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REVIEW

Abalone withering syndrome: distribution, impacts, current diagnostic methods and new findings

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ABSTRACT: Withering syndrome (WS) is a fatal disease of abalone caused by a *Rickettsiales*-like organism (WS-RLO). The causative agent, '*Candidatus* Xenohaliotis californiensis', occurs along the eastern Pacific margin of North America in California, USA, and Baja California, Mexico. However, as infected abalones have been transported to Chile, China, Taiwan, Iceland, Ireland, Israel, Spain, Thailand and Japan, the geographical range of the etiological agent is suspected to be broad, especially where California red abalones *Haliotis rufescens* are cultured or in areas where native species have been exposed to this species. Susceptibility varies among species, with up to 99% losses of black abalone *H. cracherodii* in laboratory and field studies in the USA to no losses among the small abalone *H. diversicolor supertexta* in Thailand. Some populations that have suffered catastrophic losses due to WS have developed resistance to the disease. In addition, a newly identified phage hyperparasite of the WS-RLO may reduce pathogenicity and dampen associated losses. Diagnosis of WS requires the identification of infection with the pathogen (WS-RLO detected via *in situ* hybridization or histology coupled with PCR and sequence analysis) accompanied by morphological changes that characterize this disease (e.g. pedal and digestive gland atrophy, and digestive gland metaplasia). A quantitative PCR assay was developed and may be useful in quantifying pathogen DNA. Confirmation of infection cannot be done by PCR analysis alone but can be used as a proxy for infection in areas where the agent is established and is recommended for inclusion in health examinations. Avoidance of WS is best accomplished by the establishment of a health history and multiple health examinations prior to movement of animals.

KEY WORDS: Abalone · *Haliotis* · Disease · Withering syndrome · *Rickettsia* · Phage · PCR

INTRODUCTION

Abalones are primitive marine vetigastropods of the genus *Haliotis* that inhabit the nearshore intertidal and shallow subtidal zones. They are ecologically important in engineering habitat by grazing on micro- and macroalgae, thereby maintaining open areas for recruitment of conspecifics and other benthic organisms (Geiger & Groves 1999, Roberts 2001), and also support economically valuable fisheries and aquaculture production throughout the world (Gordon & Cook 2001, 2004, Cook & Gordon 2010). Of the over 50 *Haliotis* species world-wide, 8 inhabit the northeastern Pacific (Haaker et al. 1986). They include subtidal species such as the commonly cultured red abalone *H. rufescens* and northern or pinto

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abalone *H. kamtschatkana* found in cool waters, the pink (*H. corrugata*) and green (*H. fulgens*) abalones of warmer waters, and the intertidal–shallow subtidal black abalone *H. cracherodii*. Fishing pressure and disease threaten abalone populations globally (Hobday & Tegner 2000, Rothaus et al. 2008, Tan et al. 2008, Travers 2008). Currently, 5 California species experiencing population declines receive varying levels of federal protection, ranging from 'Species of Concern' (pinto, green and pink abalones) to 'Endangered' (white and black abalones). These and other species tested to date are all susceptible to the primary established abalone disease in California, withering syndrome (WS) (OIE 2012).

WS is a fatal bacterial disease characterized by a severely shrunken body and infection with a *Rickettsiales*-like organism (RLO; Fig. 1). Friedman et al. (2000) identified and characterized a gastrointestinal RLO provisionally named '*Candidatus* Xenohaliotis californiensis' WS-RLO as the pathogen causing WS. The WS-RLO is an obligate, intracellular bacterium that infects abalone digestive epithelia and causes severe morphological abnormalities within the digestive gland, resulting in physiological starvation followed by anorexia, absorption of pedal musculature, lethargy and death (Friedman et al. 2003, Braid et al. 2005). Transmission of the WS-RLO is likely fecal–oral (Friedman et al. 2002) and initial infections are located in the post-esophagus (Fig. 1D) and, to a lesser extent, the intestine of host abalone. Subsequently, metaplasia (the substitution of one mature tissue type for another; Fig. 1F) and infection occur in the digestive gland. These digestive gland changes are associated with depletion of glycogen reserves followed by pedal catabolism, atrophy (Fig. 1H) and, finally, death (Friedman et al. 2000, Braid et al. 2005). The severity of WS-RLO infection in juvenile red abalone has been directly correlated with negative physiological functions such as decreased feeding rates, metabolism, production of feces, and energy available for growth (Kismohandaka et al. 1993, González et al. 2012).

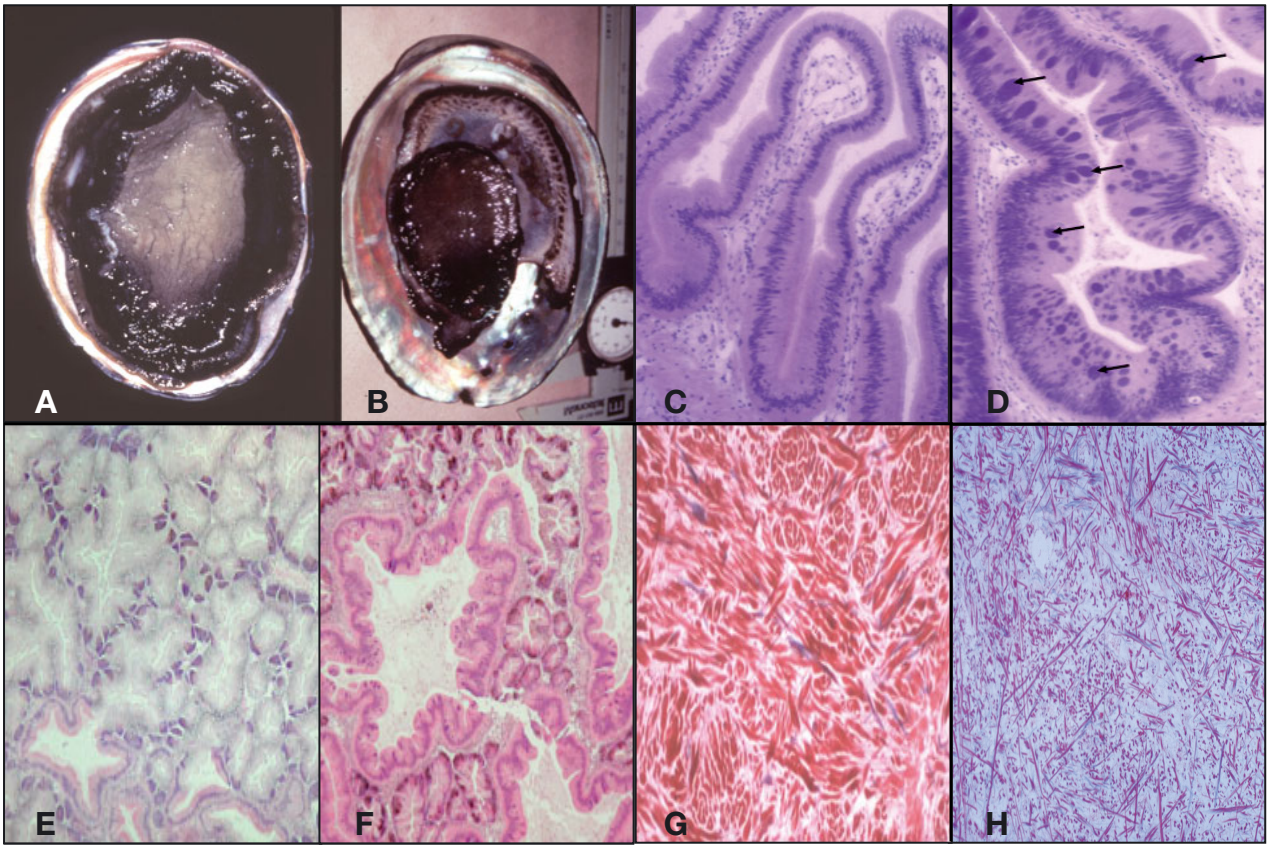

Fig. 1. Effects of withering syndrome (WS) on black abalone *Haliotis cracherodii*. (A) Uninfected abalone. (B) Severely withered WS-*Rickettsiales*-like organism (RLO)-infected abalone. (C–H) Light micrographs of abalone tissues stained with H&E. (C) Normal post-esophagus. 200× magnification. (D) WS-RLO-infected post-esophagus with arrows indicating WS-RLO cytoplasmic inclusions (bacterial colonies). 200× magnification. (E) Normal digestive gland. 100× magnification. (F) Metaplastic digestive gland. 100× magnification. (G) Normal pedal musculature. 400× magnification. (H) Pedal atrophy. 400× magnification

Two RLOs are known to infect California abalones: the WS-RLO and the stippled RLO (ST-RLO; Fig. 2, Table 1). Only '*Candidatus* Xenohaliotis californiensis' WS-RLO is known to cause WS (Friedman et al. 2000, 2003, Moore et al. 2001), while the ST-RLO appears, to date, to be non-pathogenic and is typically observed at low levels (Friedman et al. 2014b). The WS-RLO infects all members of the genus *Haliotis* examined to date including black abalone (Friedman et al. 1997, 2002), white abalone (Moore et al. 2002, Friedman et al. 2007), red abalone (Moore et al. 2000, 2001), pink abalone (Álvarez-Tinajero et al. 2002), green abalone (Álvarez-Tinajero et al. 2002), the small abalone *H. diversicolor supertexta* (Chang et al. 2008, Wetchateng et al. 2010), Japanese black abalone *H. discus discus* (Kiryu et al. 2013) and the European abalone *H. tuberculata* (Balseiro et al. 2006) in the wild or in culture facilities, as well as flat abalone *H. wallalensis* and Japanese abalone *H. discushannai* (C. S. Friedman unpubl. obs.) in laboratory challenges. WS-RLO has not been identified in any non-haliotid hosts, including limpets and snails cohabiting with WS-RLO infected abalone (Moore et al. 2002, California Department of Fish and Wildlife [CDFW] unpubl. obs.).

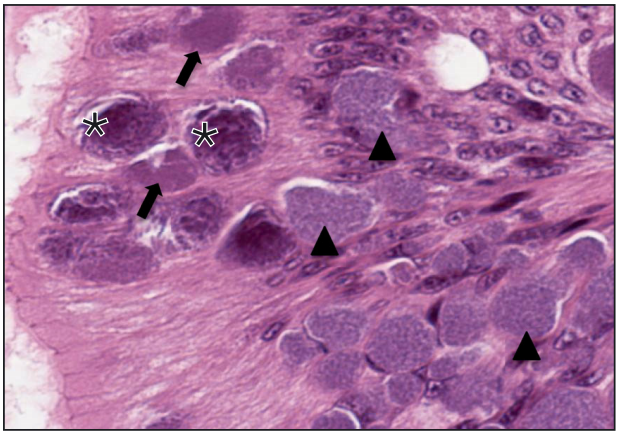

Fig. 2. Light micrograph of a withering syndrome *Rickettsiales*-like organism (WS-RLO; arrows), stippled RLO (ST-RLO; arrowheads) and RLO variant (RLOv; asterisks) inclusions infecting the posterior-esophagus epithelium of California abalone *Haliotis* spp. Individual rod to pleomorphic-shaped RLOv (phage-infected WS-RLO) are visible by light microscopy while individual WS-RLO and ST-RLO are not. Note differential H&E taining properties of RLOs. 1000× magnification

WS was first observed in black abalone populations on the south shore of Santa Cruz Island, California, USA, in 1985 shortly after the strong 1982–1983 El Niño-Southern Oscillation (ENSO) event and subsequently spread to new locations and other abalone host species. From 1986 to 1989, black abalone population declines and WS were seen at Anacapa Island followed by losses on Santa Cruz, Santa Rosa, Santa Barbara and San Miguel Islands, California (Davis et al. 1992, Tissot 1995). WS associated declines in black abalone were first observed at San Nicolas Island (SNI) in 1992 and, as in other affected areas, resulted in markedly increased population declines as compared with pre-WS losses (VanBlaricom et al. 1993, Ruediger 1999). In 1988, WS was observed in Diablo Canyon (Steinbeck et al. 1992) but was not observed elsewhere along mainland California until its discovery north of Point Conception (Altstatt et al. 1996). By 1992, evidence of infected black abalone was reported at all southern California islands except Santa Catalina, especially during seasonal warm water events (Haaker et al. 1992, Tissot 1995, Raimondi et al. 2002).

The 1997–1998 ENSO was associated with enhanced clinical signs of disease in wild abalones and also coincided with severe losses in cultured red abalone (Moore et al. 2000, Friedman et al. 2002). WS spread naturally and via anthropogenic movement of farmed red abalone (Friedman & Finley 2003, OIE 2012) throughout southern California, into the warmer waters of Baja California, Mexico (Casares-Martinez & Tinoco-Orta 2001, Álvarez-Tinajero et al. 2002, Garcia-Esquivel et al. 2007), and northward

Table 1. Light microscopy: rickettsial morphologies (means ± SD) and hematoxylin and eosin (H&E) staining properties (Friedman & Crosson 2012). Inclusion width and length were measured to the nearest 0.01 µm. RLO: *Rickettsiales*-like organism; WS: withering syndrome; RLOv: RLO variant; ST: stippled; TSTM: too small to measure; ND: not determined

RLO	H&E staining	Cellular location	Histology fixative	Inclusion width (µm)	Inclusion length (µm)	Bacterial width (µm)	Bacterial length (µm)
WS	Violet	Apical	Davidson's	14.2 ± 5.3	23.2 ± 10.4	TSTM	TSTM
	Violet	Apical	1G4F	15.4 ± 7.0	26.3 ± 11.0	TSTM	TSTM
RLOv	Navy blue	Apical	Davidson's	16.7 ± 7.8	24.1 ± 10.7	2.6 ± 1.0	3.4 ± 1.0
	Navy blue	Apical	1G4F	21.3 ± 7.7	26.1 ± 13.3	1.5 ± 0.7	3.2 ± 1.3
ST	Light blue	Basal	Davidson's	15.26 ± 7.32	19.34 ± 5.49	TSTM	TSTM
	Light blue	Basal	1G4F	ND	ND	ND	ND

from the late 1980s to the present (Lafferty & Kuris 1993, Altstatt et al. 1996, Miner et al. 2006). Both clinical disease and the WS-RLO were observed as far north as Point San Pedro (San Francisco County, California) by 1999 (Friedman & Finley 2003). Clinical WS continues to spread in a northward direction (Miner et al. 2006) and is strongly associated with declines in abundance co-occurring with increasing coastal warming and El Niño events (Tissot 1995, Altstatt et al. 1996, Raimondi et al. 2002). The WS-RLO is considered to be continuously distributed along the west coast of North America from Baja California, Mexico, to southern Sonoma County, California, including the Channel and Farallon Islands (Álvarez-Tinajero et al. 2002, Friedman & Finley 2003, CDFW unpubl. obs.). During a 1999–2000 sampling event, WS-RLO was identified in 2 red abalone populations in northern California, Van Damme State Park and Crescent City (Friedman & Finley 2003), but has not been detected at those locations since, including histological examination of over 700 red abalone from Van Damme during 2001–2009 (CDFW unpubl. obs.). The anthropogenic introductions at these locations may have failed to become established because of low temperatures. As infected abalones have been transported to Chile, PR China, Taiwan, Iceland, Ireland, Israel, Spain, Thailand (Wetchateng et al. 2010) and most recently Japan (Kiryu et al. 2013), and possibly other countries, the geographical range of the etiological agent is suspected to be broad where California red abalones are cultured or in areas where native species have been exposed to this species.

Climatic changes and short-term ocean temperature increases have the potential to significantly alter host–parasite dynamics in abalones infected with bacterial pathogens such as RLOs and make WS one of the most severe threats to abalone populations (Neuman et al. 2010). Temperature can modulate both the transmission and development of WS (Moore et al. 2000, Braid et al. 2005, Vilchis et al. 2005). Thermal induction and increased disease expression have been documented in both laboratory challenged and field RLO-infected animals including red (Vilchis et al. 2005, Moore et al. 2000, 2011) and black abalones (Tissot 1995, Friedman et al. 1997, 2002). WS-RLO transmission and subsequent WS development in red abalone were nearly negated at 12.3°C (only 1% transmission and no clinical signs of disease), while up to 94% transmission and extreme clinical signs were observed at 18.7°C (Braid et al. 2005). Although a relationship between food availability (fed or complete starvation) and WS-RLO transmission was observed (Braid et al. 2005), under more realistic feeding conditions (100, 30 and 10% feeding rates) food availability and WS-RLO transmission were not correlated, further illustrating the importance of temperature in the ecology of this disease (Vilchis et al. 2005).

Temperature appears to have a significant influence on WS in the field. Since initial observation after the 1982–1983 ENSO, WS has been repeatedly associated with seasonal or decadal thermal events (Haaker et al. 1992). Steinbeck et al. (1992) investigated mortality of black abalone within, and adjacent to, the discharge plume of the Diablo Canyon Power Plant during 1988–1989 and found that animals with clinical signs of WS were located exclusively in the thermal discharge zone where water temperatures measured up to 11°C above ambient. Lafferty & Kuris (1993) also discovered a significant correlation between WS mortality rates and warmer locations. Tissot (1995) suggested high temperature was the most important factor limiting black abalone population recovery on Santa Cruz Island. Subsequently, during the severe 1997–1998 ENSO, when markedly elevated seawater temperatures occurred throughout southern and central California, up to 70% of black abalone at surveyed field sites showed clinical signs of WS (Raimondi et al. 2002, Friedman et al. 2003). High daily temperature variability may also increase the susceptibility of black abalone to WS infection, although disease expression was not seen in abalone until temperatures exceeded thresholds known to facilitate infection (Ben-Horin et al. 2013). Since the decadal regime shift in the mid-1970s, thermal anomalies have been more common and of longer duration than during the previous 25 yr (NOAA 1998). ENSO-neutral conditions were predicted through mid-2009. However, given that annual seasonal thermal maxima in southern California typically reach 17–19°C, temperatures known to augment WS, understanding the role of both seasonal and anomalous ocean warming is crucial to understanding the ecology of marine diseases (NOAA 1998).

DIFFERENTIAL SUSCEPTIBILITY AND DISEASE RESISTANCE

While the WS-RLO infects all haliotids tested to date, susceptibility varies among species. Levels of WS range from little effect and no mortality (e.g. wild green and pink abalone: Álvarez-Tinajero et al. 2002, Moore et al. 2009) to moderate mortality (e.g. red abalone: Moore et al. 2000, 2001) to catastrophic impacts with up to 99% population mortality over a

span of several years on large spatial scales (e.g. black abalone: Altstatt et al. 1996, Moore et al. 2002, Raimondi et al. 2002, Miner et al. 2006, Friedman et al. 2007), conferring significant alterations to marine nearshore biodiversity (Haaker et al. 1992, Tissot 1995, Friedman et al. 2000, Miner et al. 2006). Vilchis et al. (2005) conducted a long-term study in which development of clinical WS was observed in red but not green abalone at elevated temperatures. These results agreed with a similar study conducted by Moore et al. (2009) in which green abalone exposed to WS-RLO were relatively resistant to disease expression under ENSO conditions. However, thermal modulation remains a key factor, as demonstrated by Garcia-Esquivel et al. (2007), who observed that green abalone held at elevated laboratory temperatures of 25°C experienced more clinical signs of WS and higher mortality then those held at 20°C. A survey of wild abalones from Baja California showed that 32% of pink and 27% of green abalones had clinical signs of WS. Few (<7%) to no abalones had advanced signs of WS, and clinical signs of WS did not correlate with WS-RLO presence (Álvarez-Tinajero et al. 2002).

In contrast, white and black abalones are highly susceptible to WS-RLO infection: up to 100% mortality (Fig. 3) (C. S. Friedman unpubl. data) and 99% (Friedman et al. 2002, Raimondi et al. 2002), respectively. White abalone captive rearing programs have experienced substantial losses after ~2–3 yr of culture when animals succumbed to WS-RLO infections and most died from the disease (Friedman et al. 2007). A laboratory study was conducted to compare the susceptibility of white and green abalones to WS when held at 18°C, and few losses (<20%) were observed in green abalone over the course of 26 wk, while 100% of white abalone died within 13 wk (Fig. 3) (C. S. Friedman unpubl. data). Initial losses of green abalone were attributed to handling stress, not WS, as no green abalone died after the first 10 wk of study.

In addition to differences in disease susceptibility among species, it was recently observed that different populations of a single species appear to respond differently to the presence of WS. Black abalone from SNI have been under significant WS pressure for over 20 yr (Fig. 4A) (VanBlaricom et al. 1993). Between 1992 and 2001, a 99.2% decline in black abalone density (11.22 to 0.095 m^2, respectively, in permanent plots sited purposely in high-density patches of abalone) occurred on SNI due to WS. From 2002 to 2012, abalone densities on SNI increased over 200% from the minimum in 2001, via recruit-

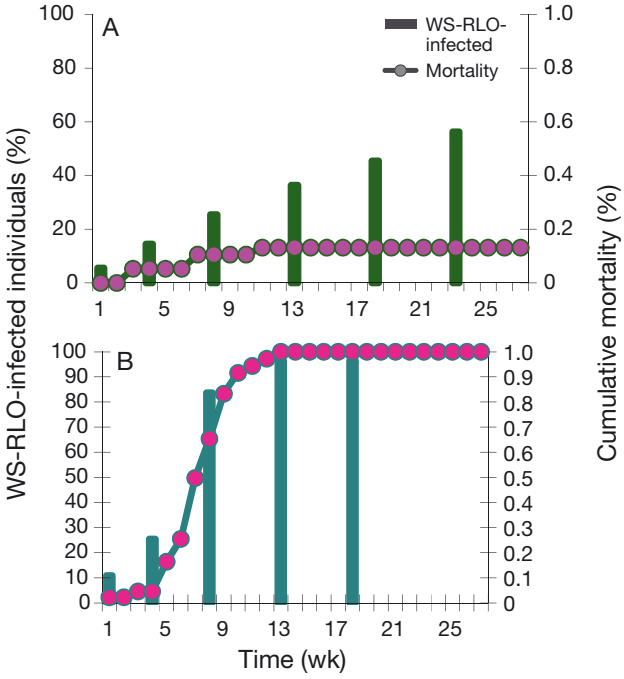

Fig. 3. Differential susceptibility of (A) green abalone *Haliotis fulgens* and (B) white abalone *H. sorenseni* to withering syndrome *Rickettsiales*-like organism (WS-RLO). Bars represent the percent of WS-RLO-infected individuals, while circles represent cumulative proportion mortality (C. S. Friedman unpubl. data)

ment events and apparently improved survival rates despite presence of the WS-RLO (Fig. 4B) (G. R. VanBlaricom unpubl. data). Thus despite catastrophic losses, a small number of black abalone survived to reproduce. The observation of population increases on SNI suggested that the 1–2% of black abalone that survived WS epidemics were more resistant to WS than populations not experiencing disease pressure (Friedman et al. 2014b). It was hypothesized that survivors were able to resist infection by mounting a sufficient immune response and/or resisting bacterial secretions thought to induce host metaplasia.

The hypothesis that abalone populations under disease pressure selected for the development of disease resistance was tested in the laboratory using progeny of surviving abalone from SNI (Site 8 animals; Fig. 4B) and 'naive' black abalone from Carmel, California, that had not been exposed to WS epidemics and thus were not under selection for improved tolerance (Friedman et al. 2014b). Upon RLO exposure at 19°C, decreased mortalities were observed in SNI abalone compared to those from Carmel (Friedman et al. 2014b). Significant differences in survival were observed among treatments ($p < 0.001$); more RLO-exposed abalone from Carmel died than did those from SNI ($p < 0.05$), while no dif-

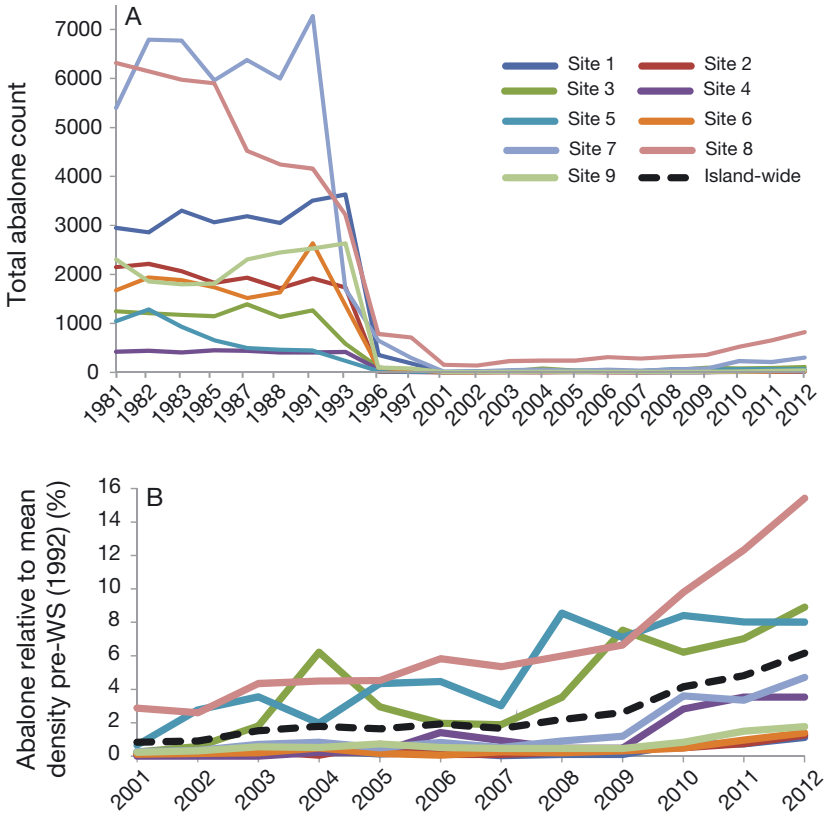

Fig. 4. *Haliotis cracherodii*. Black abalone population trends on San Nicolas Island, CA, USA. (A) Total abalone counts from 1981–2012. Note sharp population declines after withering syndrome (WS) was first observed in 1992. Declines at some sites prior to 1992 are likely due to overfishing. (B) 2001–2012 only. Proportion of abalone relative to mean abalone density pre-WS epidemics (1992). Note strong recruitment event at Site 8, where abalone for disease resistance testing were collected (VanBlaricom et al. 1993, G. R. VanBlaricom et al. unpubl. data)

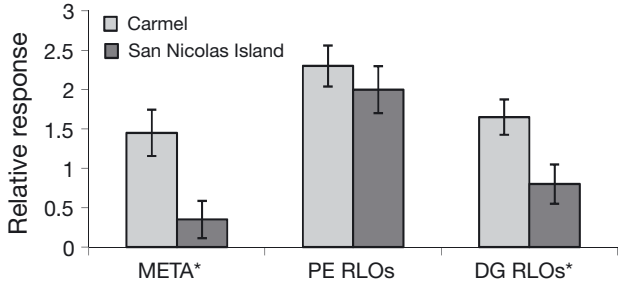

Fig. 5. *Haliotis cracherodii*. Microscopic observations of H&E stained black abalone tissues. Significant differences between San Nicolas Island (withering syndrome [WS] resistant) and Carmel (WS 'naive') animals are noted (asterisks). Error bars represent SE. Meta: metaplastic response; PE RLOs: combined *Rickettsiales*-like organism intensity in the posterior esophagus; DG RLOs: combined RLO intensity in the digestive gland (Friedman et al. 2014). See Friedman et al. (2002) for relative response scoring

ferences in survival were observed between control groups ($p > 0.05$). All RLO-exposed abalone that died exhibited clinical signs of WS, and microscopic examination suggested that resistance to WS might be more related to the host response to initial infection than to the ability to resist infection, as resistant abalone showed significantly less metaplasia and a corresponding lower RLO infection intensity in the digestive gland (Fig. 5) (Friedman et al. 2014b). Analysis of WS-RLO DNA by quantitative PCR (qPCR) of feces from both populations showed that more WS-RLO DNA was excreted from Carmel abalone compared with those from SNI, suggesting that abalone from SNI (survivors of high disease pressure) express a trait or have some characteristic that decreases the ability of RLOs to proliferate in the digestive gland (Fig. 5) (Friedman et al. 2014b). Clearly, a distinct difference in disease resistance exists among black abalone populations independent of temperature. Whether or not the observed differences are of genetic origin is currently being explored (L. M. Crosson et al. unpubl.).

PHAGE HYPERPARASITE

Friedman & Crosson (2012) recently observed a morphological variant of the WS-RLO infecting red abalone from central California and used a combination of light and electron microscopy, *in situ* hybridization and 16S rDNA sequence analysis to compare the WS-RLO and the RLO variant (RLOv). WS-RLO morphology has been consistent with its original taxonomic description (Friedman et al. 2000) and forms oblong inclusions within the abalone posterior esophagus (PE) and digestive gland (DG) tissues that contain small rod-shaped bacteria; individual bacteria within inclusions, which appear light purple upon hematoxylin and eosin (H&E) staining, cannot be discerned by light microscopy (Table 1, Fig. 2) (Friedman & Crosson 2012). Like the WS-RLO, the RLOv forms oblong inclusions in the posterior esophagus (PE) and DG but these contain large, pleomorphic bacteria that stain dark navy blue with

H&E (Table 1, Fig. 2) (Friedman & Crosson 2012). Transmission electron microscopy (TEM) examination revealed that the large pleomorphic bacteria within RLOv inclusions were infected with a spherical to icosahedral phage hyperparasite (Fig. 6) (Friedman & Crosson 2012). Binding of the WS-RLO-specific *in situ* hybridization probe to the RLOv inclusions demonstrated sequence similarity between these RLOs. In addition, sequence analysis revealed 98.9–99.4% similarity between 16S rDNA sequences of the WS-RLO and RLOv. Collectively, these data suggest that both of these RLOs infecting California abalone are '*Candidatus* Xenohaliotis californiensis' WS-RLO and that the novel variant is infected by a phage hyperparasite that induced morphological variation of its WS-RLO host.

The presence of a phage hyperparasite exhibits interesting properties that appear to affect the host–pathogen relationship between the WS-RLO and the abalone. For example, in a recent experiment with juvenile black abalone, both WS-RLO and phage-infected inclusions were statistically related to tissue pathology and mortality ($p < 0.05$; Friedman et al. 2014b). Like the WS-RLO, the phage-infected inclusions appeared to increase in prevalence and intensity with increasing temperature. Curiously, mortalities of abalone infected with all RLO types appeared to be delayed and significantly reduced relative to previous studies with the WS-RLO alone (Friedman et al. 2014b). When black abalone were exposed to WS-RLO, ST-RLO and RLOv in combination, the trial lasted 17 mo, during which 48% of the animals died. However, when abalone were exposed to the WS-RLO alone, they experienced 71% mortality in only 7 mo. In addition, when the phage (RLOv) was absent, WS-RLO loads were higher and the host metaplastic response was ~2 times that observed when the phage was co-occurring (Friedman et al. 2014b). It is likely that the presence of the phage is attenuating WS disease development and consequences of infection will vary among host species and with temperature. Current studies are underway to discern whether the

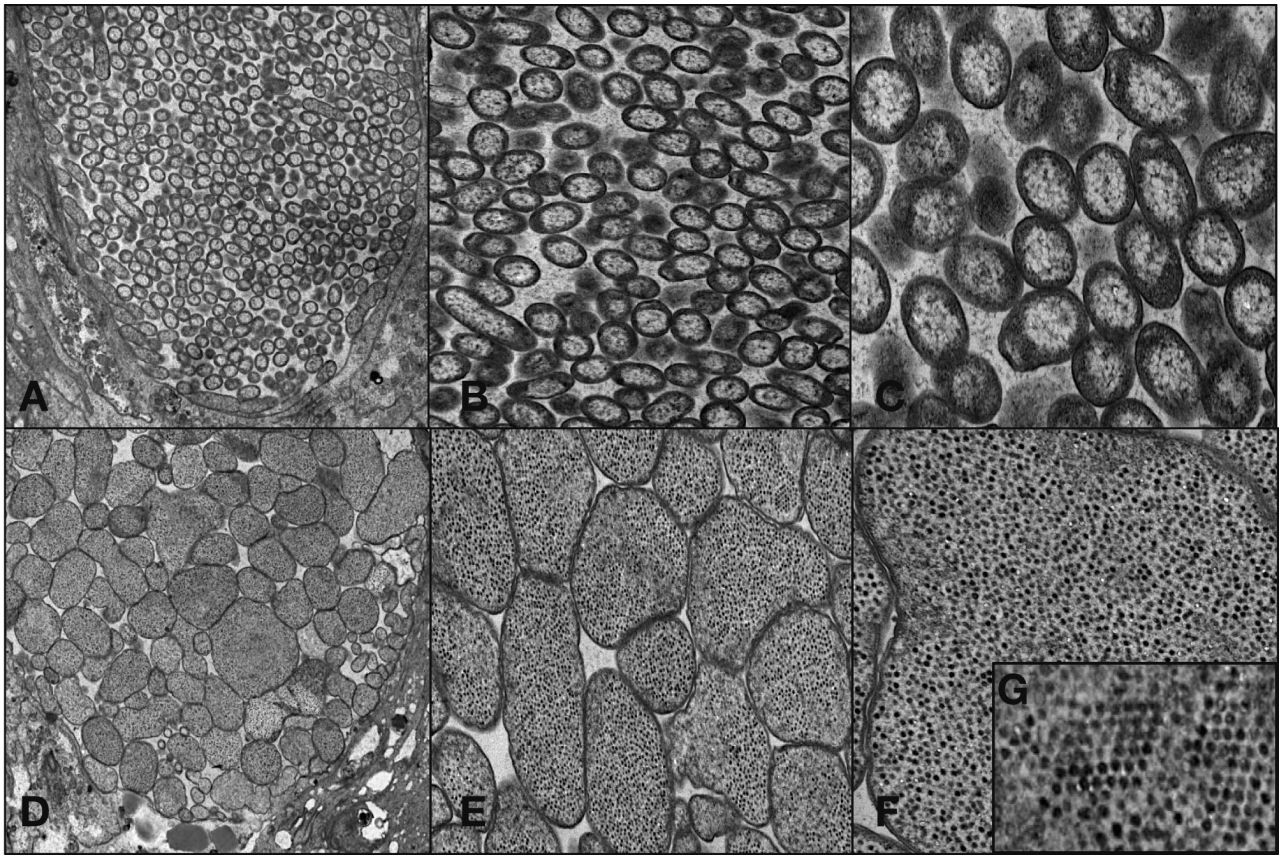

Fig. 6. Transmission electron micrographs of California red abalone *Haliotis rufescens* rickettsial inclusions following preservation in Karnovsky's solution. Withering syndrome *Rickettsiales*-like organism (WS-RLO) at (A) 6800×, (B) 13 000× and (C) 30 000× magnification. RLO variant (RLOv; phage-infected WS-RLO) at (D) 4800×, (E) 13 000× and (F) 30 000× magnification. (G) Inset illustrating phage morphology and virions in paracrystalline array (Friedman & Crosson 2012)

phage infections alter physiological processes, such as virulence and pathogenicity, in its WS-RLO host. Also of interest, red abalone farms in California experiencing seasonal losses due to WS since ~1990 (e.g. Moore et al. 2000) and with confirmed RLO-infected animals are currently reporting decreased losses in product-sized abalone relative to previous trends prior to the observation of the phage hyperparasite (R. Fields pers. comm.).

DIAGNOSTIC METHODS

Diagnosis of WS requires the identification of infection with the pathogen (WS-RLO via *in situ* hybridization or via histology coupled with PCR and sequence analysis) accompanied by morphological changes that characterize the disease (e.g. pedal and DG atrophy, and DG metaplasia). Definitive diagnosis of WS must be conducted according to World Organization of Animal Health (OIE) standards in the Manual of Diagnostic Tests for Aquatic Animals (OIE 2012). *In situ* hybridization is the method of choice for WS-RLO confirmation because it allows visualization of a specific DNA probe hybridized to the target pathogen. The *in situ* hybridization technique for WS-RLO developed by Antonio et al. (2000) is extremely useful in visualizing initial stages of infection in subclinically infected abalone. Although this method was not formally validated, tests for specificity using several bivalve and fish RLOs suggested the test was specific for WS-RLO only (Antonio et al. 2000).

A conventional PCR assay that specifically amplifies a 160 bp segment of the WS-RLO 16S rDNA sequence available in GenBank (AF133090) was developed by Andree et al. (2000) and allows for greater sensitivity than histology alone. A qPCR assay was also developed to specifically identify and enumerate bacterial loads of WS-RLO in abalone tissue, fecal and seawater samples based on 16S rDNA gene copy numbers (Friedman et al. 2014a). Both PCR assays designed to detect DNA of the WS-RLO were formally validated according to OIE (2012) standards (Friedman et al. 2014a). The conventional PCR assay limit of detection was 300 gene copies and 3 gene copies for qPCR. Thus qPCR was over 100 times more sensitive than conventional PCR in detecting target DNA (Friedman et al. 2014a). Also, the ability of qPCR to detect and quantify very small amounts of WS-RLO gene copies in a variety of sample types will enable researchers to better understand WS transmission dynamics in both farmed and natural environments while providing a useful, non-lethal tool for WS monitoring. However, it is important to note that DNA-based PCR assays do not detect a viable agent or infection and serve only as a proxy for infection or exposure. Histological examinations remain the gold standard and show clear evidence of infection but may not enable one to discern the taxonomy of the agent (Burreson 2008, OIE 2012). Both conditionally independent tests should be used collectively for proper WS diagnosis.

CONTROL AND RECOMMENDATIONS

The most effective prevention of WS is avoidance of the pathogen. Avoidance is best accomplished by the establishment of a health history and multiple health examinations prior to movement of animals. Although histology or *in situ* hybridization is required to confirm infection, PCR is able to detect small amounts of pathogen DNA and is recommended for inclusion in health examinations. Good husbandry practices are essential for control of any bacterial disease and include reducing stocking densities, avoiding grading or mixing of disparate groups or families, and rinsing hands and equipment in fresh or iodinated water between groups and/or tanks. Holding abalones at cooler temperatures (<15°C) may also reduce WS-RLO transmission (Braid et al. 2005). Infected groups should be isolated and culled or administered oral or bath treatments with oxytetracycline as per federal regulations (Friedman et al. 2003, 2007).

The ecology of RLOs in abalone disease warrants further investigation. For successful restoration and management of all abalone species, it is crucial to identify the ST-RLO and newly observed WS-RLO phage, understand the host–parasite–environment relationships, and characterize their roles in abalone disease (i.e. competition with the WS-RLO). Much research on host–parasite relationships involves interactions between a single host and one parasite/pathogen. However, evidence from a wide variety of systems suggests that mixed infections involving 2 or more parasite genotypes or species in a single host are becoming more common and, in some cases, may be the rule. Multiple pathogen infections have been examined in numerous host systems including a variety of invertebrates such as oysters (Stokes & Burreson 2001), crustaceans (Tang et al. 2003) and abalones (Hine et al. 2002, Balseiro et al. 2006). Understanding the role of abalone–RLO relationships under varying environmental conditions will be imperative to abalone resource management in the

face of global climate change. To achieve protection and sustainable use of abalone resources, we must also understand interactions among wild and farmed animals and their potential impacts on disease transmission dynamics, especially in declining and endangered species.

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