Transmission Dynamics of the Withering Syndrome Rickettsia-like Organism to Abalone in California

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Abstract

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A sentinel study was conducted to investigate the distribution of the withering syndrome (WS) pathogen by deploying modules containing live red abalone at two different field sites, one near an onshore commercial abalone farm and one in proximity to wild aggregations of abalone, both in Southern California. A newly validated quantitative polymerase chain reaction (qPCR) assay was used to quantify the withering syndrome rickettsia like organism (WS-RLO) DNA in water, tissue and fecal samples. In addition, histological screenings were conducted on tissues from all surviving abalone to understand clinical infections of the pathogen. WS-RLO DNA copies were detected in modules at the wild site but not at the site off of the abalone farm (even though WS-RLO DNA was detected in the farm's effluent; $p > 0.05$). Overall, proportions of clinical infections and WS-RLO DNA at both sites were very low and similar between sites ($p > 0.05$). Abalone infection prevalence and intensity of the WS-RLO was independent of WS-RLO DNA copy density in seawater. This study demonstrated the use of caged sentinel abalone to monitor

RLO transmission in the field. The results of this study will help managers better understand the risk of infection of abalone exposed to the WS-RLO in situ.

Acknowledgements

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The success of this sentinel study was made possible by multiple agencies and companies including the University of Washington, University of California at Santa Barbara, National Oceanic and Atmospheric Administration, The Cultured Abalone, The Abalone Farm, Inc. and the California Department of Fish and Wildlife.

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Introduction

In recent decades an increasing number of pathogenic organisms has been recognized in marine invertebrates, particularly in corals, echinoderms and molluscs, on a broad geographic scale. The emergence of previously unknown pathogens has in many cases led to detrimental losses in ecosystem function and fishery revenue associated with host species (Harvell et al., 2004; Lafferty et al., 2015). Pathogens are infectious agents that can cause disease and possibly death in host organisms. Recognition of the presence and impacts of novel marine pathogens is a significant conservation concern, especially when the origin and rate of spread by pathogens are unknown. Identifying the sources of pathogens, relative risks to potential hosts, and developing disease control measures are extremely important in a conservation context. Known factors facilitating the spread of disease among marine organisms include changes in host behavior, stress on a host, climate change, movement or range expansion of hosts, range shift of a pathogen, and rapid evolution of a pathogen (Harvell et al., 2004; Burge et al., 2014).

Pathogens are increasingly common in abalone. Abalone (*Haliotis* spp.) are marine gastropods that inhabit intertidal and shallow subtidal rocky reef habitats and are important in many marine ecosystems. In addition, abalone are economically and historically important organisms. They were a significant component of the subsistence diet in many coastal indigenous peoples of western North America over a span of at least nine millennia, and have supported major commercial and recreational fisheries since the nineteenth century. The combined effects of over-fishing and disease have negatively affected populations of abalone in many locations, and are particularly well-known in California (e.g., Karpov et al., 2000; Altstatt et al., 1996; Tan et al., 2008; Travers 2008). Natural resource management agencies have identified captive propagation and population enhancement by outplanting as potential recovery tools for abalone

depleted by emerging pathogens. However, implementation of such approaches requires a much-improved prior understanding of disease-related risks posed by introduction of abalone raised in captivity into wild populations. In the absence of such information goals for population-scale recovery of afflicted abalone species could be substantially compromised.

Seven abalone species are present in the coastal marine communities of California, USA. Substantial populations of six of the seven species have been severely depleted by overharvesting, changes in predator distribution (NOAA 2014) and disease (e.g., Friedman et al., 2000; Neuman et al., 2010). Pink, green and pinto abalone (*Haliotis corrugata, H. fulgens*, *H. kamtschatkana*, respectively) are currently designated as US Federal Species of Concern. The pinto or northern abalone (*H. kamtschatkana*) was recently formally considered for listing as "endangered" or "threatened", as defined by the US Endangered Species Act of 1973 as amended (ESA). White and black abalone (*H. sorenseni and H. cracherodii*, respectively) are currently listed as "endangered" in accordance with the ESA. Red abalone (*H. rufescens*) have declined substantially south of San Francisco, but remain moderately common and support a culturally significant recreational fishery to the north. In coastal marine habitats of California and northwestern Mexico, populations of black abalone are known to have declined considerably due to a fatal disease known as withering syndrome (WS; e.g., Haaker et al., 1992; Altstatt et al., 1996; Friedman et al., 2000, Miner et al., 2006; Neuman et al., 2010). WS may have caused significant mortality in other species of abalone off California and Mexico as well (Moore et al., 2000). WS is caused by a rickettsia-like organism (RLO) identified as '*Candidatus* Xenohaliotis californiensis' (WS-RLO), of unknown origin (Friedman et al., 2000). The WS-RLO infects digestive epithelia of host individuals and causes abnormalities within the digestive gland, resulting in malnutrition, catabolic degeneration of the muscular foot (crucial for adherence to

rocky substrata), lethargy, increased vulnerability to predators and scavengers, and eventually starvation and death (Friedman et al., 2000; Friedman et al. 2002, Braid et al., 2005).

Since WS was first discovered in the Islands off southern California in the mid-1980s, black abalone have declined by up to 95-99% in areas of observed disease outbreak (Haaker et al., 1992; VanBlaricom et al. 1993; Tissot et al. 1995). Shortly after its first appearance, the disease spread to mainland southern California (Steinbeck et al. 1992). By 1992, WS was active at all the large Islands off southern California (possibly except Santa Catalina for which few data were available) and had dispersed northward along the mainland coast into central California (Altstatt et al., 1996).

The proportion of abalone with clinical signs of WS infection varies among species and even populations, suggesting that different species and populations of abalone have different degrees of susceptibility to WS. The mechanisms of variation in host susceptibility are not well known (Crosson et al., 2014). However both resistance to and tolerance of infection have been reported (Wetchateng et al., 2010; Friedman et al., 2014b). Variable susceptibility among populations may be due to their latitudinal position along the coast. More northerly populations experience cooler water temperatures than those in along the south coast of California. Warmer seawater temperatures (above 18 ^{o} C) more typically observed along the south coast facilitate both bacterial transmission and disease development (Haaker et al., 1992; Moore et al., 2000; Friedman et al., 2002, 2014b; Braid et al., 2005; Vilchis et al., 2005; Ben-Horin et al., 2013).

Wild abalone fisheries have been damaged by overexploitation, recovery of imperiled predators such as sea otters (NOAA 2014), and, likely, by WS as well. As a result, aquaculture of abalone has become increasingly important in filling consumer demand for abalone products (e.g., Oaks

and Ponte 1996). Onshore abalone farms (i.e., those with seawater intake and effluent lines typically connected to the ocean), are found primarily in California (Friedman et al., 2000; Neuman et al., 2010), producing red abalone that are marketed for purposes of human consumption. Cultured red abalone are also susceptible to WS (Friedman et al., 2000; Moore et al., 2000; Moore et al., 2001). Abalone aquaculture facilities often experience losses due to WS, with damaged profit margins and limited ability to meet market demand for abalone as a consequence (Moore et al., 2000; Braid et al., 2005).

Recent studies have employed either a newly validated quantitative polymerase chain reaction (qPCR) assay (Friedman et al. 2014a) or a partially validated assay (Lafferty & Ben-Horin 2013) to quantify WS-RLO DNA (16S rDNA gene copy number) in both abalone tissue and environmental (e.g. seawater) samples. Examinations of environmental DNA (eDNA) using qPCR have found trends that may inform our understanding of effects of WS on black abalone populations (Crosson et al. unpubl. data). Collectively the available eDNA data suggest a latitudinal gradient of RLO prevalence and abundance (i.e., lower prevalence and abundance at higher latitudes), especially in locations adjacent to high densities of infected abalone, such as in natural aggregations or commercial abalone farms (Crosson et al. unpubl. data; Lafferty& Ben-Horin 2013).

Empirical studies are needed to evaluate the risk of an abalone becoming infected with the WS-RLO at various sites in California, and to determine if the presence of eDNA in sea water indicates the presence of viable WS-RLO, as opposed to non-viable, and therefore non-pathogenic, residual eDNA from decomposed WS-RLO cells. It is not clear how far the WS pathogen spreads from known possible point sources of the bacterium. To further investigate the

distribution of the WS pathogen I conducted a sentinel study in which we deployed modules containing live red abalone at two different field sites, one near an abalone farm and one in proximity to wild aggregations of abalone. The null hypothesis of the study was that abalone infection prevalence and intensity of the WS-RLO is independent of WS-RLO DNA copies in seawater. The alternative hypothesis was that an increase in prevalence and intensity of WS-RLO will be observed at sites with higher WS-RLO DNA copies in seawater.

Methods

Preliminary Water Samples

Crosson et al. (unpublished) evaluated WS-RLO eDNA from seawater samples collected from various locations in California, including sites near abalone farms or wild populations of abalone. I used these data to identify field sites and augmented the data with qPCR analysis of seawater from near Corona del Mar, one of my field sites, prior to full deployment of field studies (06/2015). Concentrations of eDNA in preliminary seawater samples near field sites varied from 0 to over 10^3 WS-RLO DNA copies per ml of seawater sampled.

Module Design

A total of 28 modules were constructed as shown in Fig. 1. Module frames were made of 1.9 cm diameter ("schedule 40") polyvinyl chloride (PVC) tubing and measured 46 x 36 x 38 cm (length x width x height). The modules were enclosed with plastic-coated galvanized wire mesh (square mesh 1.27 cm per side), which was small enough to prevent escape and to retain shells of animals that suffered mortality. In addition, nine perforated PVC half-rounds (7.6 x 20.3 cm) were situated within each module for use as shelters by the abalone. Wire mesh and shelters were secured to the frame by cable ties. In addition, a closeable door (17 X 17 cm) was constructed on the top surface of each module to facilitate insertion of abalone and kelp. A pilot deployment of

cages without abalone was conducted using four modules at the Corona del Mar site to verify the structural and functional integrity of the modules and earth anchors. Pairs of modules were deployed at depths of approximately 8 m and 15 m. Following similar anchoring methods as Krause (1994), the modules were anchored by divers to a sandy area on the sea floor using removable earth anchors (American Earth Anchors, Franklin, Massachusetts USA). After five weeks of deployment, modules at 8 m depth were found to be displaced, while those at 15 m depth remained securely in place with no indication of movement. For the sentinel study, modules were deployed at depths of at least 12 m to minimize the probability of displacement by waves and currents.

Fig. 1. Photograph of a module used in the field sentinel study.

Field Sentinel Study

Two field sites were selected based on quantitative polymerase chain reaction (qPCR) analysis of previously-collected seawater samples (Crosson et al., unpubl. data), habitat type, thermal regime (Fig. 2).

Site 1 near Goleta, CA (Goleta) was approximately 450-500 m offshore of an onshore commercial abalone culture facility from which seawater outflow is released onto the beach (intertidal zone). Site 2 near Corona del Mar, CA (Corona) was approximately 200 to 300 m from a population of wild green and pink abalone (Witting and Neuman, pers. obs.). Corona is not located near any abalone farms, nor any known anthropogenic source of WS-RLO DNA. The two study sites showed similar WS-RLO eDNA loads in the preliminary water samples analyzed by qPCR as described below.

I obtained live red abalone used in my study from The Abalone Farm, Inc., located near Cayucos, in San Luis Obispo County, California USA. Immediately prior to initiation of the study, I sampled 60 red abalone provided by The Abalone Farm. Animals were examined using both histological and qPCR methods as described by Friedman et al. (2002, 2014a) and were confirmed to be free of WS-RLO infection.

Modules were deployed at Corona (n=12 modules, Fig. 3) and Goleta (n=14 modules, Fig. 4) on August 7 – 10, 2015. The geographic midpoint of the array at Corona was located at 33.5859°, -119.8698°. At Goleta the midpoint was at 34.4371°, -119.9657°. Both sites have kelp cover in proximity to the modules (Fig. 3, Fig. 4). Kelp canopy cover is a reasonable proxy for locations of suitable abalone habitat.

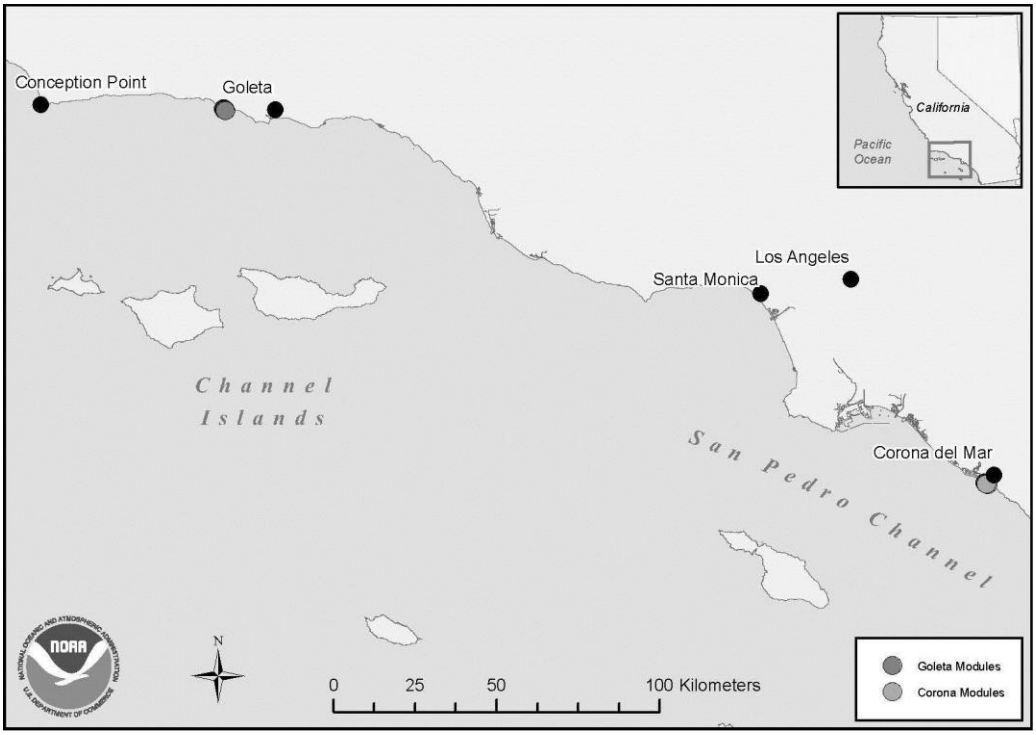

Fig. 2: Location of field study sites northwest of Goleta, in Santa Barbara County, and southeast of Corona del Mar, in Orange County, California USA (Map courtesy of Rick Morse NOAA).

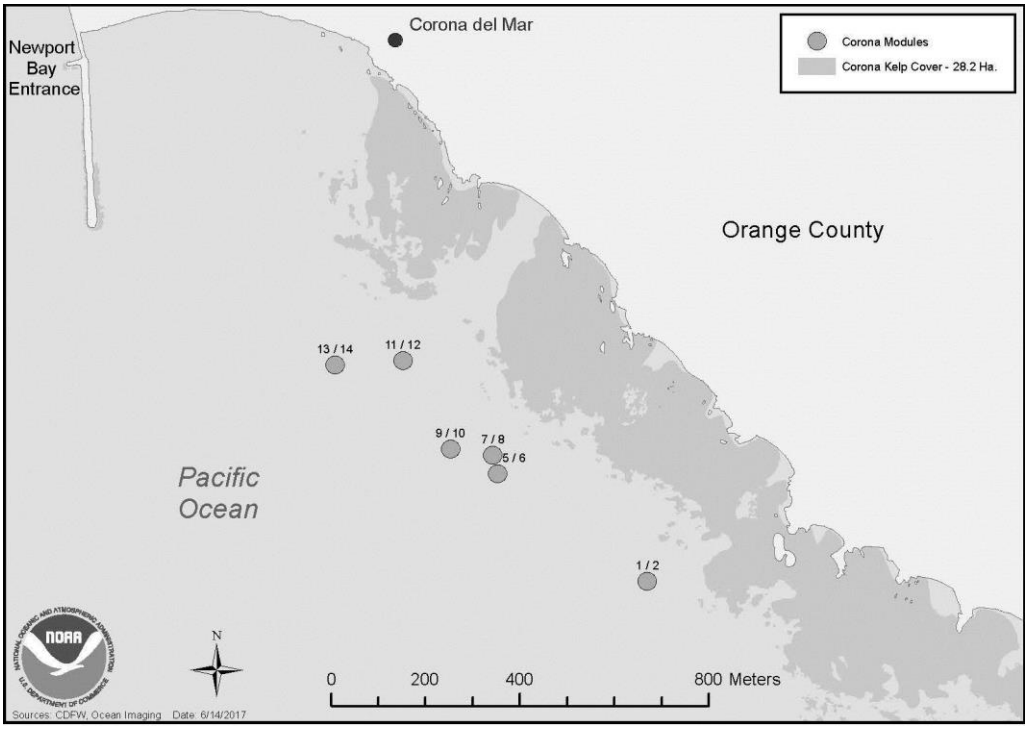

Fig. 3: Locations of module placement at Corona and kelp coverage based on one year of available data (2012). Map courtesy of Rick Morse NOAA.

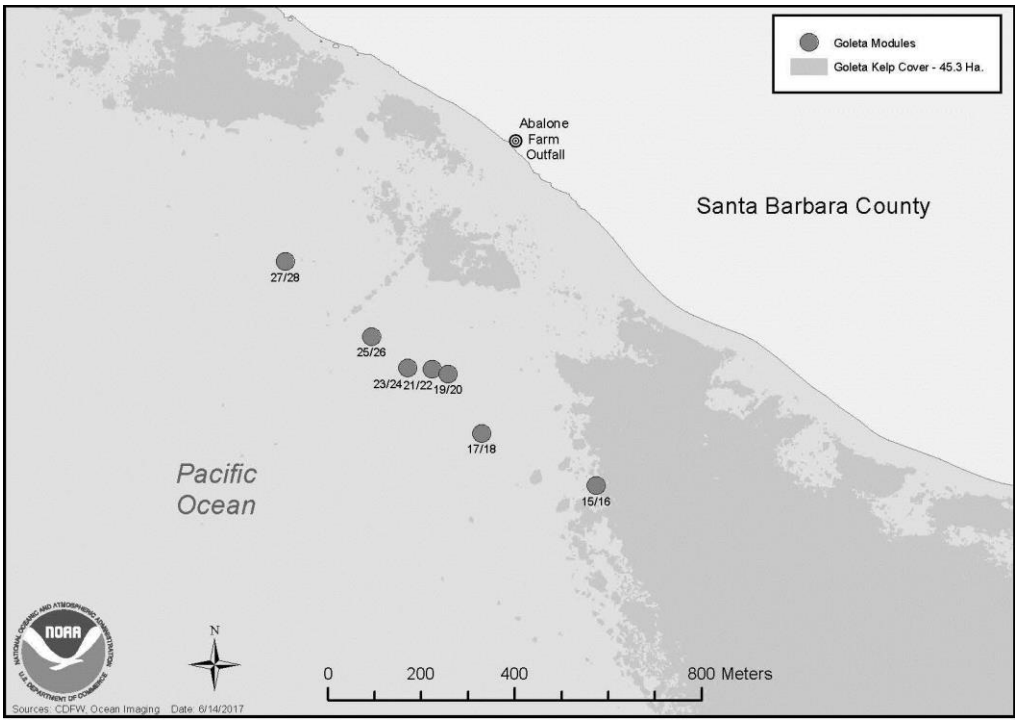

Fig. 4: Locations of module placement at Goleta and kelp coverage based on a 12 year average. Map courtesy of Rick Morse NOAA.

Modules 3 and 4 were not deployed at Corona, which limited the number of cages to 12. Duplicate pairs of modules were placed along a 710 m line approximately parallel to shoreline at an estimated depth of 12m (Fig. 5). In each duplicate pair the individual modules were separated by approximately 10 m. GPS coordinates were estimated for at least one of the duplicate modules in each pair of modules by measuring the coordinates of a buoy released to the surface from the deployed module. Red abalone (n=38-41 per module, measuring a mean of 47.6 ± 2.6 mm (maximum length of the elliptical shell) were placed in each module. At the time of deployment an average of 645 g of the kelp *Macrocystis pyrifera* was placed within each module to serve as food for the enclosed abalone. The kelp was rinsed in 2% Prepodyne (WestAgro, Kansas City, Missouri USA) prior to deployment, to remove any associated external WS-RLO. Duplicate water samples were collected adjacent to each module at deployment and processed for qPCR as described by Friedman et al. (2014a). At the Goleta site, duplicate water samples

were also collected from the seawater effluent from the nearby abalone culture facility. Temperature recorders ("ibutton", Maxium Integrated, San Jose, California USA) were installed in three modules per field site to track environmental temperature at intervals of one hour during the deployment. In addition, control abalone were held at the University of California at Santa Barbara, California USA (UCSB). Control animals were transferred directly from the commercial facility at Cayucos to UCSB, and were not exposed to field conditions while in transit. For each study site two corresponding control tanks were maintained in the laboratory, with twenty abalone in each tank from the same population as animals placed in modules in the field. Control abalone were held as described in the wet lab section below (n=80 total control animals).

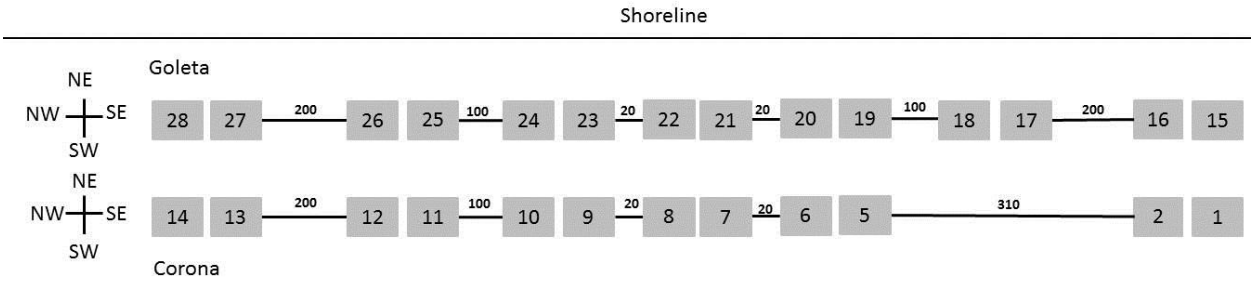

Fig. 5. Schematic map of arrays of modules placed at the two field sites. Duplicate pairs of modules were separated by 10 meters in each location within each site. The numbers shown between module pairs are distances (m) between adjacent pairs of modules. Modules 3 and 4 were not deployed at Corona because of a field logistical problem. The shoreline is oriented approximately southeast-northwest at both field locations.

After ~3 weeks of deployment, cages were inspected by divers for placement stability and to assess abalone mortality. Surviving animals were again provisioned with kelp as described above (Braid et al., 2005). Divers recorded abalone mortalities and numbers of predators (e.g., *Octopus*, crabs, fish) observed within or near the modules. Duplicate water samples were collected and processed as described above.

The abalone modules were retrieved from each site 45-46 days after deployment. Duplicate water samples were collected next to each module immediately before the modules were retrieved and from the Goleta farm effluent the day after module removal. The samples were processed as described above. Mortalities (as indicated by empty shells) and survivors were recorded for each module. The number and identity of any additional species within the cages were recorded.

Retrieved abalone were transported to a laboratory at UCSB that was equipped with continuously-running, ambient sea water (see wet lab section below). In the laboratory, half of the abalone from each module were selected for immediate tissue sampling, while the remaining abalone were placed in tanks for a three week incubation to allow any preclinical infections to progress to detectable levels. All abalone were selected for immediate sampling from modules that held 20 or less abalone. Abalone destined for sampling were shipped overnight, on ice, to a laboratory at the University of Washington at Seattle, Washington USA (UW) where they were sampled for histological and qPCR assessment of the WS-RLO and its DNA, respectively, as outlined in Friedman et al. (2000, 2014a). Groups of abalone from each of the field modules were held separately from one another through all processing that followed retrieval from the field.

Wet Lab

At UCSB, abalone (control groups and groups recovered from field modules) were placed in 10 L tanks supplied with flow-through seawater subject to 1 µm filtration and ultraviolet irradiation at ambient temperatures (range 16.6-21.8^{o}C during the study). The holding tanks were also equipped with covers and provided with aeration. Temperatures were recorded daily and tanks

and filters were cleaned biweekly. Control abalone were fed at the same time the field abalone were fed. All abalone were fed biweekly once the field-deployed abalone were placed in tanks at UCSB. Mortalities were recorded daily.

Water Filtration

Duplicate 500mL seawater samples collected at the three time points (initial deployment, mid-deployment inspection and recovery) were filtered onto 0.2 µm Whatman Supor Filters (Pall Corporation, Port Washington, New York USA). Filters were preserved by passing 5 mL of low TE buffer (10 mM Tris pH 8.0, 100 mM EDTA, 0.5 M NaCl) through each filter containing seawater sample filtrate (Friedman et al. 2014a), then flash freezing the filters in liquid nitrogen. Preserved filters were stored at -80°C prior to DNA extraction (Friedman et al. 2014a).

Fecal samples

Fecal samples were collected in the laboratory at UCSB one and three weeks after retrieval. During each sampling period fecal material was collected from each tank that held animals retrieved from the field modules. The fecal material was collected from each tank with a pipette dropper. A vacuum pump was used to place fecal material onto a filter (Whatman Supor Filters as described above). The filters and associated fecal material were flash frozen in liquid nitrogen and stored at -80°C prior to DNA extraction.

Histology and Quantitative PCR

As described by Friedman and Crosson (2012), histological samples were processed and scored from moribund or freshly dead abalone (n=630). Animals sampled were screened to identify inclusions of three morphologically distinct RLOs: Stippled RLO (ST-RLO), WS-RLO and WS-RLO variant (WS-RLOv; Friedman & Crosson 2012; Friedman et al., 2014b). Validated qPCR

assays were used to quantify WS-RLO DNA in the seawater, tissue and fecal samples (Friedman et al., 2014a). All positive WS-RLO histological screenings were subjected to qPCR analysis as well as a random selection of samples. All qPCR reactions were performed in duplicate reactions including the standard curve and negative template controls. Tissue and fecal sample loads were calculated as copies per gram and water samples were calculated as copies per milliliter. Samples were considered positive if the mean copy number of the duplicates was equal to or higher than the limit of detection (3 copies) of the subject DNA per reaction.

Data Analyses

All statistical analyses were conducted with R Software (R Core Team 2017) at α=0.05. Mean temperatures between sites were compared using analyses of variance (ANOVA). A chi-square goodness of fit test was used to compare survival between the study sites. Temperature distributions were compared using an empirical qqplot with temperature quantiles and linear regression (Wilk and Gnanadesikan 1968; Schaffler et al. 2009). Binomial general linear models (GLM) were used to test for significant differences in WS-RLO presence between sites using only tissues from randomly-selected abalone. For comparison of WS-RLO copy loads in sites, data were first submitted to a Box-Cox transformation to improve normality and heteroscedasticity, then tested using ANOVA with all positive qPCR tissue samples.

To summarize, data collected in the study fall into a number of categories under two general headings, DNA copy numbers (tissues, fecal material, and sea water) and data from histological screenings of morphologically different forms of RLO (WS pathogen, variant on the WS pathogen, and stippled type). Relationships of the various categories of data are also illustrated graphically in Fig. 6.

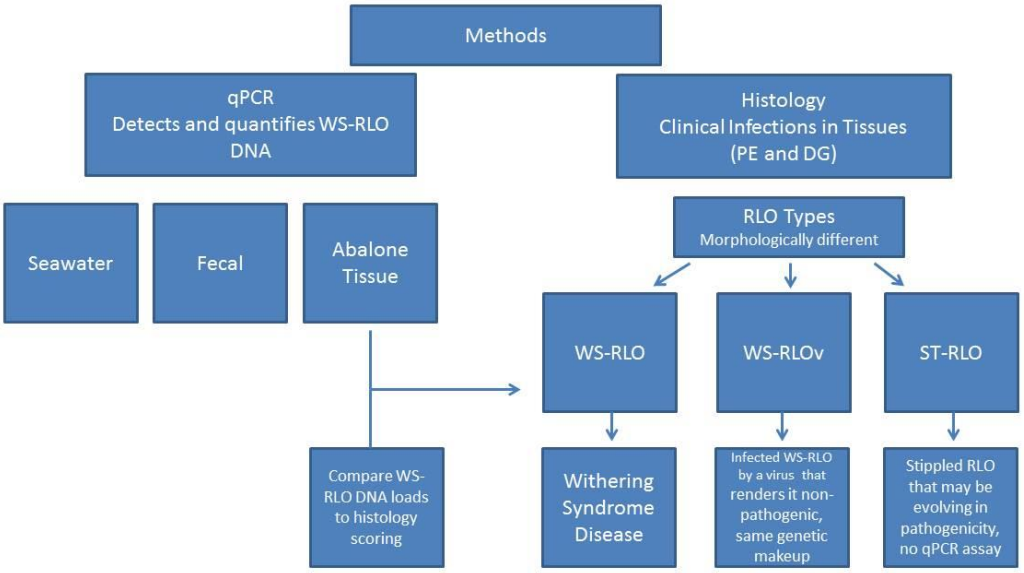

Fig. 6: Relationships of methods and data categories used for analyses.

Results

Field Study

All modules deployed at both sites were still securely in place and upright at the mid-deployment inspection. Two modules at the Corona site were not located during final retrieval, presumably due to a series of large wave events that occurred between the mid-deployment inspection and the retrieval. All modules were recovered from the Goleta site. More abalone survived at Goleta (72.4%) than at Corona (44.5%; $\chi 2=29.50$, DF=1,$p<0.0001$).

Field Temperature

Mean seawater temperatures at module depth were higher at Corona (19.2 ± 1.7 °C) than at Goleta (18.7 ± 1.8 °C ($p<0.0001$, F=33.03, $DF_1=1$, $DF_2=2134$). In addition, temperature distributions varied between the two study sites ($\beta_0 = 2.7$, $p = 0.1580$; $\beta 1 = 0.9$, $p<0.0001$) (Fig. 7). Fewer cool temperatures (<16 °C) and more warm temperatures (>22 °C) were observed at

Corona relative to Goleta. During deployment, mean temperatures exceeded 20°C on fourteen days at Corona and eight days at Goleta (Fig. 8).

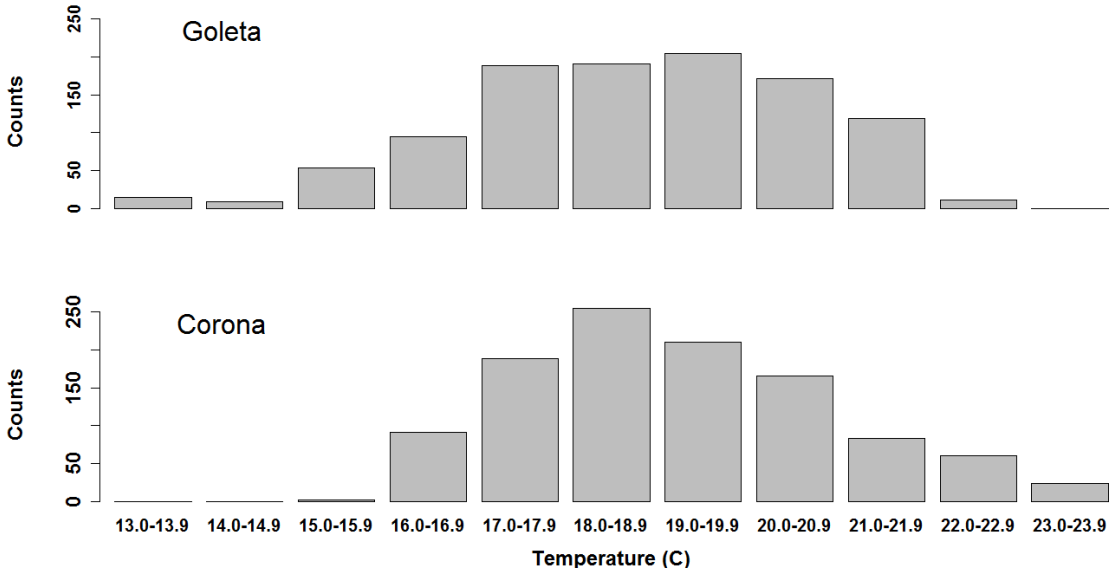

Fig. 7. Temperature distributions recorded at the study sites. Bars represent counts of temperature measurements (sampling rate was one record per h) by specified ranges during deployment. Data logged during deployment and retrieval days were not included in the bar graphs. Total sample size for Goleta was n=1056 and for Corona it was 1080.

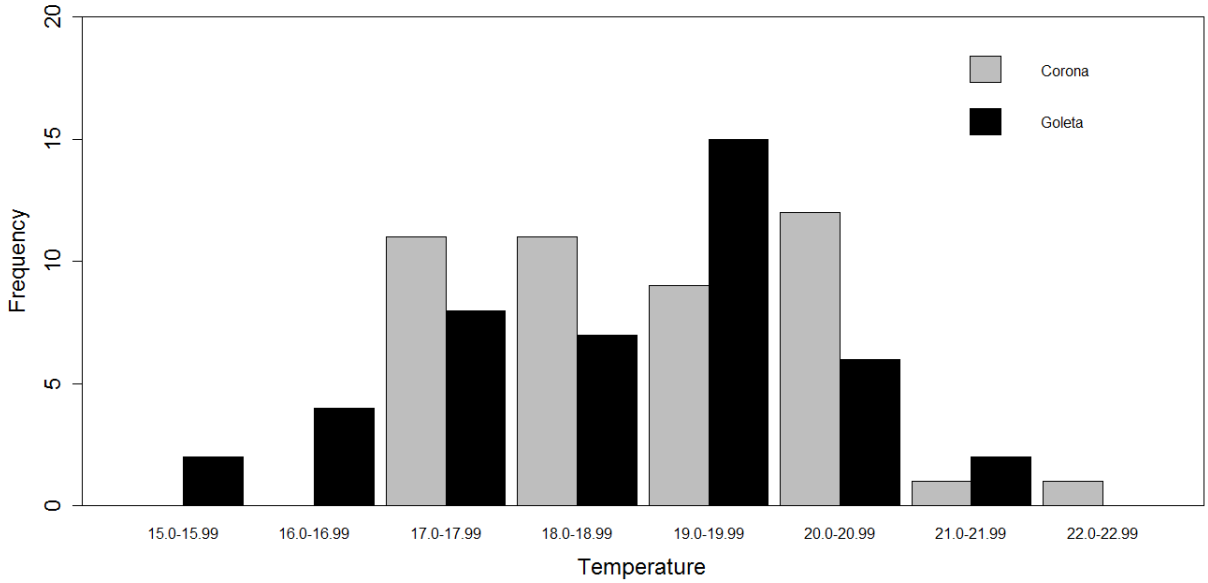

Fig. 8: Number of days (frequency) with a daily mean temperature within specified temperature intervals for each site.

WS-RLO DNA Presence in Seawater

WS-RLO DNA presence and quantities in water samples taken adjacent to modules were patchy (Fig. 9). No water samples collected at Goleta (n=84) amplified WS-RLO DNA concentrations above the limit of detection (LOD). Only three samples were found to have WS-RLO DNA amplified but at levels below the limit of detection (BLD). However, every sample taken from the onshore abalone farm effluent at Goleta (n=6) was above the LOD, with an average of 79.06 ± 66.11 copies per mL. At Corona, nine samples (of 67) amplified WS-RLO DNA above the LOD. Of the nine samples, five had one technical duplicate above the LOD while the other replicate was BLD and could not be rerun due to evaporation of the remaining sample (these were still considered positive in data analysis). An additional sixteen water samples at Corona indicated WS-RLO DNA presence, but at levels BLD. More samples contained WS-RLO DNA during the mid-deployment inspection than at module deployment or retrieval. However, all WS-RLO DNA positive samples contained very low copy loads (<1 copy/ml seawater).

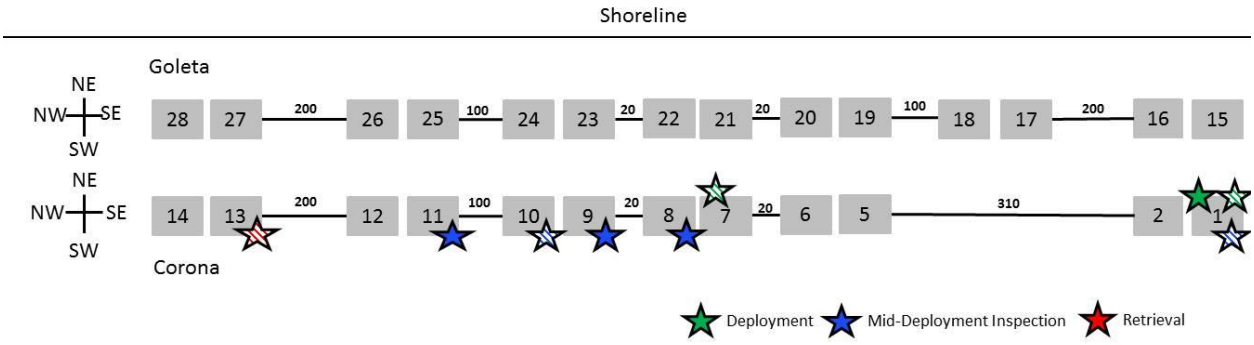

Fig. 9: Schematic map of modules with water samples registering positive for WS-RLO eDNA. Each star represents the number of positive biological samples. Star colors represent different time stamps (legend). Stars with barred color indicate that only one of the two technical duplicates (qPCR duplicate) in the sample was positive.

RLO Presence in Abalone

1. *Histology Screening*

All surviving animals were histologically screened (Control n=80, Goleta n=375, Corona n=175). In no case did tissues that scored positive for RLO inclusions during histological screening receive scores higher than 1 (on a scale of 0-3 with 1 containing a mean of 1-10 inclusions per 20x field of view), indicating low clinical infection rates. No sampled abalone were found to have RLOv inclusions, but sampled abalone from both locations did have inclusions with WS-RLO and ST-RLO. No control abalone showed inclusions of RLO. Only twelve abalone in field treatments contained WS-RLO inclusions, with similar prevalence at both sites: nine (2.40%) from Goleta and three (1.71%) from Corona (Fig. 10; Fig. 11, $p=0.6259$, $F=0.2413$, $DF1=1$, $DF2=20$). Thirty eight abalone in the field treatments contained ST-RLO inclusions between the two sites (Fig. 12). Corona had a significantly higher proportion of abalone infected with ST-RLOs than Goleta, 12.57% versus 4.53% (Fig. 11, $p=0.0169$, $F=6.7949$, $DF1=1$, $DF2=20$). Overall, ST: WS RLO ratios were 4.16 overall, 7:35 at Corona and 1.89 at Goleta. Module comparison for ST-RLO could be analyzed for the Corona abalone but not for Goleta because of an inadequate number of positive samples from the latter. Differences among modules at Corona were not significant. ($p>0.05$).

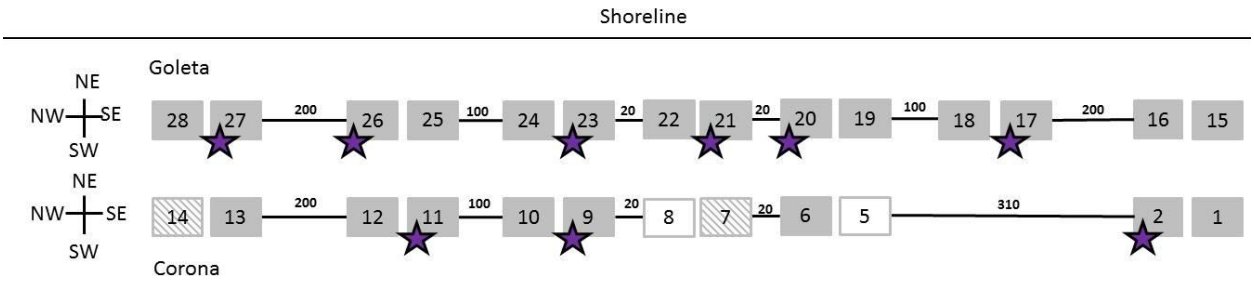

Fig. 10: Schematic map of modules containing at least one abalone with WS-RLO inclusions in tissues as determined histologically (purple stars). White boxes denote modules not recovered. Hatched boxes denote modules with zero survivors.

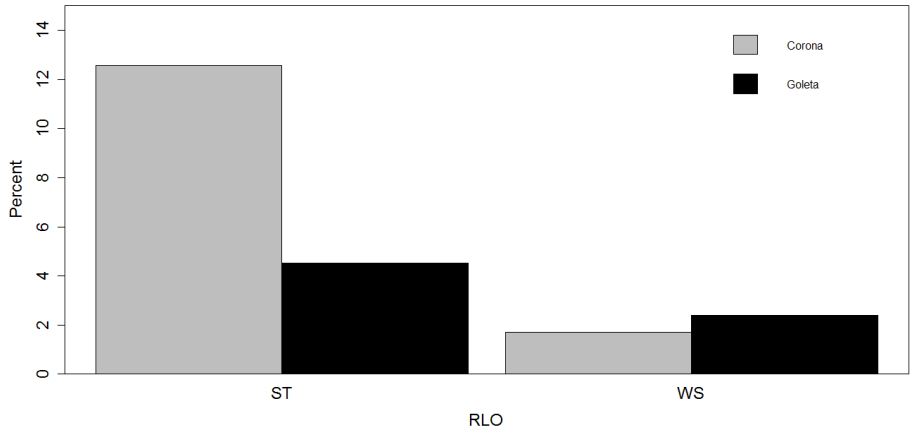

Fig. 11: Percentage of abalone with clinical infection of WS-RLO and ST-RLO for Corona (gray) and Goleta (black).

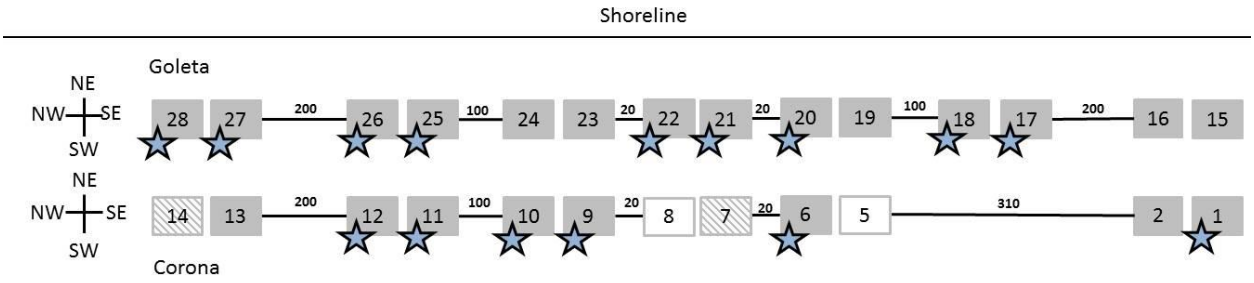

Fig. 12: Schematic map of modules containing at least one abalone with ST-RLO inclusions in tissues as determined histologically (blue stars). White boxes denote modules not recovered. Hatched boxes denote modules with zero survivors.

2. qPCR Tissues

All but two abalone with visible WS-RLO clinical infections using histology screenings (n=12) contained WS-RLO DNA upon qPCR amplification. Two tissue samples from each site showed inclusions when screened histologically, but WS-RLO DNA was not detected in qPCR data from the samples.

In addition, at least ten abalone were selected at random for qPCR evaluation from each module with survivors (module 16 from Goleta had only five remaining survivors), along with twenty-five control abalone. The proportion of abalone with WS-RLO DNA in tissues was not significantly different between sites ($p=0.2443$, $F=1.4394$, $DF_1=1$, $DF_2=20$). Since overall prevalence of tissue samples with WS-RLO DNA was low, we were unable to assess differences in prevalence among modules. WS-RLO DNA was detected in abalone tissue from both sites (Fig. 13). Tissue from a single control abalone contained low levels (4.34×10^3 copies per gram tissue) of WS-RLO DNA and, as a result, remaining tissues will be examined by qPCR (Supplemental table 1).

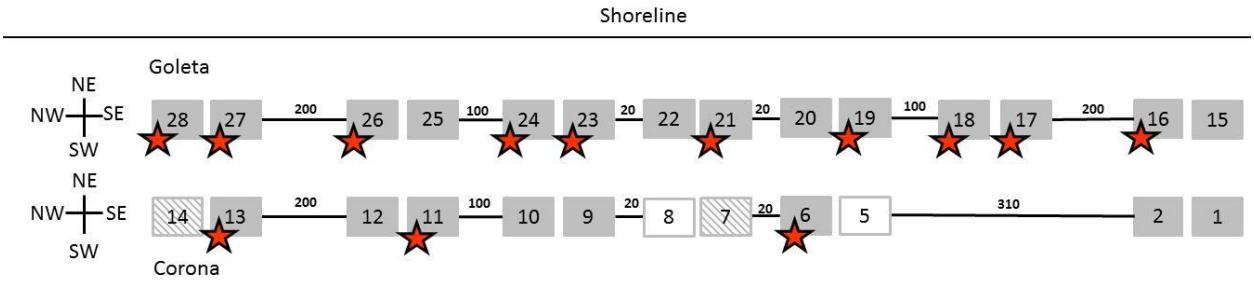

Fig. 13: Schematic map of modules containing abalone with tissues that contained WS-RLO DNA as determined by qPCR (red stars). White boxes denote modules not recovered. Hatched boxes denote modules with zero survivors.

Mean WS-RLO DNA (± standard deviation) loads were similar at both Goleta and Corona ($1.88 \times 10^7 \pm 6.11 \times 10^6$ and $5.66 \times 10^5 \pm 1.19 \times 10^6$ copies per gram tissue, respectively) (ANOVA, F-

ratio= 0.507, df_1=1, df_2=24, p=0.4830). The one control abalone with amplifiable WS-RLO DNA sampled at the end of the 3 week incubation period, contemporaneously with the abalone from the cages, contained 4.34 x 10^3 copies per gram tissue.

3. Fecal

All tanks with abalone from field modules had two fecal samples taken except for one. Only a single fecal sample was processed for module 18 as all abalone died after 10 days of holding at UCSB. WS-RLO DNA was amplified by qPCR from fecal samples for all tanks from both sites from which at least one abalone tissue contained amplifiable WS-RLO DNA or WS-RLO inclusions upon histological examination. However, WS-RLO DNA was amplified in fecal samples from three tanks holding Corona animals for which no evidence of WS-RLO infection was observed from tissue samples collected during the holding period. Module 10 lacked any signs of WS-RLO infection upon both, histology or tissue qPCR analyses, and yet the second fecal sample was qPCR positive. The second fecal sample from modules 2 and 9 also amplified WS-RLO DNA. These two modules contained WS-RLO infected abalone via histology, but those abalone were sampled from the field immediately following retrieval, and no abalone selected at a later date was found to be positive for WS-RLO (histology and qPCR).

Discussion

This study demonstrated both the use of caged sentinel abalone to monitor RLO transmission in the field and that abalone adjacent to populations of WS-RLO infected abalones (wild or farmed) are at risk of becoming infected. I accepted my null hypothesis that abalone infection prevalence and intensity of the WS-RLO is independent of WS-RLO DNA copies in seawater. I obtained evidence of substantial patchiness, both spatial and temporal, of WS-RLO DNA. I observed that more exposed abalone contained WS-RLO DNA than was apparent from numbers of abalone

with visible infections. Further, I discovered that the ST-RLO, thought to be evolving from a non-pathogenic to a pathogenic bacterium, was the most common RLO to infect our sentinel red abalone and was more prevalent at Corona than Goleta.

The use of caged sentinel animals to monitor marine disease is not new (e.g. Burge et al., 2006; 2007; Travers et al., 2008), but had not yet been used to monitor WS-RLO dynamics in the field. Sentinel studies like this allows testing of specific hypotheses that cannot be tested by simply monitoring natural abalone populations in a descriptive manner. The habitat used in my study at Goleta, adjacent to the outfall of the onshore abalone culture facility, is surrounded by unconsolidated sandy habitat for approximately 0.5 km in either along-shore direction, with no known nearby abalone populations (Fig. 4). Although kelp canopies are present inshore from the site, there is no evidence of abalone occurring in the area despite canopy presence (Ian Taniguchi, CFDW and David Witting, NOAA personal communication). As a consequence I could not assess the risk of RLO infection or WS dynamics at this site from any proximate wild population. A previous study (Lafferty & Ben-Horin 2013) implicated effluent from the Goleta culture facility as the likely source of WS-RLO DNA in seawater found in nearshore waters adjacent to the facility, and as far away as 20 km east of the facility. However, Lafferty & Ben-Horin were not able to test the potential risk to nearby abalones as no sentinel abalone were evaluated.

I anticipated that more abalone would become infected with the WS-RLO at sites in which seawater contained higher copy numbers of eDNA from the pathogen (the alternate hypothesis). However, similar numbers of abalone became infected and contained WS-RLO DNA in digestive tissues at Goleta and Corona. Thus, I accepted the null hypothesis and rejected the

alternative hypothesis. Only water samples collected from Corona contained WS-RLO DNA. This result was surprising given the known release of WS-RLO DNA from the seawater outfall at the Goleta abalone culture facility. Relatively high copy numbers (~80 copies per ml seawater) were found in effluent that flows from the Goleta facility across a sandy beach prior to entering the ocean. No seawater samples collected offshore adjacent to the modules at my Goleta site contained amplifiable WS-RLO DNA above the limit of detection (3 copies per reaction; Bustin et al., 2009; Friedman et al., 2014a).

Several environmental factors may have contributed the patterns observed in my data. Populations of wild abalone were more dispersed and closer to the array of modules at Corona as compared to the Goleta site (Melissa Neuman, NOAA personal communication). The difference may have increased the probability for any DNA shed from wild abalone populations to make contact with abalone held in the modules at Corona as compared to Goleta. In addition, more suitable abalone habitat and associated dense patches of kelp were found near the Corona modules as compared to Goleta at the time of the study. Finally, there is evidence that recovering populations of green and pink abalone were present inshore of the Corona modules during my study (Melissa Neuman, NOAA personal communication). At Goleta, suitable habitat for abalone was more scattered in proximity to most of the modules with the exception of modules 15 and 16. However, there is no evidence that wild abalone were present near modules 15 and 16 during the study (Ian Taniguchi, CDFW and David Witting, NOAA personal communication). The only known abalone present in close proximity to our Goleta study site were those held within the onshore abalone culture facility.

Lafferty & Ben-Horin (2013) found amplifiable DNA of the WS-RLO in DNA extracted from 17 of 36 seawater samples (100 ml) collected offshore of the Goleta abalone culture facility, and from 1 of 36 samples collected 20 km to the east. In both the present study and that by Lafferty & Ben-Horin, seawater was collected off shore and was filtered and preserved using similar methods. However, there were substantial differences between studies in the qPCR assays, and in the standards used to quantify WS-RLO DNA. I used the fully validated qPCR assay of Friedman et al. (2104a) that employs a hydrolysis probe for enhanced specificity, and uses a plasmid containing a single copy of the target WS-RLO DNA gene to accurately quantify copies of the target gene. In contrast, Lafferty & Ben-Horin (2013) modified a widely used conventional PCR assay (Andre et al., 2000; OIE 2014) modified into a SYBR-based qPCR assay and used a dilution of infected tissue to create a standard curve, precluding quantification of target DNA and a true limit of detection (Bustin et al., 2009). These differences make it difficult to compare data between the two studies and to determine the number of samples from Lafferty & Ben-Horin that are above the theoretical limit of detection of 3 copies per reaction for a qPCR assay (Bustin et al., 2009). In a previous study seawater samples were collected near the seawater outfall (e.g. along the shoreline and not off-shore) of the Goleta culture facility on two dates and analyzed using the same qPCR assay I used in my study (Friedman et al., 2014a). Amplifiable WS-RLO DNA was found only in samples taken within 25 m of the outfall (parallel to the shoreline) on one date, and within 300 to 500 m of the outfall on the second date (Crosson et al., unpubl. data). These data demonstrate the variability of WS-RLO DNA presence between sampling periods, the apparent spatial patchiness of WS-RLO DNA in seawater in the vicinity of the Goleta site, and the likely rapid dilution of eDNA once the facility's effluent reaches the ocean, particularly with increasing distance offshore. Empirically-observed coastal ocean currents off southern

California, and in the nearshore ocean in general, reflect a strong influence from tidal forces (e.g., Winant 1979; Gelfenbaum 2005). On average, tidally-induced flows are strongest during mid-tide for both flood and ebb phases, when lower frequency forcing of nearshore flows by other mechanisms may be largely masked by tidal flows. During mid-tide the tidally-induced current vectors are approximately parallel to the shoreline. Near times of maximum and minimum tidally-mediated water level, current vectors typically are approximately perpendicular to shore, but with much smaller magnitudes than during mid-tide periods. For the case of dispersal of eDNA from the onshore ocean outfall at the Goleta abalone culture facility, dominance by tidally-forced nearshore currents should disperse eDNA in the effluent parallel to the shoreline much more quickly and over greater distances than dispersal perpendicular to the shoreline. I suggest that the apparently substantial temporal and spatial variability in eDNA concentrations at my Goleta study site are facilitated, at least in part, by weak and uncertain tidally-induced transport in the offshore direction from the culture facility outfall, exacerbated by strong and predictable tidally-induced flow parallel to shore and away from my study site. Differences in dispersal of eDNA between Goleta and Corona may also be influenced by differences in coastline geomorphology. It is recognized that coastline irregularities such as capes, points, and submerged ridges may interact with geostrophic density structure in the ocean to facilitate semi-permanent eddies on a broad range of spatial scales near the shoreline. Such eddies can result in increased and persistent retention of water in association with coastal geological features (e.g., Hickey 1979; Lagerlof 1992; Marchesiello et al., 2003). Topographically-linked increases in coastal water retention are known to have important effects on dispersal patterns of small particles such as algal spores and larvae of invertebrates and fish, with significant consequences for nearshore ecology (e.g., Largier 2003; McCulloch

and Shanks 2003; Muchlin et al., 2008). Although specific data are lacking to my knowledge, the configuration of the coastline appears somewhat more irregular near Corona as compared to Goleta. It follows that differences in topographically-induced rates of local-scale water retention might have influenced patterns in my data relating to eDNA concentrations.

Our results further demonstrate the need to use fully validated molecular assays (Burrenson 2008) to study eDNA dynamics and that more frequent and spatially expansive water sampling is needed to fully understand trends of WS-RLO eDNA in the marine environment. In addition, it is important to note that neither fully validated nor modified conventional assays differentiate quantification of rickettsial DNA from live versus dead bacteria, which is important to consider when using qPCR as a proxy for pathogen presence (Burreson 2008; Lafferty & Ben-Horin 2013; Friedman et al., 2014a).

Molecular assays, such as conventional PCR (cPCR) and qPCR, commonly detect target DNA in more tissues than are confirmed by visual methods (e.g. histology) due to the higher sensitivity of molecular assays relative visible detection methods (Bustin et al., 2009; Burreson 2008; White et al. 2013; Friedman et al., 2014a). For example, over 1.5 times more walleye pollock contained DNA of the protist, *Ichthyophonus*, using a qPCR assay than visible infections using histology (White et al., 2013). As expected, we observed more abalone with WS-RLO DNA relative to those with visible infections (bacterial inclusions). In prior studies more WS-RLO DNA was detected in both experimentally exposed red and white abalones using cPCR relative to microscopic examination of stained tissue sections (Friedman et al., 2007; Rosenblum et al., 2008). In fact, 2.15 times more red abalone contained WS-RLO DNA than visible infections (Rosenblum et al. 2008). Thirty percent of exposed white abalone that lacked visual infections

contained amplifiable WS-RLO DNA in exposed individuals (Friedman et al. 2007). qPCR assays are typically more sensitive than conventional PCR assays. For example, similar numbers of walleye Pollock contained *Ichthyophonus* DNA using either cPCR or visible infections, while qPCR detected over 1.5 times more target DNA (White et al., 2013). In addition, comparison of cPCR and qPCR assays demonstrated a 100x higher limit of detection for the WS-RLO DNA when using the cPCR versus qPCR methods (Friedman et al., unbubl data). In previous studies, using a suite of abalone species, observed visible WS-RLO inclusions (score of 1 on scale of 0-3 as described above, Friedman et al. 2002) are typically associated with tissue loads of $1.01 \times 10^6 \pm 3.46 \times 10^5$ copies per gram tissue (Friedman et al., 2014a), which is similar to the loads quantified in tissues for which I observed visible infections. Similarly, tissues from the field exposed abalones lacking visible WS-RLO infections contained a mean of $5.06 \times 10^2 \pm 2.76 \times 10^2$ copies per gram (Friedman et al., 2014a), which is lower than loads I observed in qPCR positive but histology negative abalones. However, like Friedman et al. (2014a), tissue loads of abalone lacking visual signs of infection in my study were over 100 times less than those containing bacterial inclusions. The observation of a single control abalone from tanks at UCSB with low levels of WS-RLO DNA (4.34×10^3 copies per gram) was likely due to cross contamination from nearby tanks that held field-exposed abalones from the cages at Corona and Goleta. The low copy number and high water temperature, combined with the fact that they were held in isolation at UCSB for 6 weeks prior to the introduction of tanks holding field-exposed animals (9 weeks total holding period), suggest a recent exposure. In a previous study in which red abalone were exposed to infected abalone, 83-100% of the animals contained WS-RLO DNA 30 days after cessation of exposure when held at 17.4 ^{o}C (e.g. Rosenblum et al. 2008).

The undescribed ST-RLO was the most commonly observed RLO in abalone tissues overall with more abalone from Corona than Goleta infected by this bacterium. The taxonomy of the ST-RLO is unknown but its cellular location, morphology and tinctorial characteristics diverge from that of the WS-RLO. The ST-RLO (vs WS-RLO) forms round (vs oblong) inclusions located basally (vs apically) in the host cell. Individual ST-RLO cells are visible at the light microscope level (vs only at the electron microscopic level) and stain a faded blue (vs violet; Friedman et al., 2000; Crosson et al., 2014). In addition, the 16S rDNA sequence of ST-RLO differs from that of the WS-RLO based on the inability of the WS-RLO specific *in situ* hybridization assay of Antonio et al. (2000) to bind to the ST-RLO (Crosson & Friedman 2017). The host specificity and environmental physiology of the ST-RLO, which are not well characterized, may have facilitated the observed difference in ST:WS RLO ratios observed at Corona (>7x) versus Goleta (<2x). Resident pink and green abalones are known to exist in Corona and red abalone are reared in the culture facility near the Goleta site, but only red abalones are known to contract high levels of ST-RLO. A recent study demonstrated low infectivity of the ST-RLO for pink abalone (Crosson & Friedman 2017) and previous studies revealed that green abalone are highly refractory to RLO infection (Álvarez-Tinajero et al., 2002; Vilchis et al., 2005; Crosson et al., 2014). The ST-RLO appears to be evolving into a pathogen for red abalone based on a recent study that demonstrated preferential infection of red over pink or pinto abalone and that ST-RLO infections correlated with pedal atrophy only in red abalone (Crosson & Friedman 2017).

The cited recent studies demonstrate the high susceptibility of red abalone to the ST-RLO but fail to explain why more abalone were infected with this bacterium in Corona (which is largely devoid of red abalone) relative to Goleta (where large numbers of red abalone are cultured nearby). It is plausible that observed differences in thermal exposure between my two field sites

may have influenced viability of the RLOs. The higher temperatures observed at Corona may have facilitated ST-RLO infection. Bacterial virulence and host susceptibility are known to be linked to temperature (e.g. Harvell et al. 2002). A clear link between infection with the WS-RLO and temperature of many abalone species has been demonstrated (Moore et al., 2000; Braid et al., 2005; Vilchis et al., 2005; Ben-Horin et al., 2013; Crosson & Friedman 2017). Susceptibility of the European abalone *H. tuberculata* to a vibrio bacterium was modulated by both host physiology and environmental temperature (Travers et al., 2008, 2009). An increase in temperature of 1^{o}C resulted in an increase of vibrio-induced mortalities from 0 to 80% (Travers et al., 2009). Further studies are needed to characterize the field dynamics of RLO infections and the link between environmental temperature and infection by the ST as well as the WS-RLO and RLOv.

My data suggest that WS-RLO DNA was present at higher concentrations in seawater at Corona as compared to Goleta. However, seawater loads of WS-RLO DNA at Corona (and not at Goleta) did not correlate with a significantly higher percentage of abalone with clinical signs of infections at this site as compared to Goleta. These seemingly incongruous patterns could be a result of a generally warmer temperature distribution at Corona as compared to Goleta during field exposure of study animals. The optimal temperature range for the WS-RLO bacterium is unknown. In a laboratory exposure study involving two western Pacific species of abalone, *Haliotis gigantea* and *H. discus discus*, more exposed abalone contained WS-RLO DNA when held at a water temperature of 20°C relative to those held at 25°C (Ikunari Kiryu, National Research Institute of Aquaculture personal communication). Thus, temperatures above a threshold level may be detrimental to the WS-RLO and host-RLO interactions may influence the

relationship, with a potential benefit for host abalone species with temperature tolerances higher than those of the pathogen.

Mortality rates for subject abalone in my field exposure trial were relatively high at both field sites with a significantly higher percentage of mortality at Corona relative to Goleta. Measured seawater temperatures at the modules often exceeded the known preferred temperature range of red abalones (18-19°C; Diaz et al., 2000). Laboratory studies have shown that 5% of red abalone show evidence of thermal stress by beginning to detach from the substratum at temperatures >21 °C, with increasing numbers detaching at temperatures >24°C (Diaz et al., 2000). The summer of 2015 saw development of one of the two strongest El Niño events observed since 1950, and contributed to unusually high temperature anomalies in the coastal marine waters of southern California (Climate Prediction Center, National Weather Service, U.S. National Oceanic and Atmospheric Administration, http://www.cpc.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml). In addition, an aberrant thermal anomaly in the temperate northeastern Pacific Ocean, known as "the blob", emerged in late 2013 and persisted into late 2015 (e.g., Bond et al. 2015), contributing to elevated ocean temperatures in much of the northeastern Pacific Ocean including southern California. Deployment of my field experiment occurred within the time span of both the extreme 2015-2016 El Niño event and the 2013-2015 "Blob" event. More frequent warmer temperatures and less frequent cooler temperatures at my Corona study site, as compared to Goleta, may have resulted in higher mortality of test animals at Corona as a direct result of thermal stress during my field study

I suggest that the results of this study will help managers better understand the risk of infection of abalone exposed to the WS-RLO *in situ*. Abalone managers in California are currently planning and implementing restoration projects to help abalone populations in decline for various reasons including disease impacts. During the discourse on abalone restoration strategies onshore abalone culture facilities have been implicated in dispersing the WS-RLO DNA into the "wild" and potentially harming wild abalone populations (Lafferty & Ben-Horin 2013). My finding that infection rate of abalone was not higher at Goleta than at Corona suggests that both wild and farmed abalone infected with the WS-RLO may serve as sources of infectious RLO for nearby uninfected abalone, and that farmed and wild congeners appear to pose comparable infection risk. However, two important points must be considered. First, The WS-RLO DNA released into the ocean from culture facility effluent may not be associated with live WS-RLO cells, and may therefore not be viable in the context of infecting healthy wild abalone. Second, there is evidence that different abalone species in the coastal ocean of California vary in susceptibility to infection by WS-RLO and, as a consequence, may experience variable survival rates in restoration locations with WS-RLO DNA present. The more susceptible black (Alstatt et al., 1996; Friedman et al., 2014b) and white abalones (Crosson et al., 2014) would be less likely to survive if viable WS-RLOs numbers are above the (currently unknown) infectious dose within a targeted restoration site. Species moderately susceptible to infection, such as red abalone (Moore et al., 2000; Braid et al. 2005) or refractory to infection such as green abalone (Alvarez-Tinajero et al., 2002; Moore et al., 2009; Crosson et al., 2014) may have a higher chance of surviving if viable WS-RLO DNA is present in designated restoration zones but may serve as reservoirs of WS-RLO to nearby susceptible abalones..

As noted above, my study was conducted during an extreme El Niño event that coincided with the final months of the "Blob" event in the northeastern Pacific. Given the recognized sensitivity of WS infections to ocean temperatures, it is clear that additional field studies, occurring during average and cooler ocean temperatures, are essential in understanding linkages of thermal variation to risks of infection by WS-RLO in abalone. Several laboratory studies have shown that abalone respond differently under different temperatures to the WS-RLO pathogen (Braid et al., 2005; Ben-Horin et al. 2013; Crosson and Friedman 2017). Laboratory studies are often conducted over longer time periods than that used in my field work. In laboratory studies extending from several months to over a year in duration, abalone can be sampled at different time points, providing improved insights to thermal effects on infection and mortality rates. If resources allow and mortalities from unfavorable environmental factors or natural predators are not a strong concern, then extended time frames would provide valuable additional information regarding infection and mortality of abalone by WS-RLO in the wild.

In this study, water samples were only taken at depth next to the modules. Future studies would benefit from more spatially comprehensive water sampling, with sample arrays throughout the water column (from the surface to the bottom) and inclusion of samples from an increased number of locations. Sampling of transects perpendicular to the shoreline, and at different distances away from a point source such as an onshore aquarium, marine lab or abalone culture facility, would be of particular interest. An increased sampling effort across time should also be pursued in future studies. Of particular interest would be sampling across different tidal phases in order to better understand patterns of potential dispersal of the pathogen from known point sources as driven by currents.

A more detailed understanding of the potential effects of the WS pathogen on abalone restoration potential would also be facilitated by implementation of a sentinel study in proximity to sites known to be occupied by infected abalone of several species. The black abalone populations at San Nicolas Island, California USA (SNI) have provided a long-term record of apparent impacts from withering syndrome. Although SNI is relatively far from shore and is highly exposed to ocean wave activity (providing possible logistical challenges to module deployment), it would nevertheless be a useful site for further studies of likely WS impacts on future abalone restoration activities.

Literature Cited

Altstatt, J. M., Ambrose, R. F., Engle, J. M., Haaker, P. L., Lafferty, K. D., & Raimondi, P. T. (1996). Recent declines of black abolone Haliotis cracherodii on the mainland coast of central California. *Marine Ecology Progress Series*, *142*(13), 185–192. Retrieved from http://www.jstor.org/stable/24857235

Álvarez, T., Cáceres-Martínez, J., & Gonzáles, A. (2002). Histopathological evaluation of the yellow abalone Haliotis corrugata and the blue abalone Haliotis fulgens from Baja California, México. *Journal of Shellfish Research*, *21*(2), 825-830.

Andree, K. B., Friedman, C. S., Moore, J. D., & Hedrick, R. P. (2000). A polymerase chain reaction assay for the detection of genomic DNA of a Rickettsiales-like prokaryote associated with withering syndrome in California abalone. *Journal of Shellfish Research*, *19*(1), 213-218.

Antonio, D. B., Andree, K. B., Moore, J. D., Friedman, C. S., & Hedrick, R. P. (2000). Detection of Rickettsiales-like prokaryotes by in situ hybridization in black abalone, Haliotis cracherodii, with withering syndrome. *Journal of invertebrate pathology*, *75*(2), 180-182.

Ben-Horin, T., Lenihan, H. S., & Lafferty, K. D. (2013). Variable intertidal temperature explains why disease endangers black abalone. *Ecology*. WileyEcological Society of America. https://doi.org/10.2307/23435678

Bond, N. A., Cronin, M. F., Freeland, H., & Mantua, N. (2015). Causes and impacts of the 2014 warm anomaly in the NE Pacific. *Geophysical Research Letters*, *42*(9), 3414-3420.

Braid, B. A., Moore, J. D., Robbins, T. T., Hedrick, R. P., Tjeerdema, R. S., & Friedman, C. S. (2005). Health and survival of red abalone, Haliotis rufescens, under varying temperature, food supply, and exposure to the agent of withering syndrome. *Journal of Invertebrate Pathology*, *89*, 219–231. https://doi.org/10.1016/j.jip.2005.06.004

Burge, C. A., Griffin, F. J., & Friedman, C. S. (2006). Mortality and herpesvirus infections of the Pacific oyster Crassostrea gigas in Tomales Bay, California, USA. *Diseases of aquatic organisms*, *72*(1), 31-43.

Burge, C. A., Judah, L. R., Conquest, L. L., Griffin, F. J., Cheney, D. P., Suhrbier, A., et al. (2007). Summer seed mortality of the Pacific oyster, Crassostrea gigas Thunberg grown in Tomales Bay, California, USA: the influence of oyster stock, planting time, pathogens, and environmental stressors. *Journal of Shellfish Research*, *26*(1), 163-172.

Burge, C. A., Mark Eakin, C., Friedman, C. S., Froelich, B., Hershberger, P. K., Hofmann, E. E., et al. (2014). Climate Change Influences on Marine Infectious Diseases: Implications for Management and Society. *Annual Review of Marine Science*, *6*(1), 249–277. https://doi.org/10.1146/annurev-marine-010213-135029

Burreson, E. M. (2008). Misuse of PCR assay for diagnosis of mollusc protistan infections. *Diseases of aquatic organisms*, *80*(1), 81-83.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., ... & Vandesompele, J. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical chemistry*, *55*(4), 611-622.

Crosson, L., Wight, N., VanBlaricom, G., Kiryu, I., Moore, J., & Friedman, C. (2014). Abalone withering syndrome: distribution, impacts, current diagnostic methods and new findings. *Diseases of Aquatic Organisms*, *108*(3), 261–270. https://doi.org/10.3354/dao02713

Crosson L and Friedman C.S. (2017). Withering syndrome susceptibility corresponds with phylogeny of Northeastern Pacific abalones. Journal of Invertebrate Pathology. In Review.

Díaz, F., del Río-Portílla, M. A., Sierra, E., Aguilar, M., & Re-Araujo, A. D. (2000). Preferred temperature and critical thermal maxima of red abalone Haliotis rufescens. *Journal of Thermal Biology*, *25*(3), 257-261.

Friedman, C. S., Andree, K. B., Beauchamp, K. a, Moore, J. D., Robbins, T. T., Shields, J. D., & Hedrick, R. P. (2000). "Candidatus Xehohaliotis californiensis", a newly described pathogen of abalone, Haliotis spp., along the west coast of North America. *Int. J. Syst. Evol. Microbiol.*, *50*(2000), 847–855. https://doi.org/10.1099/00207713-50-2-847

Friedman, C. S., Biggs, W., Shields, J. D., & Hedrick, R. P. (2002). Transmission of withering syndrome in black abalone, Haliotis cracherodii Leach. *Journal of Shellfish Research*, *21*(2), 817-824.

Friedman, C. S., Scott, B. B., Strenge, R. E., Vadopalas, B., & McCormick, T. B. (2007). Oxytetracycline as a tool to manage and prevent losses of the endangered white abalone, Haliotis sorenseni, caused by withering syndrome. *Journal of Shellfish Research*, *26*(3), 877-885.

Friedman, C. S., & Crosson, L. M. (2012). Putative phage hyperparasite in the rickettsial pathogen of abalone,"Candidatus Xenohaliotis californiensis". *Microbial ecology*, *64*(4), 1064-1072.

Friedman, C. S., Wight, N., Crosson, L. M., White, S. J., & Strenge, R. M. (2014a). Validation of a quantitative PCR assay for detection and quantification of "Candidatus Xenohaliotis californiensis." *Diseases of Aquatic Organisms*. https://doi.org/10.3354/dao02720

Friedman, C. S., Wight, N., Crosson, L. M., VanBlaricom, G. R., & Lafferty, K. D. (2014b). Reduced disease in black abalone following mass mortality: Phage therapy and natural selection. *Frontiers in Microbiology*, *5*(MAR), 1–10. https://doi.org/10.3389/fmicb.2014.00078

Gelfenbaum, G. (2005). Types of coastal currents. Pages 259-260 in M.L. Schwartz (editor). Encyclopedia of coastal science. Springer, Dordrecht, The Netherlands.

Haaker, P. L., Parker, D. O., Togstad, H., Richards, D. V., Davis, G. E., & Friedman, C. S. (1992). Mass mortality and withering syndrome in black abalone, Haliotis cracherodii. In *California. In: Shepherd SA, Tegner MJ, Gusman del Proo SA (eds) Abalone of the world: biology, fisheries and culture. Proceedings of the first international symposium on abalone. University Press, Cambridge* (pp. 214-224).

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., & Samuel, M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science*, *296*(5576), 2158-2162.

Harvell, D., Aronson, R., Baron, N., Connell, J., Dobson, A., Ellner, S., et al. (2004). The Rising Tide of Ocean Diseases: Unsolved Problems and Research Priorities. *Frontiers in Ecology and the Environment*, *2*(7), 375. https://doi.org/10.2307/3868363

Hickey, B. M. (1979). The California current system—hypotheses and facts. *Progress in Oceanography*, *8*(4), 191-279.

Karpov, K. A, Haaker, P. L., Taniguchi, I. K., & Rogers-Bennett, L. (2000). Serial depletion and the collapse of the California abalone (Haliotis spp .) fishery. *Workshop on Rebuilding Abalone Stocks in British Columbia*, *200*, 11–24.

Krause, P. R. (1994). Effects of an oil production effluent on gametogenesis and gamete performance in the purple sea urchin (Strongylocentrotus purpuratus stimpson). *Environmental Toxicology and Chemistry*. https://doi.org/10.1002/etc.5620130717

Lafferty, K. D., & Ben-Horin, T. (2013). Abalone farm discharges the withering syndrome pathogen into the wild. *Frontiers in microbiology*, *4*.

Lafferty, K. D., Harvell, C. D., Conrad, J. M., Friedman, C. S., Kent, M. L., Kuris, A. M., et al. (2015). Infectious Diseases Affect Marine Fisheries and Aquaculture Economics. *Annual Review of Marine Science*, *7*(1), 471–496. https://doi.org/10.1146/annurev-marine-010814-015646

Lagerloef, G. S. (1992). The Point Arena eddy: A recurring summer anticyclone in the California Current. *Journal of Geophysical Research: Oceans*, *97*(C8), 12557-12568.

Largier, J. L. (2003). Considerations in estimating larval dispersal distances from oceanographic data. *Ecological Applications*, S71-S89.

Marchesiello, P., McWilliams, J. C., & Shchepetkin, A. (2003). Equilibrium structure and dynamics of the California Current System. *Journal of Physical Oceanography*, *33*(4), 753-783.

McCulloch, A., & Shanks, A. L. (2003). Topographically generated fronts, very nearshore oceanography and the distribution and settlement of mussel larvae and barnacle cyprids. *Journal of Plankton Research*, *25*(11), 1427-1439.

Miner, C. M., Altstatt, J. M., Raimondi, P. T., & Minchinton, T. E. (2006). Recruitment failure and shifts in community structure following mass mortality limit recovery prospects of black abalone. *Marine Ecology Progress Series*, *327*, 107-117. Inter-Research Science Center. https://doi.org/10.2307/24870722

Moore, J. D., Robbins, T. T., & Friedman, C. S. (2000). Withering syndrome in farmed red abalone Haliotis rufescens: Thermal induction and association with a gastrointestinal Rickettsiales-like prokaryote. *Journal of Aquatic Animal Health*, *12*(1), 26–34. https://doi.org/10.1577/1548-8667(2000)012

Moore, J. D., Robbins, T. T., Hedrick, R. P., & Friedman, C. S. (2001). Transmission of the rickettsiales-like prokaryote" Candidatus Xenohaliotis californiensis" and its role in withering syndrome of California abalone, Haliotis spp. *Journal of Shellfish Research*, *20*(2), 867-874.

Muhlin, J. F., Engel, C. R., Stessel, R., Weatherbee, R. A., & Brawley, S. H. (2008). The influence of coastal topography, circulation patterns, and rafting in structuring populations of an intertidal alga. *Molecular Ecology*, *17*(5), 1198-1210.

Moore, J. D., Juhasz, C. I., Robbins, T. T., & Vilchis, L. I. (2009). Green abalone, Haliotis fulgens infected with the agent of withering syndrome do not express disease signs under a temperature regime permissive for red abalone, Haliotis rufescens. *Marine biology*, *156*(11), 2325-2330.

Neuman, M., Tissot, B., & Vanblaricom, G. (2010). Overall Status and Threats Assessment of Black Abalone (Haliotis Cracherodii Leach, 1814) Populations in California. *Source: Journal of Shellfish Research*, *29*(3), 577–586. https://doi.org/10.2983/035.029.0305

NOAA (2014). Status Review Report for Pinto Abalone (*Haliotis kamtschatkana*). 240 pp.

Oakes, F. R., & Ponte, R. D. (1996). The abalone market: opportunities for cultured abalone. *Aquaculture*, *140*(1), 187-195.

Office Internationale des Epizooties (OIE) (2014). Infection with *Xenohaliotis californiensis. In:* Diagnostic manual of aquatic animal diseases, 12th edition. World Animal Health Organization. Paris, France. *http://www.oie.int/eng/normes/fmanual/A_summry.htm*

Rosenblum, E. S., Robbins, T. T., Scott, B. B., Nelson, S., Juhasz, C., Craigmill, A., et al. (2008). Efficacy, tissue distribution, and residue depletion of oxytetracycline in WS-RLP infected California red abalone Haliotis rufescens. *Aquaculture*, *277*(3), 138-148.

Schaffler, J. J., Reiss, C. S., & Jones, C. M. (2009). Patterns of larval Atlantic croaker ingress into Chesapeake Bay, USA. *Marine Ecology Progress Series*, *378*, 187–197. https://doi.org/10.3354/meps07861

Steinbeck J.R., Groff J.M., Friedman C.S., McDowell T., Hedrick R.P. (1992) Investigations into a mortality among populations of the California black abalone, *Haliotis cra cherodii*, on the central coast of California. In: Shepard SA, Tegner MJ, Guzmán del Próo SA (eds) Abalone of the world: biology, fisheries and culture. Fishing News Books, Cambridge, MA, p 203–213.

Tan, J., Lancaster, M., Hyatt, A., Van Driel, R., Wong, F., & Warner, S. (2008). Purification of a herpes-like virus from abalone (Haliotis spp.) with ganglioneuritis and detection by transmission electron microscopy. *Journal of Virological Methods*, *149*, 338–341. https://doi.org/10.1016/j.jviromet.2007.12.019

Tissot B.N. (1995). Recruitment, growth, and survivorship of black abalone on Santa Cruz Island following mass mortality. *Bulletin Southern California Academy of Sciences* 94(3):179–189.

Travers, M. A., Le Goïc, N., Huchette, S., Koken, M., & Paillard, C. (2008). Summer immune depression associated with increased susceptibility of the European abalone, Haliotis tuberculata to Vibrio harveyi infection. *Fish & shellfish immunology*, *25*(6), 800-808

Travers, M. A., Basuyaux, O., Le Goïc, N., Huchette, S., NICOLAS, J. L., Koken, M., & Paillard, C. (2009). Influence of temperature and spawning effort on Haliotis tuberculata mortalities caused by Vibrio harveyi: an example of emerging vibriosis linked to global warming. *Global Change Biology*, *15*(6), 1365-1376.

VanBlaricom G.R., Ruediger J.L., Friedman C.S., Woodard D.D., Hedrick R.P. (1993). Discovery of withering syndrome among black abalone *Haliotis cracherodii* Leach, 1814, populations at San Nicolas Island, California. *Journal of Shellfish Research* 12(2): 185−188.

Vilchis, L. I., Tegner, M. J., Moore, J. D., Friedman, C. S., Riser, K. L., Robbins, T. T., & Dayton, P. K. (2005). Ocean Warming Effects on Growth, Reproduction, and Survivorship of Southern California Abalone. *Ecological Applications*. WileyEcological Society of America. https://doi.org/10.2307/4543367

Wetchateng, T., Friedman, C., Wight, N., Lee, P., Teng, P., Sriurairattana, S., et al. (2010). Withering syndrome in the abalone Haliotis diversicolor supertexta. *Diseases of Aquatic Organisms*, *90*(1), 69–76. https://doi.org/10.3354/dao02221

White, V. C., Morado, J. F., Crosson, L. M., Vadopalas, B., & Friedman, C. S. (2013). Development and validation of a quantitative PCR assay for Ichthyophonus spp. *Diseases of aquatic organisms*, *104*(1), 69-81.

Wilk M.B., Gnanadesikan R (1968) Probability plotting methods for the analysis of data. *Biometrika* 55:1–17

Winant, C.D. (1979) Coastal current observations. Review of Geophysics and Space Physics 17: 89-98.

Supplemental Tables

In order to compare our two metrics for WS-RLO presence, the number of tissue samples analyzed by histology and qPCR (as described in the methods section) were compared in table form. Table columns included the number of samples containing visible evidence of infection (histology +) or absence of infection (histology-) and rows included the number of samples within each histology category that either amplified (qPCR +) or failed to amplify (qPCR-) WS-RLO DNA.

Table S1. Comparison of histology and qPCR analysis of abalone for which both assays were performed from Corona (A), Goleta (B) and Control (C) abalones.

A	Corona (n=89)	
	Histo "+"	Histo"-"
qPCR "+"	1	4
qPCR "-"	2	82

B	Goleta (n=144)	
	Histo "+"	Histo"-"
qPCR "+"	7	14
qPCR "-"	2	121

C	Control (n=25)	
	Histo "+"	Histo"-"
qPCR "+"	0	1
qPCR "-"	0	25