflammatory response in infected tissues; the potential of these parasites to influence host health in not known.

Seasonal fluctuations have been long known to influence endoparasite presence in marine hosts (Noble 1957, Rohde 1984, Couch 1985). Relatively high-intensity microsporidian infections were observed in geoduck siphons and intestinal epithelia year-round; no clear temporal or spatial environmental driver was detected. The greatest prevalence of SLO infections was observed in geoducks during colder months (February through May), whereas SLO parasites in warmer months were rarely observed. This observation is consistent with the annual oocyte maturation cycle in geoducks (Goodwin et al. 1979). Gametogenesis begins in spring months and peaks in June and July (Goodwin 1976, Sloan & Robinson 1984, Campbell & Ming 2003). The female spawning season is reported to be shorter compared with males, occurring August through October (Goodwin 1976); however, recent observations suggest that reproduction starts in late winter with evidence of spawning in March followed by simultaneous spawning of both male and female geoducks in Puget Sound in June and July (Friedman & Vadopalas, unpubl. data). Of geoduck cases with SLO parasites, infection intensity was generally low, possibly because of elimination by the host when oocytes are released during spawning. Vertical transmission of *Steinhausia* is suspected to occur in *Mytilus galloprovincialis*, which may explain the perpetuation of infection in the geoduck population year after year (Bower et al. 1994).

Spatial Distribution of Geoduck Parasites

The Puget Sound is a series of interconnected, fjord-type channels connected to the Northeast Pacific Ocean by the Strait of Juan de Fuca. This large estuarine environment has a massive

land–water interface with fluctuations in freshwater, organic matter, nutrients, and sediments from land and urbanized areas (Emmett et al. 2000). The sites selected for this study represent geoduck populations from two of the five major basins of the Sound—Thorndyke Bay (Hood Canal) and Totten Inlet (South Sound)—and one site from the Strait de Juan de Fuca: Freshwater Bay. Seawater conditions vary among these sites (Herlinveaux & Tully 1961, Thompson 1994, Newton et al. 2002, Moore et al. 2008).

Spatial differences in parasite communities were evident, especially between Freshwater Bay and Totten Inlet. Freshwater Bay and Totten Inlet exhibited the greatest differences in parasite abundance and infection intensity of the parasite taxa described in this study; although, in general, Thorndyke Bay exhibited intermediate parasite abundance and infection intensity. Intestinal MLO and metazoan parasites were observed in greatest prevalence at Totten Inlet (mean, 63%), and they showed the lowest abundance at Freshwater Bay (mean, 9%). In contrast, trends in RLO prevalence were the inverse of those observed for metazoan and intestinal microsporidia; Totten Inlet exhibited the lowest RLO prevalence (mean, 19%), whereas RLOs were commonly observed in Freshwater Bay (mean, 62%). Sample site did not influence the presence of the SLO, which was limited to reproductively active female geoducks regardless of site. Similarly, siphon muscle microsporidian parasites were generally of low prevalence or absent at all sites. Drivers of the distinct spatial patterns observed among the locations sampled in this study are unclear, but may be linked to environmental and hydrographic conditions unique to these locales.

In addition to physiological tolerances of these parasites to environmental variation, host density and spatial population aggregation can influence parasite dispersal in marine species (Blower & Roughgarden 1989). Geoducks are commonly found in discontinuous aggregate populations that vary in population density (Goodwin & Pease 1991), which could affect parasite ranges and distribution within Puget Sound. Furthermore, host factors such as feeding rate and diet may also contribute to the variation in parasite distribution and accumulation in filter-feeding bivalves (Ford & Tripp 1996, Ford et al. 1999).

CONCLUSIONS

The presence of several previously unreported parasites in Puget Sound geoducks was reported. Parasite presence in geoduck populations was influenced significantly by spatiotemporal differences in Puget Sound. Reasons for the differences in parasite assemblages may be attributed to host physiology and density, seasonality of infective stages of parasites, temperature shifts, or localized environmental factors (e.g., currents, freshwater input, mixing, nutrient availability) at each sampling location.

Parasite presence is ultimately dependent on both the environment of the host and the microenvironment of the parasite. Management of future disease outbreaks in geoducks will benefit from the baseline knowledge gathered in this study. To assess the potential risks of geoduck diseases more completely, continued exploration of individual parasite distributions, virulence, and physiological tolerances is needed. Gathering additional information about geoduck endosymbiont life cycles and host–parasite interactions can assist in future fishery management decisions regarding geoduck aquaculture and stock movement.

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