Report to the World Organization for Animal Health: Recommended measures to control *Candidatus Xenohaliotis californiensis*, the causative agent of abalone withering syndrome

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Overview

In recent decades, abalone (*Haliotis* spp.) populations in some areas of the west coast of North America have experienced declines in abundance in excess of 90% due to widespread infection with the Rickettsia-like organism (RLO) *Candidatus Xenohaliotis californiensis*, which causes the intense pathology commonly called withering syndrome (WS; Crosson et al., 2014). Past and continued transport of infected *Haliotis rufescens* from California aquaculture facilities to parts of Europe, Southeast Asia, South America, and Oceania may have introduced this pathogen to previously unaffected abalone populations, making an understanding of its epidemiology and pathology increasingly important (Kiryu et al., 2013; Wetchatang et al., 2010). New evidence suggests that a recently discovered hyperparasitic phage targeting RLO may act to combat the pathology of these infections and have a net therapeutic effect on the abalone that its host infects (Vater et al., 2018). Here, I explore the roles of each member of this three species interaction and the potential use of augmentation of the hyperparasitic phage as a conservation tool to control the impacts of RLO on the abalone stocks it affects, finally presenting a list of management recommendations to control the threat of WS RLO to global abalone populations.

Background

Ecological and Anthropogenic Importance of Haliotis *spp*.

Abalone are large marine gastropods in the genus *Haliotis*. Worldwide there are approximately 50 species and of those that have been exposed to RLO, all are viable hosts (Crosson et al., 2014). They typically inhabit intertidal and subtidal zones where they feed primarily on macroalgae, both as grazers and drift feeders (Crosson et al., 2014). In doing so they modify their habitat significantly, maintaining different macroalgal assemblages than those that grow in the presence of other grazers, creating unique microhabitats in areas where they are dominant in the grazing community (Wing et al., 2015). Adult abalone are important food sources for coastal marine mammal species, primarily otters, while juveniles are common and locally important prey for a number of fish and invertebrate species (Chades et al., 2012; Hofmeister et al., 2018).

Abalone also provide important fisheries and cultural services to humans. Abalone shells are the primary source of mother of pearl, a material that once served as currency and jewelry in multiple indigenous cultures and continues to be used commonly for fine inlay and jewelry (Cook, 2014). Harvest for meat takes place or historically took place throughout the range of all known species and, despite general declines in abundance in most *Haliotis* species, still sustains important wild capture fisheries, notably in Southern Africa (Troell at al., 2006), Southern Australia (Mayfield et al., 2011), New Zealand (Egger and Kahui, 2013), and, until very recently, the coast of California (Tegner et al., 2001). Declines in wild populations caused by disease and, more commonly, overharvest have led to domestication of a number of species and large scale

aquaculture, especially of the red abalone (*Haliotis rufescens*) on the Eastern Pacific coast, the Japanese abalone (*H. discus*) throughout east Asia, and the South African species *H. midae* (Vivanco-Aranda et al., 2011, Body, 1987, Rhode et al., 2012). Globally, wild capture and aquaculture together comprise a fishery annually worth almost 1 billion USD (Cook, 2014).

Candidatus Xenohaliotis californiensis *and Associated Withering Syndrome Pathology*

Ca. X. californiensis is a prokaryote in the family Rickettsiaceae. It forms intracellular inclusions in the digestive epithelium of its abalone host, primarily in the post-esophagus and less commonly in the digestive gland and intestine (Crosson et al., 2014). It was first suspected as the causative agent of WS in the mid-1990s and confirmed as such in 2000; prior to that point the source of the disease was largely a mystery to abalone managers and researchers (Friedman et al., 2000).

The pathology of WS is characterized by starvation-like symptoms including progressive decrease in body mass, weakness and inability to adhere or self-right, metaplasia of digestive tissues, and atrophy of pedal muscle fibers (Crosson et al., 2014). Once symptoms appear the disease is progressive and causes almost 100% mortality in all *Haliotis* spp. thus far examined (Crosson et al., 2014). Pathology is more common at higher water temperatures and is directly related to the thermal tolerance range of the infected species—that is, symptoms generally appear only when hosts experience thermal stress (Crosson and Friedman, 2018). Abalone species are differentially susceptible; in the well-studied north-eastern Pacific species, susceptibility corelates negatively to thermal tolerance (Moore et al., 2009, Gonzales et al., 2014, Crosson and Friedman, 2018).

Transmission is thought to be accomplished entirely through fecal-oral routes, though this can include suspended feces, meaning abalone need not come in direct contact with infected individuals to contract WS RLO (Crosson et al., 2014). Factors affecting rate or nature of transmission are the topic of current research and have not yet been fully characterized.

Where and when the RLO responsible for WS appeared is not entirely clear. The earliest die-offs that have been attributed to WS occurred in black abalone (*H. cracherodii*) in 1985 off the coast of Santa Cruz island in Southern California following a major El Nino event (Crosson et al., 2014). In subsequent years, it has been responsible for widespread mortality and pathology in both cultured and wild abalone and is distributed continuously along the North American west coast from Sonoma County, California southward to Baja California, Mexico (Álvarez Tinajero et al., 2002, Friedman and Finley, 2003). Accidental introductions in transported cultured *H. rufescens* have led to possible establishments in Chile, China, Taiwan, Iceland, Ireland, Israel, Spain, and Thailand (Kiryu et al., 2013; Wetchatang et al., 2010) with confirmed transmission in wild and cultured abalone in Western Europe (Balseiro et al., 2006). Thermal barriers apparently prevent range expansion; for example, on the North American west coast a northward bound to the its range exists despite continued availability of potential hosts further north. However, what WS RLO's exact thermal tolerance is has not yet been studied (Friedman and Finley, 2003).

Current Treatments for WS RLO

The current standard treatment for WS RLO in cultured abalone is the antibiotic oxytetracycline (Friedman et al., 2003). Injection and oral administration are both very effective at lowering

infection rate, preventing mortality, and improving growth rate of infected animals (Friedman et al., 2003). Oxytetracycline dips have been proposed and tested in a series of recent studies but have not proved successful in any trials to date (Winkler et al., 2018). Thermal control is also an effective means of preventing pathology, though specific temperatures at which WS pathology will or will not occur vary among species (Moore et al., 2000). No means of control in a wild population has yet been developed; the only currently effective means of stopping the spread of WS RLO on a population scale is prevention of new introductions (Crosson et al., 2014).

WS RLO Phage

The phage hyperparasitizing WS RLO was first discovered by Friedman and Crosson (2012) in morphologically changed RLO inclusions. The presence of the phage has been linked to decreased pathogenicity and mortality from WS RLO infection in *H. rufescens* and *H. cracherodii* while in *H. sorenseni* phage infection has no effect on WS pathology (Vater et al., 2018, Friedman et al., 2014). How and why these differences in therapeutic effects occur is not yet known, nor is the existence or extent of such effects on other *Haliotis* spp.

Recommended management actions

Prevention

Prevention of further spread of WS RLO must be prioritized. Current levels of stringency in transporting live abalone from infected populations have not been enough to prevent spread—indeed, no consistent international protocol currently exists for transporting live abalone from infected areas—and repeatedly artificial introduction has been responsible for WS RLO's

dissemination. As suggested by Crosson et al. (2014), establishment of veterinary health records and pre-transport screening should become universally implemented for any abalone being moved from areas of known infection. I suggest a preventative system involving preemptive treatment of any animal to be transported with continual oral administration of oxytetracycline in feed over a 14-day period as described in Friedman et al. (2003). This would be followed by qPCR analysis of fecal material using the assay described in Friedman et al. (2014) to determine whether infection persisted; any animals which continue to shed RLO would undergo repeated treatments until qPCR results showed infection was successfully cleared.

Local eradication

Where possible, ubiquitous treatment of cultured abalone with known antibiotic methods to 1) prevent further spread of infected cultured abalone 2) increase growth and lower mortality of cultured abalone and 3) prevent avoidable transmission from infected farmed abalone to wild populations. Treatment would mirror the plan described in the previous section; all animals displaying WS pathology would be culled prior to beginning treatment as they already face near certain mortality (Crosson et al., 2014). This is only a feasible measure in regions where WS RLO infection is limited to cultured animals at a known number of facilities to ensure 100% of infected animals are treated. If infection has spread to wild populations or has become widespread throughout cultured animals, this measure becomes increasingly less realistic.

Further research

As of yet, relatively few species' reactions to WS RLO have been fully characterized; better understanding its pathology and infectiveness on unstudied species could better inform how and where its spread will be problematic or of relatively low concern. Specifically, this would involve expanding the number of species which have been subjected to WS RLO challenge trials to determine other species susceptibility to pathology and rates of mortality, allowing those most at risk to be given greater protection priority.

Characterizing the environmental tolerances of WS RLO could also be highly informative in determining the path of its spread and what effects changing climate might have on its distribution. Studies should be done examining the greatest extremes of pH and temperature within its host's tolerance range at which WS RLO is able to survive. This will aid in determining which, if any, abalone populations will be newly at risk of infection. Additionally, as pathology does not occur at cooler temperatures (Crosson et al., 2014), the thermal threshold at which it does begin to occur in abalone species vulnerable to WS RLO exposure should be determined to inform which populations will actually face consequence in the event of infection.

Very little is known of the effects of the WS RLO phage in controlling its hosts prevalence or pathology. Considerably more research is needed to determine it effects on WS RLO and related pathology in more *Haliotis* spp. to better understand its potential as a conservation tool.

Bacteriophage therapy has already been established as a viable means of treating at least one other abalone disease, vibriosis, supporting the possibility of its usefulness in combating WS RLO (Wang et al., 2017). The phage treatment used by Wang et al. (2017) to

treat vibriosis involved dipping infected animals into a broth containing cultured phage and is thus only effective for application to cultured animals in its current form. In order to realize the potential benefits of phage therapy in wild populations, new techniques will need to be developed to administer phage in the field, where animals are relatively dispersed in comparison to cultured abalone. Currently, no comparable application of phage therapy exists from which to base a population level treatment plan. The possibility of genetically engineering a more potent WS RLO phage has similarly not been explored, despite being a relatively well-developed strategy in treatment of some human diseases (Housby and Mann, 2009). Considerable research and testing remains to be done before application of the phages therapeutic effects in the field is possible.

Conclusion

Abalone hold great ecological, economic, and social importance in coastal regions throughout the world. Increasingly widespread WS RLO and a changing ocean imperil them; new management solutions must be found to control this pathogen. To prevent further spread, I recommend greater regulation of the transport of live abalone via establishment of veterinary health records and preliminary antibiotic treatment. Anthropogenic introductions of WS RLO which are discovered prior to spreading to wild abalone populations should be stopped by a combination of antibiotic treatment and culls to prevent further range increase. In order to better predict where new introductions of WS RLO will have the greatest impact, more research must be done to characterize its environmental tolerances and effects on previously unexposed abalone species. To stop the high levels of mortality facing populations already afflicted with

widespread WS RLO infection, new treatments must be developed and WS RLO's naturally occurring hyperparasitic phage may prove key in doing so.

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