

# Variable intertidal temperature explains why disease endangers black abalone

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**Abstract.** Epidemiological theory suggests that pathogens will not cause host extinctions because agents of disease should fade out when the host population is driven below a threshold density. Nevertheless, infectious diseases have threatened species with extinction on local scales by maintaining high incidence and the ability to spread efficiently even as host populations decline. Intertidal black abalone (*Haliotis cracherodii*), but not other abalone species, went extinct locally throughout much of southern California following the emergence of a Rickettsiales-like pathogen in the mid-1980s. The rickettsial disease, a condition known as withering syndrome (WS), and associated mortality occur at elevated water temperatures. We measured abalone body temperatures in the field and experimentally manipulated intertidal environmental conditions in the laboratory, testing the influence of mean temperature and daily temperature variability on key epizootiological processes of WS. Daily temperature variability increased the susceptibility of black abalone to infection, but disease expression occurred only at warm water temperatures and was independent of temperature variability. These results imply that high thermal variation of the marine intertidal zone allows the pathogen to readily infect black abalone, but infected individuals remain asymptomatic until water temperatures periodically exceed thresholds modulating WS. Mass mortalities can therefore occur before pathogen transmission is limited by density-dependent factors.

**Key words:** *fade out; Haliotis cracherodii; infectious disease; local extinction; microclimate; temperature variation; time lag.*

## INTRODUCTION

Epidemiological theory holds that host-specific infectious diseases will rarely drive hosts to extinction because transmission eventually breaks down due to a decline in susceptible hosts (Anderson and May 1992, de Castro and Bolker 2005). This phenomenon, known as epizootic fade out, implies that a disease will die out when the host population falls below a threshold density (Lloyd-Smith et al. 2005). Accordingly, infectious diseases have played only a minor role in global species loss, and have rarely provided the sole cause of threat for species listed under either the U.S. Endangered Species Act or IUCN Red List of Threatened Species (Smith et al. 2006). Yet there are cases of infectious diseases threatening species with extinction on local scales (McCallum et al. 2009). In these cases, pathogens maintain high incidence and the ability to spread efficiently even as the susceptible host population declines, either through frequency-dependent transmission or the presence of a reservoir host species (de Castro and Bolker 2005). Here, we consider a less-appreciated pathway to host extinction, a disease that

spreads rapidly through a tolerant host population followed by host mortality linked to an environmental stressor.

Beginning in the mid-1980s, abundant populations of intertidal black abalone (*Haliotis cracherodii*) experienced widespread declines and localized extinctions throughout southern and central California following the emergence of a Rickettsiales-like organism (WS-RLO) and its associated disease known as withering syndrome (WS). This disease has been observed in all five southern California abalone species (Friedman et al. 2002) and contributed to the closure of the southern California abalone fishery. Although all California abalone species are susceptible to WS-RLO infection, black abalone have shown the highest susceptibility to disease-induced population declines, and WS was the cardinal threat leading to the listing of black abalone to the U.S. Endangered Species List (Neuman et al. 2010). As black abalone have disappeared, the intertidal communities they once dominated have changed significantly, and perhaps irreversibly, from open space and calcareous algae, to increased cover of sessile invertebrates and sea urchins (Miner et al. 2006). Remaining populations of black abalone have failed to recover since initial WS epizootics, yet the disease agent continues to infect susceptible hosts and remains present in seawater throughout the Southern California Bight (T. Ben-

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Horin, unpublished data). These factors suggest that the incidence of WS-RLO infection is maintained even as black abalone are driven toward extinction.

Black abalone population declines correspond with warm temperatures. Transmission appears to occur in warm and cold years alike (Lafferty and Kuris 1993), but exposure to elevated ocean temperatures over a period of months to years increases the expression and severity of WS (Braid et al. 2005, Vilchis et al. 2005, Moore et al. 2011), and mass mortalities happen faster during warmer years (Lafferty and Kuris 1993, Raimondi et al. 2002). Although critical temperatures driving the expression of WS vary by abalone species (Moore et al. 2000, 2009, Vilchis et al. 2005), the clinical signs of WS diminish in cooler climates, even in abalone maintaining moderate WS-RLO infections (Moore et al. 2000, 2011, Friedman and Finley 2003). As a result, the initial disease-induced mass mortalities of black abalone followed a prolonged period of elevated sea surface temperature associated with the strong El Niño-Southern Oscillation (ENSO) of 1982–1983 (the initial source of the pathogen is unknown).

It remains a mystery why black abalone populations are particularly susceptible to WS-induced declines. Here, we hypothesize that the environmental conditions of the intertidal zone, specifically its temperature regime, enhance the impact of WS on black abalone populations relative to conditions in the subtidal. The geographic range of black abalone overlaps with all other California abalone species, but black abalone are unique because they occur primarily in rocky intertidal habitats: all other southern California abalone species may infrequently inhabit the intertidal zone but are primarily found in subtidal habitats (Cox 1962). Why black abalone evolved to inhabit primarily the intertidal has not yet been tested. A key distinction of intertidal microclimates, particularly in temperate regions, is the daily fluctuation in temperature driven by the semi-diurnal rising and falling of tides (Helmuth et al. 2006). Such temperature changes can be extreme, and short-term changes in temperature have shown to dramatically influence the dynamics of temperature-sensitive diseases, particularly diseases of small-bodied, ectotherm hosts (Raffel et al. 2006), and mosquito-transmitted diseases such as malaria and dengue fever (Paaijmans et al. 2009, Lambrechts et al. 2011). Given this, we considered the independent effects of the mean body temperatures abalone experience, and daily temperature variability on WS in black abalone.

To address the influence of mean body temperature and daily temperature variability on key epizootiological processes of WS, we quantified abalone body temperatures at field sites where abalone were previously abundant but are now rare, and these data were used to identify the appropriate temperature regime to recreate in the laboratory. We then mimicked intertidal environmental conditions in controlled mesocosms to manipulate the body temperatures of abalone exposed

to WS-RLO and examined the role of stable and variable thermal conditions in local extinctions of black abalone throughout southern California.

## METHODS

### *Study system*

Withering syndrome is caused by infection with WS-RLO, which occurs as intra-cytoplasmic inclusions of gastrointestinal epithelial cells in the posterior esophagus, digestive gland and, to a lesser extent, intestine (Friedman et al. 2000). WS is manifested as morphological changes to the digestive gland, including degeneration and metaplasia of the digestive tubules, accompanied by anorexia, depletion of glycogen reserves, and a loss in body mass and subsequent death (Friedman et al. 2000, Braid et al. 2005).

As with most gastrointestinal pathogens, transmission is likely to be via a fecal-oral route. Nearly all terrestrial Rickettsiales and Rickettsiales-like prokaryotes are obligate endosymbionts and cannot survive even transient desiccation (Warner et al. 2010), however, studies of WS-RLO in abalone suggest this pathogen can survive in seawater (Friedman et al. 2002). Consequently, the transmission of WS-RLO is likely to occur by direct contact with infectious abalone and through exposure to contaminated seawater.

### *Field temperatures*

To determine the fine-scale (i.e., microclimatic) variation in temperature experienced by wild abalone, we measured the body temperatures black abalone might attain in intertidal microhabitats (i.e., fully exposed rock and crevices located both in and out of the splash zone) by randomly placing 62 thermally matched temperature loggers at four rocky intertidal sites (Willows Anchorage and Forney Cove on Santa Cruz Island, Point Sierra Nevada and Rocky Point along the central California coast; Appendix A: Fig. A1). Details of the design of the temperature loggers are described in Appendix A. Temperature loggers were deployed from fall 2009 to summer 2010, and were programmed to record temperature every 30 minutes. Temperature variability was quantified by describing the maximum range in daily temperature fluctuations. Daily maximum and minimum temperatures were extracted from time histories of natural temperatures, and the difference was used as an independent observation of temperature variability. Temperature variability observations from each logger were described by exponential distributions, and the daily temperature range (DTR) of each logger was defined as the 95th percentile of the fitted distributions. We chose this metric to approximate the magnitude of extremes in body temperature and to describe the frequency and time history of these events. We determined the longer-term seasonal and interannual variation in sea surface temperatures off western Santa Cruz Island, California, from 1981 to 2006 using data compiled by the AVHRR Pathfinder Version 5.2

SST Project and acquired from the NOAA National Oceanographic Data Center (Casey et al. 2010; data available online).<sup>4</sup>

#### Laboratory experiment

Adult *Haliotis cracherodii* were collected from intertidal rocky reefs south of Carmel, California (36.54° N, 121.89° W). WS-RLO had been detected in that region and we found abalone collected from this region to be susceptible to WS (T. Ben-Horin, unpublished data). Abalone were transported to the University of California, Santa Barbara and treated with the antibiotic oxytetracycline (Acros Organics, Geel, Belgium) by water bath (T. McCormick, unpublished protocol) to eliminate any preexisting WS-RLO infections. All abalone were held in a 155-L fiberglass tank with ambient flow-through seawater for two months following antibiotic treatment. All individuals tested negative for WS-RLO infection at the start of the experiment.

Sixty experimental tanks were constructed to mimic subtidal and intertidal environmental conditions. We used a 2 × 2 × 2 full factorial experiment with water temperature, tide, and substrate as factors. Complete details of our experimental design are provided in Appendix B. Briefly, we manipulated the subtidal thermal environment by chilling and warming incoming seawater in cool and warm water temperature treatments (water temperature effect). We produced daily temperature fluctuations by manipulating tidal exchange in intertidal treatments within both water temperature treatments (tide effect), and we manipulated exposure to radiative heating (from infrared heat lamps) and cooling at low tide by providing and withholding protective substrate (masonry blocks; substrate effect).

We used incoming seawater as the source of exposure to WS-RLO. We have detected new incidences of WS-RLO and clinical signs of WS in quarantined black abalone previously held at our facility (T. Ben-Horin, unpublished data). Individual abalone were weighed and measured and randomly assigned to the 60 tanks ( $N = 1$  abalone per tank) and each tank was equipped with a thermally matched temperature logger programmed to record temperature hourly. Loggers were affixed in the experimental tanks, immediately adjacent to the settled position of each black abalone, with a thin layer of heat-conductive paste. Loggers were repositioned if live abalone moved. We used two measures to describe the temperature regime in each mesocosm: mean body temperature (MBT) and daily temperature range (DTR). We defined MBT as the mean of all hourly temperature observations. DTR, defined previously, described the frequency and magnitude of daily temperature variability.

One week following assignment to laboratory tanks, two 200-mg samples of expelled feces were collected

from each abalone and processed using a QIAamp Stool Sample Kit (QIAGEN, Venlo, Netherlands). We modified the manufacturer's protocol to account for low WS-RLO DNA yield by eluting DNA with 100 µL Buffer AE (QIAGEN, Venlo, The Netherlands). Infection with WS-RLO was determined using the PCR assay described by Andree et al. (2000). All abalone tested negative for WS-RLO infection at this time. Each tank was examined at least once daily throughout the experiment and all individuals were fed freshwater-rinsed *Macrocystis pyrifera* and *Egretta menziensi*, collected offshore our aquaria, once per week. We have not detected WS-RLO to be associated with *M. pyrifera* and *E. menziensi* collected from this region (Appendix B). At one month intervals, all abalone were weighed and measured and samples of expelled feces were collected and processed for PCR of eluted DNA. The experiment was terminated after the 12th monthly sample was collected (day 376), and all abalone were treated with the antibiotic oxytetracycline and returned to a 155-L flow-through tank with ambient seawater.

All PCR reactions were run in duplicate with positive and negative controls. We used WS-RLO positive tissue and fecal samples as positive controls and DNA-free water as a negative control. We defined WS-RLO infection as two positive PCR amplifications in each of the two fecal samples taken from each tank monthly. The PCR assay is a presumptive test of WS-RLO infection and does not indicate the presence of live bacteria or actual infection. However, presumptive tests are commonly used as a proxy for infection (Burreson 2008, Burge et al. 2011). All PCR reactions with discrepancies in either sample or tank replicates were rerun, and we were able to classify all discrepancies as false positives or false negatives. We defined false positives as one or more but less than four positive PCR reactions among the initial sample and tank replicates, but four negative PCR reactions in the subsequent sample and tank replicates. False negatives were one or more but less than four negative PCR reactions among the initial sample and tank replicates, but four positive PCR reactions in the subsequent sample and tank replicates.

#### Statistical analysis

Analysis of variance (ANOVA) was used to assess the effects of water temperature, tide, substrate, and their interaction on MBT and DTR. We used a post hoc ANOVA contrast to test the effect of substrate on MBT and DTR in subtidal and intertidal tanks. A Cox proportional hazard model was used to relate the time until WS-RLO infection to MBT and DTR, as well as the experimental factors (tide and substrate) and initial body mass. We did this to allow for interpretations of our results in the context of temperature variability estimated from the field observations, as well as account for the unforeseen effects of emersion, initial body mass, and other unforeseen experimental artifacts. We esti-

<sup>4</sup> <http://www.nodc.noaa.gov/SatelliteData/pathfinder4km/>

mated the hazard ratio for each covariate, calculated as  $e^{\beta}$ , where  $\beta$  is the regression coefficient for each covariate. Here, the hazard ratio described the change in risk of WS-RLO infection over the study period, given a one-unit increase in MBT, DTR, and initial body mass. For the categorical covariates tide and substrate, the hazard ratio described the change in risk of WS-RLO infection between the two groups within each treatment. We used logistic regression to assess the influence of MBT and DTR on the final prevalence of WS-RLO infection.

We described clinical signs of WS as the loss in body mass, proportional to initial body mass, over the study period. Exposure to elevated water temperatures alone does not result in biochemical changes to the foot muscle nor a subsequent loss in body mass, particularly over the range of mean temperatures explored here (Rosenblum et al. 2005), justifying the use of this metric as a description of disease expression. The effect of MBT and DTR on clinical signs of WS was evaluated using linear regression, while including the experimental factors (tide and substrate) and initial body mass as covariates. All analyses were conducted using Matlab (Version R2010a; The Mathworks, Natick, Massachusetts, USA).

## RESULTS

Hourly abalone body temperature observations varied from 9.86°C to 19.02°C in the cool subtidal tanks and 11.91°C to 20.14°C in the warm subtidal tanks. MBT ranged from 12.11°C to 12.17°C in cool tanks and 14.10°C to 14.25°C in the warm tanks (Fig. 1A). Hourly body temperature observations in the intertidal tanks displayed greater variability, ranging from 7.77°C to 24.32°C in the cool tanks (Fig. 1B and C) and 7.81°C to 26.28°C in the warm tanks (Fig. 1D and E). MBT differed between warm and cool tanks (Appendix C: Fig. C1; three-way ANOVA,  $F_{1,53} = 23\,992.88$ ,  $P < 0.001$ ; Table C2A), however, tide and substrate availability did not significantly influence MBT (Fig. C1; three-way ANOVA, in both cases  $P \geq 0.10$ ; Table C2A) and we did not observe significant interaction between the factors. The MBT (mean  $\pm$  SE) recorded in the cool tanks was  $12.14 \pm 0.02^\circ\text{C}$  and  $14.18 \pm 0.03^\circ\text{C}$  in the warm tanks, and we classified MBT as cool or warm for all subsequent analyses. DTR was influenced by tide (three-way ANOVA; tide,  $F_{1,52} = 409.12$ ,  $P < 0.001$ ; Table C2D), substrate (three-way ANOVA; substrate,  $F_{1,52} = 47.50$ ,  $P < 0.001$ ; Table C2D), and water temperature (three-way ANOVA; water temperature,  $F_{1,52} = 19.73$ ,  $P < 0.001$ ; Table C2D). We did not observe an effect of substrate on DTR in the subtidal tanks (post hoc contrast, substrate when tide is subtidal;  $F_{1,53} = 0.00$ ,  $P = 0.99$ ; Table C2E), but substrate did influence DTR in the intertidal tanks (post-hoc contrast, substrate when tide is intertidal;  $F_{1,53} = 286.45$ ,  $P < 0.001$ ; Table C2F). DTRs ranged from 4.91°C to 11.09°C in the tanks (Fig. C2A). Intertidal tanks without substrate had the largest DTRs (5.68–11.09°C), and

DTRs varied from 4.91°C to 6.59°C in intertidal tanks with substrate. In contrast, the DTRs in the subtidal tanks ranged from 2.35°C to 2.43°C. This result is consistent with observations of DTR in water temperatures in the Santa Barbara Channel (the DTR of water temperatures recorded from the West Channel Buoy, 70 km west-southwest of Santa Barbara, California is 2.38°C). DTR varied from 3.03°C to 19.94°C in the wild (Fig. C2B) and DTRs did not differ between the sampling locations (pairwise KS tests; in all cases  $P \geq 0.24$ ).

The relative risk of WS-RLO infection increased significantly with DTR (Cox proportional hazard model, DTR; hazard ratio = 1.87, SE = 0.44,  $z = 2.68$ ,  $P < 0.001$ ; Table C2G). A hazard ratio close to one suggests infection with WS-RLO was just as likely to occur among abalone in the cool MBT group as abalone in the warm MBT group (Cox proportional hazard model, MBT; hazard ratio = 0.92, SE = 0.16,  $z = -2.08$ ,  $P = 0.04$ ; Table C2G). We found a significant effect of the interaction between MBT and DTR on the relative risk of WS-RLO infection (Cox proportional hazard model, DTR  $\times$  MBT; hazard ratio = 1.10, SE = 0.05,  $z = 1.98$ ,  $P = 0.04$ ; Table C2G), probably due to the significant, positive effect of water temperature on DTR (Table C2D). The DTR recorded in cool tanks varied from 4.91°C to 10.39°C, but increased to 5.07°C to 11.09°C in warm tanks. The tide and substrate treatments, and initial body mass, did not significantly influence the relative risk of WS-RLO infection (Cox proportional hazard model; in all cases  $P \geq 0.51$ ).

The final prevalence of WS-RLO infection was 0.17 in subtidal abalone, but increased to 0.45 in the intertidal abalone in tanks at cool water temperatures with substrate, and 0.38 in abalone in intertidal tanks at warm water temperatures with substrate. Prevalence increased to 0.67 in the intertidal abalone in tanks at cool water temperatures without substrate, and 0.75 in the abalone in intertidal tanks at warm water temperatures without substrate. Final WS-RLO prevalence increased with DTR (Fig. 2A; logistic regression, DTR;  $\beta = 0.35$ , SE = 0.18,  $t = 1.89$ ,  $P = 0.05$ ; Table C2H) but we did not observe a significant effect of MBT (Fig. 2A; logistic regression, MBT;  $\beta = -0.06$ , SE = 1.74,  $t = -0.61$ ,  $P = 0.54$ ; Table C2H). We did not observe a significant effect of the interaction between DTR and MBT on the final prevalence of WS-RLO (logistic regression; DTR  $\times$  MBT;  $\beta = 0.18$ , SE = 0.28,  $t = 0.63$ ,  $P = 0.52$ ; Table C2H).

We observed no significant effect of DTR on the loss in body mass over the experimental period (linear regression, DTR;  $\beta = -0.01$ , SE = 0.09,  $t = -1.08$ ,  $P = 0.29$ ; Table C2I). Although clinical signs of WS were only observed in abalone infected with WS-RLO, body mass loss was not a direct consequence of WS-RLO infection (linear regression, WS-RLO;  $\beta = 0.02$ , SE = 0.03,  $t = 0.91$ ,  $P = 0.37$ ; Table C2I). Rather, we observed a loss in body mass in WS-RLO infected abalone only in

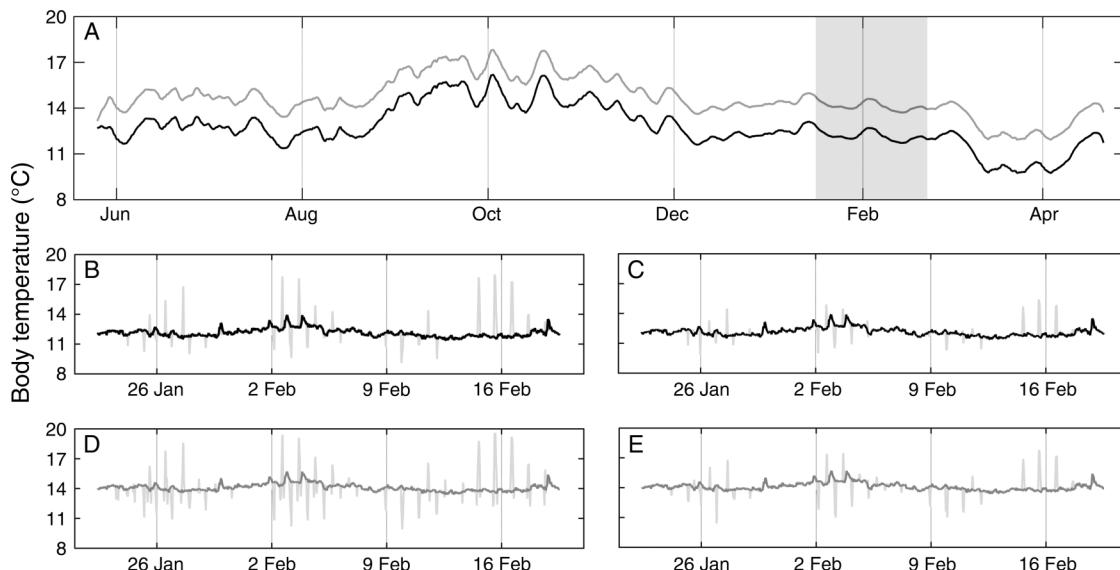


FIG. 1. (A) One-week moving average of abalone body temperatures recorded in cool (black line) and warm (dark gray line) subtidal tanks. (B–E) Hourly abalone body temperatures (light gray lines) recorded over the time period indicated by the shaded area in panel A, in a cool intertidal tank (B) without substrate and (C) with substrate, and a warm intertidal tank (D) without substrate and (E) with substrate. Heavy lines in B–E demonstrate body temperatures recorded in cool (black lines) and warm (dark gray lines) subtidal tanks.

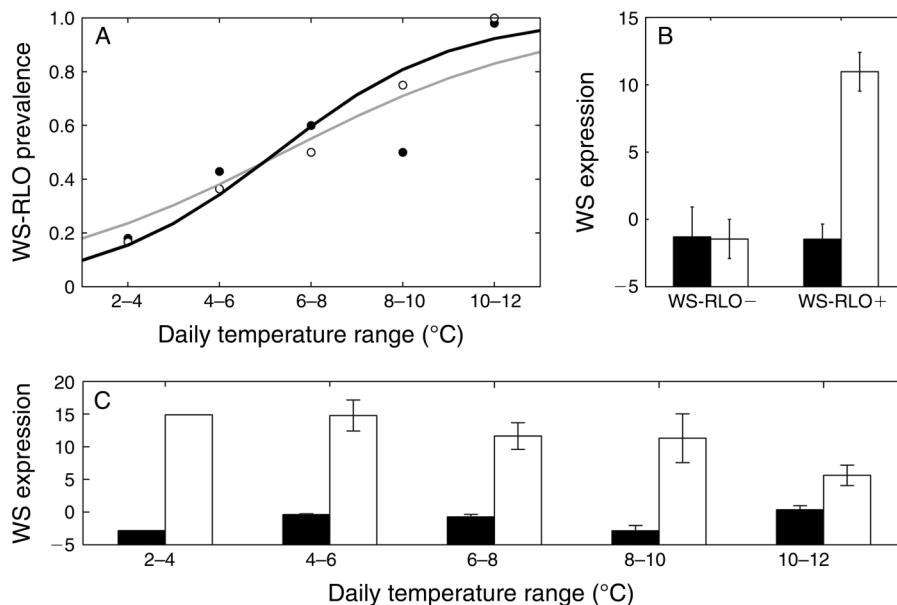


FIG. 2. (A) Daily temperature range (DTR) and the final prevalence of withering syndrome, the prevalence of Rickettsiales-like organism (WS-RLO) infection among cool (solid points, gray line) and warm (open points, black line) MBT groups. All subtidal tanks fell in the 2–4°C DTR group. DTRs of the intertidal tanks were all greater than 4°C. In the cool mean body temperature (MBT) group, MBT  $\pm$  SE was  $12.13 \pm 0.02^\circ\text{C}$  for 2–4°C DTR,  $12.16 \pm 0.03^\circ\text{C}$  for 4–6°C DTR,  $12.13 \pm 0.04^\circ\text{C}$  for 6–8°C DTR,  $12.15 \pm 0.05^\circ\text{C}$  for 8–10°C DTR, and  $12.14 \pm 0.03^\circ\text{C}$  for 10–12°C DTR. In the warm MBT group, MBT was  $14.18 \pm 0.02^\circ\text{C}$  for 2–4°C DTR,  $14.15 \pm 0.04^\circ\text{C}$  for 4–6°C DTR,  $14.21 \pm 0.02^\circ\text{C}$  for 6–8°C DTR,  $14.18 \pm 0.04^\circ\text{C}$  for 8–10°C DTR, and  $14.19 \pm 0.04^\circ\text{C}$  for 10–12°C DTR. (B) The expression of withering syndrome (WS), measured as a proportional loss in body mass (mean  $\pm$  SE), among uninfected black abalone (WS-RLO–), and black abalone infected with WS-RLO (WS-RLO+) over the study period in cool (solid bars) and warm (open bars) MBT groups. MBT was  $12.14 \pm 0.02^\circ\text{C}$  in the cool MBT group and  $14.18 \pm 0.03^\circ\text{C}$  in the warm MBT group. (C) DTR and WS expression among WS-RLO infected abalone in cool (solid bars) and warm (open bars) MBT groups.

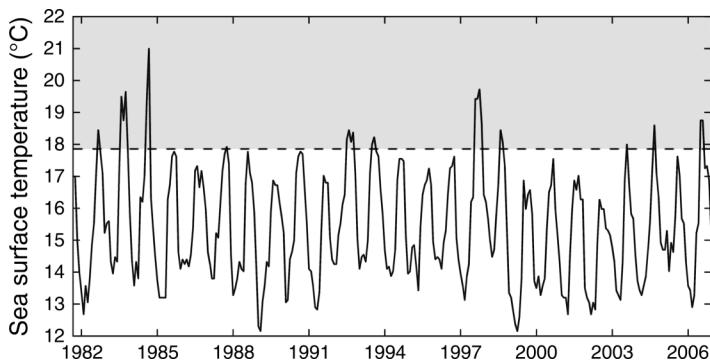


FIG. 3. One-week average sea surface temperatures recorded off western Santa Cruz Island, California, USA, from 1981 to 2006. The dashed line and shaded area indicate the maximum one-week moving average of water temperatures recorded in warm experimental tanks ( $17.84^{\circ}\text{C}$ ). Strong ENSO conditions (multivariate Southern Oscillation Index  $\leq -3$ ) occurred in 1982–1983, 1990, 1997–1998, and 2004–2005.

the warm MBT group (Fig. 2B and C; linear regression, WS-RLO  $\times$  MBT;  $\beta = 0.08$ ,  $\text{SE} = 0.04$ ,  $t = 2.02$ ,  $P = 0.04$ ; Table C2I). Tide, substrate, and initial body mass did not influence the expression of WS (linear regression; in all cases  $P \geq 0.19$ ; Table C2I).

#### DISCUSSION

Our results indicate that the risk of WS-RLO infection is minimized when black abalone are exposed to relatively low daily temperature variation (Fig. 2A), which is characteristic of subtidal habitats. Abalone exposed to high temperature variation, such as those in intertidal habitats, are at much greater risk of WS-RLO infection. Abalone in exposed microhabitats, lacking features such as crevices and overhangs that shade incoming solar radiation, are particularly susceptible to WS-RLO infection. We found that the susceptibility of black abalone to WS-RLO infection increased with daily temperature variability without increasing the associated expression of WS (Fig. 2). Increased susceptibility to WS-RLO infection, without increased mortality, allows WS-RLO to spread easily through black abalone populations, as long as water temperatures remain cool enough to prohibit the expression of WS.

Several laboratory studies have manipulated water temperatures in subtidal mesocosms, and found that WS-RLO transmission and the expression of WS increase with water temperature (Moore et al. 2000, 2011, Braid et al. 2005, Vilchis et al. 2005; but see Moore et al. 2009). WS-RLO transmission in subtidal abalone populations is probably maximized when water temperatures are warm. However, these conditions also favor WS expression and mortality, and the potential for ongoing transmission is therefore small. In contrast, WS-RLO transmission can be maintained in intertidal abalone without fading out due to host population declines, when water temperatures remain cool. Estimates of WS-RLO prevalence in intertidal black abalone are often near 100%, even in the absence of apparent signs of WS (Friedman and Finley 2003; T. Ben-Horin, unpublished data). The prevalence of WS-RLO infection in subtidal abalone species ranges from 0% to 83% (Moore et al. 2000, Friedman and Finley 2003), but can approach 100% in green abalone (del Carmen Alvarez

Tinajero et al. 2002), a species that has shown tolerance to clinical WS at temperatures modulating WS in other California abalone species (Vilchis et al. 2005, Moore et al. 2009). Ocean warming above the maximum temperatures observed in our warm tanks occurs seasonally at irregular intervals of 2–7 yr, usually with ENSO (Fig. 3), and clinical WS and its associated mortality manifest in WS-RLO infected black abalone following these periods (Raimondi et al. 2002). The high prevalence of WS-RLO infection in intertidal black abalone suggests large population declines when water temperatures periodically warm. Populations of subtidal abalone species (*H. rufescens*, *H. fulgens*, *H. corrugata*, and *H. sorenseni*), although vulnerable to WS-RLO infection and WS-induced mortality as water temperatures increase (Moore et al. 2003, 2011, Braid et al. 2005, Vilchis et al. 2005, Friedman et al. 2007), are probably less susceptible to WS-induced population crashes because the environmental conditions favoring WS-RLO transmission also favor epizootic fadeout.

Time-delays, such as the lag between WS-RLO infection and the expression of WS in black abalone, have a destabilizing influence in many ecological contexts (Maynard-Smith 1968). In host–pathogen systems, such time delays allow pathogens to overwhelm host populations before transmission is limited by declines in the host population. For example, Vredenberg et al. (2010) found the prevalence of infection with the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) in mountain yellow-legged frogs (*Rana* spp.) to rapidly approach 100% following its introduction to naïve populations. Frog mass mortalities occurred only after infection intensities reached critical thresholds, and therefore lagged behind the spread of Bd. The time delay between infection and disease-induced mortality, occurring over only weeks to months, explains frog extinctions within alpine lake basins. The presence of vagile, sympatric amphibians, insects and birds, capable of transporting Bd between alpine lake basins, clarifies the widespread decline of mountain yellow-legged frogs throughout California's Sierra Nevada. For WS-RLO, the time delay is not related to disease dynamics but is, instead, a consequence of the periodicity of extreme environmental events.

How has WS-RLO repeatedly spread among disjunct black abalone populations (Lafferty and Kuris 1993, Altstatt et al. 1996)? WS-RLO transmission within a black abalone population results when susceptible hosts encounter infectious particles deposited in the environment by neighboring infected black abalone (Friedman et al. 2002). Transmission between populations, and sustained mass mortalities, likely require a reservoir host and a means of long-distance dispersal. All southern California abalone species, in addition to black abalone, are susceptible to WS-RLO infection and can therefore serve as reservoirs. The ability of WS-RLO to survive in seawater, even briefly, may be what allows the pathogen to spread from subtidal to intertidal abalone populations and, more rarely, from island to island. Outflow from abalone culture facilities is another potential source of WS-RLO in the environment, so should be carefully considered when developing restoration plans for black abalone under the Endangered Species Act.

Global climate change and the unprecedented rate of infectious disease emergence represent two of the most formidable ecological problems of our time. Our results are consistent with mass mortalities of corals (Rosenberg and Ben-Haim 2002) and fishes (Monette et al. 2006) that have been linked to bacterial infections that cause mortality during extreme temperatures. The link between climate and infectious disease, as with most aspects of ecology, is complex and requires careful and thorough evaluation (Lafferty 2009). Our results demonstrate this complexity, particularly the effect of temperature variability, and highlight the importance of understanding the response of climate-disease interactions to all moments of climate and ocean temperature change.

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## SUPPLEMENTAL MATERIAL

### Appendix A

Details of thermally matched temperature loggers and field temperature surveys ([Ecological Archives E094-014-A1](#)).

### Appendix B

Experimental design and laboratory tank system ([Ecological Archives E094-014-A2](#)).

### Appendix C

Results of all statistical tests ([Ecological Archives E094-014-A3](#)).