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Impact of temperature on an emerging parasitic association between a sperm-feeding scuticociliate and Northeast Pacific sea stars

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ABSTRACT

Global warming has important implications for the dynamics and ecological impacts of emerging diseases. We investigated temperature effects on scuticociliate, *Orchitophrya* cf. *stellarum*, infections in ripe testes of two Pacific northeast sea stars (*Asterina miniata* Brandt and *Pisaster ochraceus* Brandt) using laboratory and field approaches. We predicted that a small increase in temperature would result in higher ciliate growth rates and heightened infection intensities. To test this we (1) cultured free-living *O. stellarum* at 10 and 15 °C and quantified ciliate abundance after 3 days, and (2) housed sea stars of both species at 10 and 15 °C for durations varying from 4 to 21 days and then measured the infection intensity. Ciliate densities in cultures were two orders of magnitude higher in the warmer treatment. Infection intensity was also temperature sensitive: greater proportions of testes were infected and infection stage was more advanced at 15 versus 10 °C, leading to a reduction in spermatozoa and regression of the germinal layer within three weeks. In seven field populations surveyed, we found a tight linear correlation between infection prevalence (percent infected sea stars) and infection intensity (proportion of infected testes per individual and mean infection score). However, 45% of *P. ochraceus* testes exhibited heavy infections versus 8% of *A. miniata* testes, which may relate to the different thermal habitat of each species: *P. ochraceus* occurs higher on the shore and likely reaches higher body temperatures at low tide. While the sex ratio of *A. miniata* is unbiased, *P. ochraceus* populations are consistently female-biased and show no relationship to infection prevalence (ranged from 30 to 90%). *O.* cf. *stellarum* infections of testes of both sea stars are prevalent in field populations, are highly temperature sensitive, and lead to rapid loss of reproductive potential.

1. Introduction

Increased intensity and frequency of disease has been reported in a range of marine taxa over the past twenty years (Harvell et al., 1999; Ward and Lafferty, 2004; Lester et al., 2007). Climate change has been implicated as a critical agent driving the emergence of disease and several compelling examples show a positive association between increased environmental temperatures and the intensity of microbial infections in marine invertebrates. For example, the spread of withering foot syndrome and subsequent die-offs in abalone are correlated with warmer than average periods (Lafferty and Kuris, 1993; Raimondi et al., 2002). Growing evidence also indicates that climate variability strongly increases the sensitivity of marine species to parasitic diseases yielding community changes in response to shifts in the strength of species interactions, productivity and/or food web structure (e.g., Mouritsen and Poulin, 2002). In particular, the transmission and developmental rates of many parasites are positively related to temperature leading to higher prevalence (percent of the population with the infection), infection intensity and, at the extreme, host mortality (e.g., Harvell et al., 1999). For instance, Mouritsen et al. (2005) developed a simulation model that predicts a parasite-induced collapse in populations of coastal amphipods in response to a 3.8 °C increase in seawater temperature. However, empirical data quantifying temperature-related impacts on parasitic diseases are limited (e.g., Daszak et al., 2000).

Orchitophrya stellarum Cépède, 1907 is a ciliated protozoan (Order Scuticociliata) that infects sea stars (Family Asteriidae) and feeds on sperm when testes are ripe in multiple hosts from the North Atlantic and Pacific (e.g., Leighton et al., 1991; Claereboudt and Bouland, 1994; Byrne et al., 1997; Stickle et al., 2001b, 2007a). The presence of the parasite in the testes is linked to degeneration of the germinal tissue and reduced reproductive output. *O. stellarum* has recently been detected in several species along the Pacific coast of North America, leading Boom (1988) to hypothesize that it was inadvertently introduced to the Northeast Pacific in the late 1980s. Furthermore,

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Stickle et al. (2007b) found that the growth rate of the ciliate in culture conditions varied with temperature, indicating that the development of the infection in living hosts may also be temperature dependent. Thus, the sea star–ciliate association presents a unique opportunity to study the consequences of a temperature regime on an emerging infectious parasite in co-occurring hosts.

Among the Northeast Pacific hosts is the original keystone species (Paine, 1969), *Pisaster ochraceus* Brandt, 1835. Circumstantial evidence suggests that the consequences of the infection may be severe in *P. ochraceus* as inter-annual declines in the proportion of males have occurred following detection of the disease (Leighton et al., 1991), and recently surveyed populations are heavily female-biased (Bamfield, B.C.: Stickle and Kozloff, 2008). *O. stellarum*-like ciliates also infect the testes of a co-occurring species, *Asterina miniata* Brandt, 1835 (Sunday et al., 2008); this represents the first host record in Family Asterinidae.

The main objective of this study was to quantify the influence of temperature regime on the intensity of *O.* cf. *stellarum* infections in male *A. miniata* and *P. ochraceus*. We hypothesized that an increase in temperature will result in significantly higher ciliate growth rates and heightened infection intensities. To test this hypothesis we (1) cultured free-living *O. stellarum* at 10 and 15 °C and (2) housed sea stars of both species in sea water at these two temperatures, and then measured proportion of testes infected and infection stage. In addition, we quantified prevalence, population sex ratio and natural levels of infection intensity at seven sites in the field.

2. Materials and methods

2.1. Study location

A. miniata (>3.0 cm radius) and *P. ochraceus* (>8.0 cm radius) were collected or surveyed from locations near the Bamfield Marine Sciences Centre (Barkley Sound, British Columbia, Canada: 48°50′17″N, 125°08′26″W) from 0.2 to 1.0 m above astronomical tide as estimated by the Canadian Hydrographic Service chart datum during low tides from May to June 2008 (Fig. 1). In all cases, males were identified by examining the gonad through an incision (~1 cm in length) at the junction of two rays.

2.2. Laboratory experiments

2.2.1. In-vitro growth rates of ciliates at different temperatures

To obtain ciliates for in-vitro culture experiments, one infected testis was removed from three individuals of each species (collected from the Ross Islets on June 10). 1 µl of fluid from each parasitized testis was diluted with sperm-rich exudate from an uninfected testis (of the same species) to a final concentration of <3 ciliates ml^{-1}. 1 ml of this culture fluid was then transferred to eppendorf tubes: 6 replicate tubes per individual and 18 tubes total per species were included in each temperature treatment as follows. Eppendorf tubes were loosely capped and suspended in a 10 or 15 °C waterbath for 72 h. At 24-hour intervals, a 10 or 100 µl aliquot (depending upon ciliate density) was dispensed on a glass slide overlying a 0.01 mm grid. 1 µl of formalin was added to immobilize the ciliates for counts.

2.2.2. Infection progression at different temperatures

To quantify the influence of temperature on infection intensity of *O. stellarum* in sea stars, natural populations with ciliate infections were sub-sampled and experimental animals were held at temperatures coherent with what is measured in the field: 10 °C (ambient seawater temperature) and 15 °C (maximum temperature attained using a flow-through heating design). Animals were contained individually in 5 L PVC buckets. The inflow was directed through a 10 m length of Tygon tubing (1 cm diameter) coiled in a seawater bath maintained at 10 or 25 °C (a 25 °C waterbath achieved a 15 °C

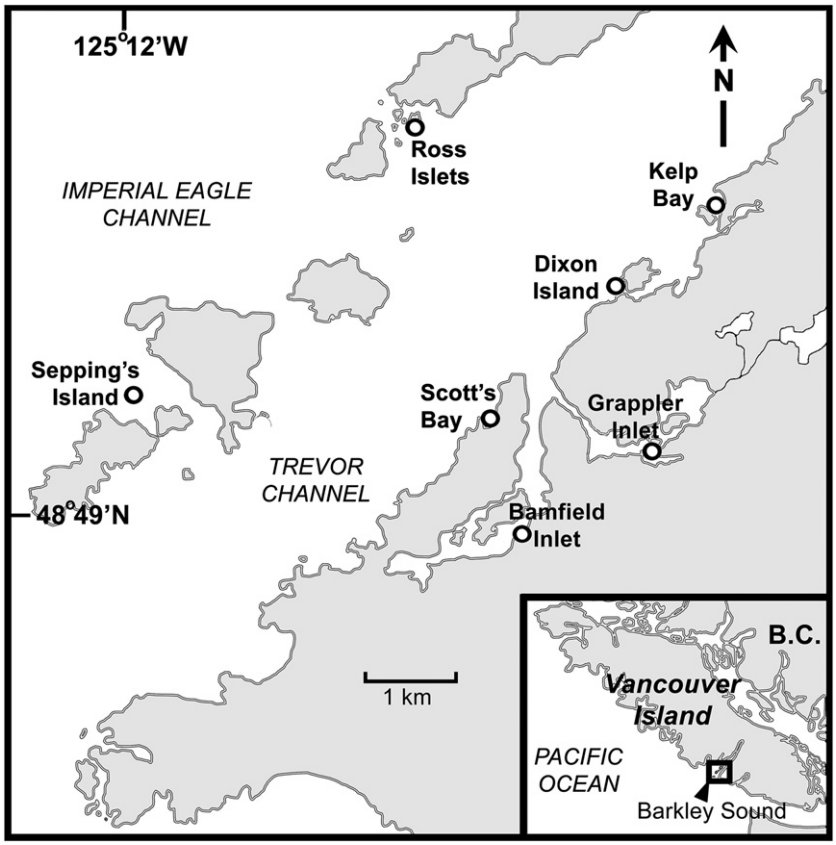

Fig. 1. Map showing rocky shore sampling localities in Barkley Sound on the west coast of Vancouver Island, British Columbia, Canada.

outflow temperature). Ambient or heated seawater was subsequently re-directed using y-junctions and tubing (0.25 cm diameter) to each bucket (as dictated by treatment type) at flows 0.1 ± 0.03 L min^{-1}. Air was bubbled into each bucket to minimize differences in the dissolved oxygen concentration between the two temperature treatments.

Two short-term experiments were conducted with *A. miniata* for 4 (June 6–10) and 6 days (May 30–June 5) (hereafter, A4: Scott's Bay, $n = 30$ and A6: Grappler Inlet, $n = 48$) and one with *P. ochraceus* for 4 days (June 11–15) days (hereafter, P4, Scott's Bay: $n = 32$). Males were identified in the field and then divided evenly into three groups. The first group, distinguished as the pre-experiment group, was dissected to quantify infection intensity parameters prior to the initiation of the experiments. The second and third groups were used in the 10 and 15 °C experimental treatments, respectively. In these experiments, >80% of sea stars were infected so we consequently included all animals in calculation of proportion testes infected.

We did not conduct long-term experiments with *P. ochraceus* because some of the animals held in the lab for longer than 4 days showed signs of flesh wasting disease (Eckert et al., 1999; Bates et al., 2009). However, *A. miniata* was healthy in laboratory conditions after 21 (May 5–26) days (hereafter, A21: Bamfield Inlet, $n = 48$). Because we were interested in temperature-dependent infection intensity, as well as the impact of the ciliates on testis condition, we selected a population with ~50% prevalence for experiments so that we had uninfected sea stars to control for natural change in testis mass over the experiment duration. Males were divided evenly between the 10 and 15 °C treatments and after 21 days animals were dissected and divided into two groups: infected or uninfected. Sample sizes for each treatment were as follows: 10 °C infected (7) or uninfected (9); 15 °C infected (8) or uninfected (8). At the completion of each experiment testes were removed from each animal and three response parameters were measured: proportion of testes infected, infection intensity and testis condition index.

2.2.3. Testis condition index

The duration of the 21-day temperature experiment allowed us to compare the testis condition of *A. miniata* with and without the parasite (testis mass is highly variable and is not a feasible parameter for short-term experiments). We randomly selected five intact testes per animal, dried these testes overnight (40 °C) in aluminum trays, and recorded the total mass using an analytical balance (±0.0001 g). This mass was then divided by five to obtain a mean. Because *A. miniata* radii (ranged from 4.0 to 6.0 cm) did not differ significantly among treatments (one-way ANOVA, $p > 0.05$), we report mean testis mass and do not standardize values by sea star size.

2.3. Field data

In the first week of June (2008), sites where both sea star species were abundant intertidally (0.2 to 1.0 m) were sampled: Bamfield Inlet, Dixon Island, Grappler Inlet, Kelp Bay, Ross Islets, Scott's Bay and Sepping's Island. Animals were sexed to obtain 15 males of each species at each locality; sex ratio was recorded (# males/# females). Males were transported to the lab and the testes were dissected from each individual and examined to quantify prevalence: # individuals infected with live ciliates/total # individuals examined with reproductive testes, and infection intensity.

2.4. Characterization of infection intensity

Two measures of infection intensity for each sea star included in experiments and field surveys were quantified: (1) proportion testes infected = # testes infected/total # testes examined, and (2) mean infection score = sum of the stage scores (1 to 5) for all infected testes/total # infected testes. Because we were interested in the state of infection within infected testes specifically, the latter score incorporates infected testes only and does not represent a mean score for all testes.

Testes were classed as ripe, spawned out or infected based on examination of the fluid exudate extracted from the testis tissue and qualitative observations of testis colouration and morphology determined visually under a light microscope. Sperm abundance and motility in the exudate were highly bimodal: either abundant and motile or rare and immotile. Because we examined 3600 testes in total it was not practical to count sperm and we opted to use qualitative descriptors of sperm abundance and motility (high or low). Ciliates were counted as described above.

Infected testes were further scored into 5 stages of the disease, identified prior to the study by preliminary surveys where testes from three locations were repeatedly sampled every two weeks from mid April to late May (2008). Stages 1 to 3 testes were white or yellow with white exudate that was packed with motile sperm. Ciliate density alone distinguished the first three stages. Ciliates were present in stage 1 testes at low densities (<1 ciliate μl^{-1} of testis exudate). Testes with higher ciliate densities were scored as stage 2 (1 to 99 ciliates μl^{-1} testis exudate) and stage 3 (>100). Stages 4 and 5 testes were separated from early infection stages (1 to 3) by exudate that was transulent or off-white in colour (e.g., brown or orange hued), markedly reduced sperm abundance and motility, and testes that were tan to dark brown in colour. Although the testis colouration and morphology were similar in both stages 4 and 5, ciliates were still present in testes scored as stage 4 (<100 μl^{-1} testis exudate), while live ciliates were not observed in stage 5 testes (although shriveled ciliate skeletons were sometimes observed: ~5% of cases). Histological sections confirmed whether patterns in sperm and ciliate abundance in the testis exudate were similar to patterns at the tubule scale (see Appendix A for histological methods and a description of the infection progression).

Stage 5 testes were distinguished from spawned out testes (*P. ochraceus* is spring-spawning (e.g., Farmanfarmaian et al., 1958)) by the following criteria: (1) colouration of the testis (tan to dark brown = stage 5, white to cream = spawned out) and exudate (stage 5 = transulent or off-white; spawned out = white), and (2) morphology (for a description of the effect of ciliate infections on the testes see Leighton et al., 1991: *P. ochraceus* and Sunday et al., 2008: *A. miniata*). Infection status was further verified by histological analyses (Appendix A). To be conservative, any suspected stage 5 testis that could not be scored with high confidence as infected was excluded from analyses.

2.5. Statistics

All statistical analyses were conducted in NCSS 2007 with alpha = 0.05. ANOVAs (data were log-transformed to meet assumptions of equal variance where required) tested for differences among treatments and interaction effects (where appropriate) in all laboratory experiments followed by Tukey–Kramer multiple comparisons tests. When assumptions of equal variance and normality were not met by transformation, a Kruskal–Wallis ANOVA on ranks test was followed by Dunn's multiple comparison tests. Pearson's Product Moment correlations tested for significant associations between prevalence, proportion testes infected and mean infection score in field populations of the two sea stars.

3. Results

3.1. In-vitro growth rate at different temperatures

Ciliates cultured from the testes of *A. miniata* and *P. ochraceus* (three isolates per species) exhibit temperature-dependent exponential growth (Fig. 4). The mean doubling time of ciliates isolated from both species is 7 h at 15 °C versus 24 h at 10 °C (Fig. 2). As a consequence, after 3 days, ciliate densities in cultures isolated from

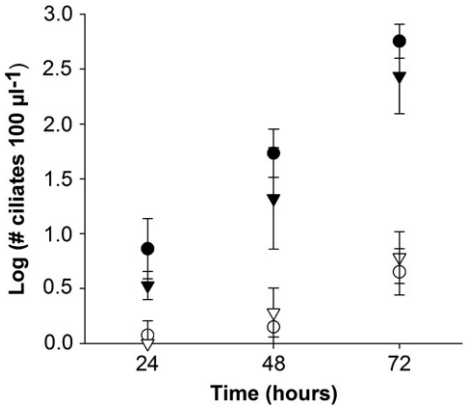

Fig. 2. Mean (±1 SD) density of ciliates in cultures measured daily at 10 °C (white) and 15 °C (black) on a log scale. Ciliates were isolated from *A. miniata* (circles) and *P. ochraceus* (triangles) testes. Growth is exponential: after 3 days ciliate densities are 2 orders of magnitude higher than at 15 vs. 10 °C. This leads to significantly higher densities of ciliates cultured at 72 h for both sea stars (see text for details on statistical analyses).

the two species are two orders of magnitude higher at 15 °C (*A. miniata* = (mean ± 1 SD $m1^{-1}$) 6046 ± 2277, *P. ochraceus* = 3637 ± 2503) than 10 °C (*A. miniata* = 50 ± 22, *P. ochraceus* = 68 ± 27) (two-way ANOVA on log-transformed data, temperature: $df = 1$, $F = 291.76$, $p < 0.000001$). Neither the effect of isolate nor the temperature × isolate interaction were significant (isolate: $df = 1$, $F = 0.71$, $p = 0.41$; interaction: $df = 1$, $F = 4.18$, $p = 0.054$).

3.2. Infection progression at different temperatures

Holding infected male sea stars at warmer seawater temperatures results in higher infection intensities in both species within 4 days. In all experiments conducted with *A. miniata* and *P. ochraceus*, the number of testes infected per individual is significantly higher in sea stars held at 15 °C in comparison to both the 10 °C treatment and pre-experiment group (Fig. 3A) (P4, A4, A6: Table 1 and A21: Kruskal–Wallis test: $H = 18.12$, $p = 0.038$). Mean infection score of *A. miniata* testes is also significantly higher at 15 °C after 6 days (Table 1) and 21 days (Kruskal–Wallis test: $H = 15.23$, $p = 0.038$) (Fig. 3B). Furthermore, after 21 days, the proportion of testes infected and mean infection score is significantly higher in both temperature treatments versus the pre-experiment group (identified by Dunn's multiple comparisons tests).

There was no difference in the testis mass of infected *A. miniata* housed at 10 °C for 21 days and uninfected sea stars held at 10 and 15 °C. However, the testis mass of infected *A. miniata* from the warm treatment is significantly reduced (Fig. 3C, Kruskal–Wallis test: $H = 8.41$, $p = 0.038$).

3.3. Field data

Prevalence of the ciliate parasite in co-occurring populations of the two sea stars ranged from 30 to 90%, did not differ between the two species (two-tailed paired *t*-test: $p = 0.41$), and exhibits a positive linear relationship (Fig. 4A). Prevalence is correlated with proportion of testes infected and infection score in both species (Fig. 4B and C). Thus populations with high infection prevalence are comprised of individuals with relatively high infection intensities. However, ciliate infections are more severe in *P. ochraceus*. The mean proportion of testes infected in *P. ochraceus* is significantly higher than in *A. miniata* (one-tailed paired *t*-test: $t = 3.01$, $p = 0.012$) (Fig. 4B). Mean infection score is also significantly higher in *P. ochraceus* in comparison to *A. miniata* from the same localities (one-tailed paired *t*-test: $t = 9.46$, $p = 0.000041$) (Fig. 4C).

Prevalence does not predict sex ratio in populations of either species in June (linear regression analysis for *P. ochraceus*: $r^2 = 0.12$, slope (±1SE) = 0.13 ± 0.15, $p = 0.44$ and *A. miniata*: $r^2 = 0.028$, slope = −0.061 ± 0.16, $p = 0.72$). *P. ochraceus* populations are highly female-biased for the seven sites surveyed in this study: mean (±1 SD) sex ratio (# males/# females) is 0.29 ± 0.06. In comparison, the sex ratio of *A. miniata* at the same sites is unbiased: 0.50 ± 0.08.

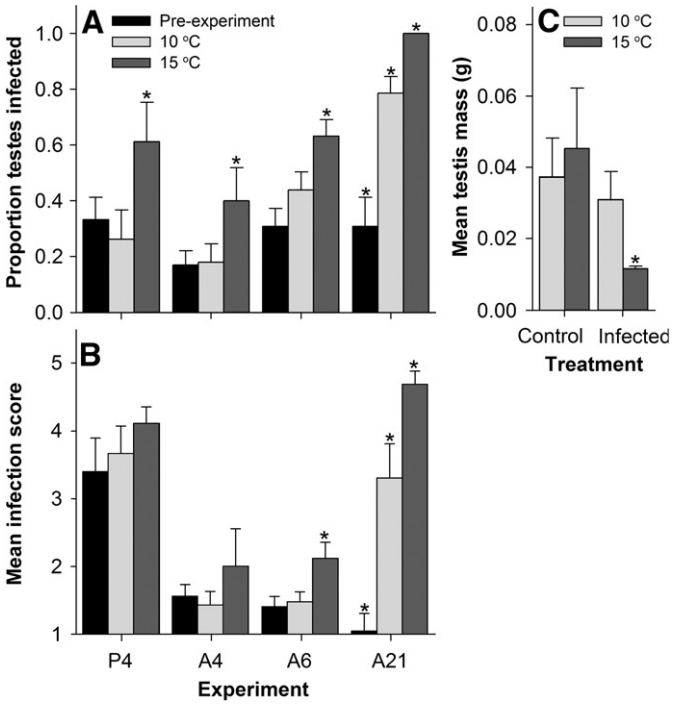

Fig. 3. (A) Mean (±1 SE) proportion testes infected and (B) mean (±1 SE) infection score in *A. miniata* and *P. ochraceus* initially (pre-experiment) and following exposure to 10 and 15 °C. Experiment duration in days is 4 (A4 and P4), 6 (A6) and 21 (A21). Stars indicate treatments where proportion testes infected and infection score are significantly ($p < 0.05$) different. Each experiment was analysed independently using a one-way ANOVA (P4, A4 and A6) or Kruskal–Wallis one-way ANOVA on ranks (A21): see Table 1 and text for results of statistical analyses. (C) Mean (±1 SD) testis mass (g) for healthy (control) and parasitized (infected) *A. miniata* after 21 days (A 21) in the two temperature treatments. Significantly different treatments are indicated by a star (see text for details on statistical analyses). Number of sea stars per treatment in each experiment: P4: 8, A4: 10, A6: 16 and A21: 7, 8 or 9.

4. Discussion

Multiple approaches in the field and lab indicate that thermal conditions influence the prevalence and intensity of *O.* cf. *stellarum* infections in the testes of intertidal *A. miniata* and *P. ochraceus*. The progression of the ciliate infection appears similar for the two sea star species and can proceed to completion within weeks: all of the testes of *A. miniata* housed at 15 °C displayed advanced stages of the

Table 1
One-way ANOVA results for infection progression at different temperatures.

			ss	df	Mean-square	F	p
Proportion testes infected	P4	Temperature	0.73	2	0.36	3.91	0.036
		Error	1.95	21	0.0093		
	A4	Temperature	0.73	2	0.37	5.17	0.013
		Error	1.92	27	0.0071		
	A6	Temperature	0.82	2	0.41	6.61	0.0031
		Error	2.71	44	0.0062		
Mean infection score	P4	Temperature	2.65	2	1.33	0.91	0.42
		Error	20.33	16	1.45		
	A4	Temperature	2.13	2	1.06	1.14	0.34
		Error	14.95	19	0.93		
	A6	Temperature	4.63	2	2.31	4.36	0.019
		Error	21.75	41	0.53		

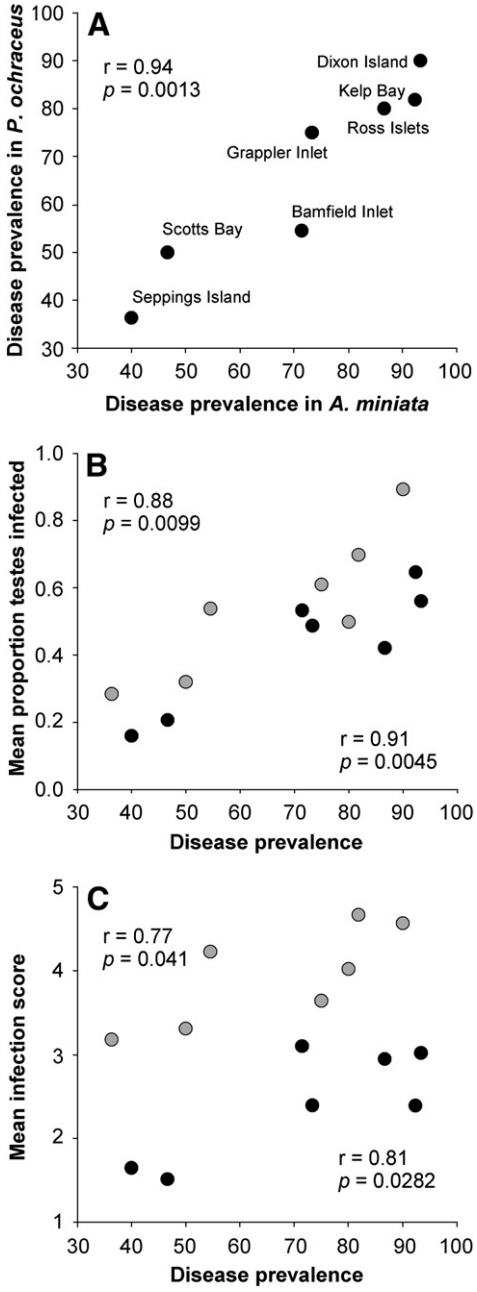

Fig. 4. (A) Prevalence of the ciliate infection in *A. miniata* and *P. ochraceus* is correlated at seven sites near Bamfield, B.C. (B) Proportion testes infected and (C) mean infection score are correlated with prevalence: *A. miniata* = black and *P. ochraceus* = gray. 15 specimens per species were sampled at each site; *r* = Pearson's product moment coefficient.

infection after 21 days. The ciliate increases in density within each tubule leading to a reduction in spermatozoa within the tubule lumen. At advanced stages of the disease live ciliates and motile sperm are rare or absent, spermatozoa show reduced abundance and the germinal epithelium is reduced, as has been reported for other sea star species (e.g., *Asteria*: Vevers, 1951; Bouland and Jangoux, 1988; Byrne et al., 1997; *Leptasterias*: Stickle et al., 2001a, 2007a). Thus, the infection can lead to partial or total loss of male reproductive potential in both species.

4.1. Temperature effects

The population doubling time of *O.* cf. *stellarum* is temperature dependent. When cultured at 10 and 15 °C in a sperm-rich seawater medium, ciliates reach two orders of magnitude higher densities at 15 °C. While we report growth rates of ciliates in culture at 15 °C that are comparable to the data of Stickle et al. (2007b) (on the order of 10000 ciliates ml^{-1} after 48 h), at 10 °C, ciliates displayed markedly lower growth rates in comparison to ciliates cultured at the same temperature by Stickle et al. (2007b). Differences in methodology may provide an explanation for why we measured highly reduced growth rates of ciliates at 10 °C. We provided ciliates with a sperm-rich media in capped eppendorf tubes (to simulate conditions within testes), while Stickle et al. (2007b) cultured ciliates on bacterized sea water in 10 ml vials with an airspace above the culture medium. However, why the two studies returned dissimilar ciliate growth rates at 10 °C but similar values at 15 °C remains a mystery and suggests that future studies should quantify the growth rate of ciliates over a range of temperatures with oxygen level and diet as factors to test for interaction effects.

The lower growth rate of the ciliate at 10 °C in cultures mirrors our results for temperature-related differences in the infection intensity of testes: the progression of the disease is highly temperature dependent. After only four days, the proportion of testes infected is significantly higher in both sea star species. Higher temperature also led to a more rapid development of the disease and in those testes that were infected: mean infection stage is significantly higher after six days at 15 versus 10 °C. The lack of significant difference in mean infection stage at the two temperatures after 4 days likely reflects the time it takes for newly infected testes to reach later stages. Our data further show that warmer temperatures can result in highly reduced reproductive output after 3 weeks as all the infected *A. miniata* included in the 15 °C treatment displayed advanced stages of the ciliate infection.

While laboratory results on temperature effects on the ciliate's growth rate and infection of testes provide an excellent basis for formulating hypotheses, the exact role of thermal variation in driving natural patterns in disease prevalence and intensity in the field remains poorly understood. However, there is some evidence that spatial and temporal patterns in prevalence of the ciliate may relate to thermal conditions. Winter spawning *Leptasterias* spp. are less severely impacted than co-occurring spring-spawning species (Stickle and Kozloff, 2008), potentially due to warmer seawater temperatures when ciliates infect and grow in the testes. Furthermore, Stickle and Kozloff (2008) found that the prevalence of *O. stellarum* infections is minimal in Alaska in comparison to California and Washington localities (for *Evasterias troschelii, Leptasterias* spp. and *P. ochraceus*), probably because winter minimal seawater temperatures fall near to or below the ciliate's low temperature limit (3 °C: Stickle et al., 2007b). Temperature may also explain infection intensity differences between intertidally foraging *P. ochraceus* and subtidally foraging *A. miniata* (see next section). Finally, shifts in the prevalence of the ciliate may relate to temporal changes in the thermal regime. For example, *Asteria amurensis* re-sampled for reproductive studies over many decades from one location in Tokyo Bay (Japan) suddenly dropped in testes output in the early 1990s and showed 100% infection prevalence of the ciliate in 1996 during its winter spawning period (Byrne et al., 1997). Interestingly, a temperature increase in the winter sea water temperature (note that *A. amurensis* is winter spawning) of 1.5 °C per decade occurred from 1976 to 1997 as a consequence of diverted freshwater runoff (Yanagi, 2008): this temperature increase corresponds to the first detection of the ciliate in Tokyo Bay.

4.2. Prevalence and infection intensity

Prevalence of *O.* cf. *stellarum* was high in field populations of *A. miniata* and *P. ochraceus*, ranging from ~30 to 90% and was similar for the two species on a site-by-site basis. This result was unexpected, because the overall availability of testes with sperm was higher in

A. miniata versus *P. ochraceus* (mean proportion gonads with sperm, respectively: 0.86 and 0.35). Many of the *P. ochraceus* we examined displayed signs that the ciliate infection had proceeded to completion and testes had very low sperm abundance. Furthermore, *P. ochraceus* was at the end of its spawning period in June (e.g., sea stars were observed with spawned out testes), while *A. miniata* was fully reproductive at this time (spawned out testes were not observed). Stickle and Kozloff (2008) also observed similar prevalence values for male *Evasterias troschelii* and *P. ochraceus* collected at the same time and location (respectively, 54 and 59% at a Washington location and 7 and 11% at an Alaskan location). Thus, the ciliate is probably not selecting one host species over the other and appears to be opportunistic in nature.

Prevalence and infection intensity are correlated, suggesting a link in the mechanism driving transmission among individuals at a population level and among testes at an individual level. While the mode of transmission for *O. stellarum* is currently unknown, the ciliate is found on the aboral surfaces of both male and female asteroid sea stars from Pacific northeast locations throughout the year (Stickle and Kozloff, 2008) and also thrives in culture conditions (Stickle et al., 2007a). Ciliates probably enter the testis through the gonopore because infected testes are not always adjacent to one another and the number and extent of the damage among parasitized testes varies from one male sea star to the next (Burrows, 1936; Vevers, 1951; Bouland and Jangoux, 1988; Goggin and Bouland, 1997; this study). We observed ciliates at high densities (>5000 individuals ml^{-1}: stage 3 infections) emerging in the spawn that can presumably infect other animals or testes of the same animal. Therefore the link between prevalence and infection intensity reported here may simply reflect the growth rate of the ciliate *in hospite*: when ciliate densities are high, there is an increased probability that nearby testes on the same or different sea stars (regardless of species) will become infected.

While the prevalence of the infection is similar for the two species, infection intensities are more severe in *P. ochraceus*: both the number of parasitized testes and infection scores are higher. Although it is possible that our estimates of late stage infections are biased because we incorrectly scored spawned out testes as stage 5, spawned out testes have distinct characteristics in histological sections that are consistent with our observations of intact testes. For instance, tubule diameter is reduced and cell layers are structured, as occurs for testes in post-spawning recovery stages in other echinoderm species (e.g., *Paracentrotus lividus*: Byrne, 1990). Furthermore, when stage 5 testes are removed from analyses, *P. ochraceus* infection intensity scores remain higher than *A. miniata*. Because prevalence is similar for the two species and *A. miniata* held at 15 °C for three weeks were heavily parasitized, we hypothesize that the growth rate of the ciliate is faster in field populations of *P. ochraceus*. As we observed a strong relationship between temperature and ciliate growth rates in lab experiments, this result may relate to the respective thermal niche of each species. *P. ochraceus*, which forages intertidally, likely experiences higher mean and maximum body temperatures than *A. miniata*, which forages subtidally.

4.3. Consequences of infection

By holding *A. miniata* with uninfected and infected testes at 10 and 15 °C for three weeks, we show that late infection stages display a significantly lower testis mass, presumably due to a reduction in spermatozoa and germinal epithelium and drop in abundance of ciliates. Whether testes regain reproductive potential following heavy ciliate infestations is currently unknown. Nevertheless, the reduced testes output in 30 to 90% of both sea star species at different locations leads to the prediction that reproductive success may be limited at local scales on a seasonal basis, as has been suggested for other sea stars infected by *O. stellarum* (e.g., Claereboudt and Bouland, 1994; Byrne et al., 1997). A large body of research indicates that successful fertilization in free spawning invertebrates decreases markedly at low sperm concentrations (reviewed Levitan and Peterson, 2000). In fact, Vevers (1951) attributed significant reductions in recruitment of *Asteria rubens* in the years following heavy infestation of males (20%) by *O. stellarum*. However, the impact of the ciliate on recruitment success of the Pacific Northeast sea stars remains to be determined.

While there has been no direct evidence that the ciliate causes host mortality, several authors report female-biased sex ratios in populations where the ciliate occurs (Leighton et al., 1991; Claereboudt and Bouland, 1994; Byrne et al., 1997; Stickle et al., 2001b; Stickle and Kozloff, 2008). In contrast, we observed an unbiased sex ratio in *A. miniata*, even in populations with high ciliate infection intensities. In comparison, *P. ochraceus* is heavily female-biased at the seven sampled locations, although variation in sex-ratio does not relate to infection intensity. It is possible that differential habitat use in space or time by the sexes can explain the skewed sex ratios observed in *P. ochraceus*. For example, Kim (1968) observed seasonal variation from 36 to 75% in the percentage of males in a population of uninfected *Asteria amurensis*. Repeated sampling at one locale indicated no change in the sex ratio of *A. amurensis* in 30 years, despite the presence of the ciliate (Goggin and Bouland, 1997). While it is also possible that cumulative mortality from previous years may be causing male-biased mortality in *P. ochraceus*, it remains an open question whether the ciliate directly causes mortality. In fact, *Asteria forbesi* (northwest Atlantic) can recover from ciliate infections within days in the laboratory (Bang, 1976).

4.4. Implications

This study presents experimental evidence that *O.* cf. *stellarum* infections of *P. ochraceus* and *A. miniata* testes are highly temperature sensitive. Thus, short-term elevated air temperatures (e.g., summer low tides occur during the day on the west coast of Vancouver Island: Helmuth et al., 2002; Pincebourde et al., 2008) or periods of upwelling relaxation (Sanford, 1999) could lead to a severe reduction in the reproductive output of sea stars infected with ciliates resulting in reduced fertilization success for populations of sea stars at local scales. Our results emphasize that occasional, short-duration thermal stress events can have important long-term consequences for benthic marine populations (Harley and Paine, 2009). The high disease prevalence and intensities reported for Pacific Northeast sea stars provides a platform for researchers to examine the consequences of a temperature-sensitive emerging infectious disease in multiple hosts, each host occurring in a different thermal regime.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jembe.2009.12.001.

References

Bang, F.B., 1976. Disease processes in seastars: a metchnikovian challenge. Biol. Bull. 162, 135–148.

Bates, A.E., Hilton, B., Harley, C.D.G., 2009. Effects of temperature, season and locality on wasting disease in the keystone predatory sea star, *Pisaster ochraceus*. Dis. Aquat. Org. 86, 245–251.

Boom, J.D.G., 1988. Actin gene transcription during spermatogenesis in the sea star *Pisaster ochraceus* (Brandt). Masters thesis, Simon Fraser University, British Columbia.
Bouland, C., Jangoux, M., 1988. Infestation of *Asteria rubens* (Echinodermata) by the ciliate *Orchitophrya stellarum*: effect on gonads and host reaction. Dis. Aquat. Org. 5, 239–242.
Burrows, R.B., 1936. Further observations on parasitism in the starfish. Science 84, 329.
Byrne, M., 1990. Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. Mar. Biol. 104, 275–289.
Byrne, M., Cerra, A., Nishigaki, T., Hoshi, M., 1997. Infestation of the testes of the Japanese sea star *Asteria amurensis* by the ciliate *Orchitophrya stellarum*: a caution against the use of this ciliate for biological control. Dis. Aquat. Org. 28, 235–239.
Claereboudt, M.R., Bouland, C., 1994. The effect of parasitic castration by a ciliate on a population of *Asteria vulgaris*. J. Invert. Pathol. 63, 172–177.
Daszak, P., Cunningham, A.A., Hyatt, A.D., 2000. Emerging infectious diseases of wildlife-threats to biodiversity and human health. Science 287, 443–449.
Eckert, G.L., Engle, J.M., Kushner, D.J., 1999. Sea star disease and population declines at the Channel Islands. Proc. 5th California Island Sym. 5, 390–393.
Farmanfarmaian, A., Giese, A.C., Boolootian, R.A., Bennett, J., 1958. Annual reproductive cycles in four species of West Coast starfishes. J. Exp. Zool. 138, 358–367.
Goggin, C.L., Bouland, C., 1997. The ciliate *Orchitophrya* cf. *stellarum* and other parasites and commensals of the northern pacific seastar *Asteria amurensis* from Japan. Int. J. Parasitol. 27, 1415–1418.
Harley, C.D.G., Paine, R.T., 2009. Contingencies and compounded rare perturbations dictate sudden distributional shifts during periods of gradual climate change. Proc. Natl. Acad. Sci. U. S. A. 106 (27), 11172–11176.
Harvell, C.D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R., Grimes, D.J., Hofmann, E.E., Lipp, E.K., Osterhaus, A.D.M.E., Overstreet, R.M., Porter, J.W., Smith, G.W., Vasta, G.R., 1999. Emerging marine diseases — climate links and anthropogengic factors. Science 285, 1505–1510.
Helmuth, B., Harley, C.D.G., Halpin, P.M., O'Donnell, M., Hofman, G.E., Blanchette, C.A., 2002. Climate change and latitudinal patterns of intertidal thermal stress. Science 298, 1015–1017.
Kim, Y.S., 1968. Histological observations of the annual change in the gonad of the starfish, *Asterias amurensis* Lütken. Bull. Fac. Fish. Hokkaido Univ. 19, 97–108.
Lafferty, K.D., Kuris, A.M., 1993. Mass mortality of abalone *Haliotis cracherodii* on the California Channel Islands: tests of epidemiological hypothesis. Mar. Ecol. Prog. Ser. 96, 239–248.
Leighton, B.J., Boom, J.D.G., Bouland, C., Hartwick, E.B., Smith, M.J., 1991. Castration and mortality in *Pisaster ochraceus* parasitized by *Orchitophrya stellarum* (Ciliophora). Dis. Aquat. Org. 10, 71–73.
Lester, S.E., Tobin, E.D., Behrens, M.D., 2007. Disease dynamics and the potential role of thermal stress in the sea urchin, *Strongylocentrotus purpuratus*. Can. J. Fish. Aquat. Sci. 64, 314–323.
Levitan, D.R., Peterson, C., 2000. Sperm limitation in the sea. Trends Ecol. Evol. 10, 228–231.
Mouritsen, K.N., Poulin, R., 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology 124, S101–S117.
Mouritsen, K.N., Tompkins, D., Poulin, R., 2005. Climate warming may cause a parasite-induced collapse in coastal amphipod populations. Oecologia 146, 476–483.
Paine, R.T., 1969. A note on trophic complexity and community stability. Am. Nat. 103, 91–93.
Pincebourde, S., Sanford, B., Helmuth, B., 2008. Body temperature during low tide alters the feeding performance of a top intertidal predator. Limnol. Oceanogr. 53, 1562–1573.
Raimondi, P.T., Wilson, C.M., Ambrose, R.F., Engel, J.M., Minchinton, T.E., 2002. El Niño and the continued declines of black abalone along the coast of California. Mar. Ecol. Prog. Ser. 242, 143–152.
Sanford, E., 1999. Regulation of keystone predation by small changes in ocean temperature. Science 283, 2095–2097.
Stickle, W.B., Kozloff, E.N., 2008. Association and distribution of the ciliate *Orchitophrya stellarum* with asteriid sea stars on the west coast of North America. Dis. Aquat. Org. 80, 37–43.
Stickle, W.B., Weidner, E.H., Kozloff, E.N., 2001a. Parasitism of *Leptasterias* spp. by the ciliated protozoan *Orchitophrya stellarum*. Invertebr. Biol. 120, 88–95.
Stickle, W.B., Rathbone, E.N., Story, S., 2001b. Parasitism of sea stars from Puget Sound, Washington, by *Orchitophyra stellarum*. In: Barker, M. (Ed.), Echinoderms 2000. Swets and Zeitinger, Lisse, pp. 221–226.
Stickle, W.B., Kozloff, E.N., Henk, M.C., 2007a. The ciliate *Orchitophrya stellarum* viewed as a facultative ciliate parasite of asteriid sea stars. Cah. Biol. Mar. 48, 9–16.
Stickle, W.B., Kozloff, E.N., Story, S., 2007b. Physiology of the ciliate *Orchitophrya stellarum* and its experimental infection of *Leptasterias* spp. Can. J. Zool. 85, 201–206.
Sunday, J., Raeburn, L., Hart, M.W., 2008. Emerging infectious disease in sea stars: castrating ciliate parasites in *Asterina*. Dis. Aquat. Org. 81, 173–176.
Vevers, H.G., 1951. The biology of *Asteria rubens* L. II. Parasitization of the gonads by the ciliate *Orchitophrya stellarum* Cépède. J. Mar. Biol. Soc. UK 29, 619–624.
Ward, J.R., Laffarty, K.D., 2004. The elusive baseline of marine disease: are diseases in ocean ecosystems increasing? PLoS Biol. 2, 542–547.
Yanagi, T., 2008. Great water temperature changes of 1.5 °C per decade in Tokyo Bay, Japan — its causes and consequences. J. Disas. Res. 3, 113–114.