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**Research Summaries** 

#### Title

Restoration of Endangered White Abalone: Resource Assessment, Genetics, Disease and Culture of Captive Abalone

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# Restoration of Endangered White Abalone: Resource Assessment, Genetics, Disease and Culture of Captive Abalone

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### BACKGROUND

Once harvested commercially, the white abalone (Haliotis sorenseni) is in danger of extinction and since 2002 has been under the protection of the federal Endangered Species Act. Recent failed efforts to find remnant populations in the species' historical deep-seafloor habitats underscore the animal's scarcity. In the time between when the grant for the project was written and funded, NOAA Fisheries halted the collection of wild white abalone for research. It now appears that species recovery is either going to happen on its own over the course of several or more decades, or captive-bred white abalone will have to be outplanted in the wild. Outplanting is not without its own set of risks, as it may erode genetic diversity or introduce the withering syndrome pathogen into previously uninfected deep-water refuges. Just as worrisome is the potential to unknowingly introduce previously unknown pathogens.

The goal of this project was to collect life history, genetic, disease susceptibility and treatment data to support recovery. Each component of the project was led by one of four investigators with expertise in the particular, relevant subject area. Their findings are summarized, in brief, below. The interested reader is encouraged to obtain the full report to Sea Grant available at the University of California eScholarship Repository at http://repositories.cdlib.org/ csgc/rcr/Fisheries07\_02 and/or the peer-reviewed articles listed on the back of this page.

## **SURVIVAL AND GROWTH**

The optimal temperature range for white abalone larvae was found to be between 12 °C and 15 °C. At 12 °C, larvae were competent to settle in seven days. At 15 °C and 18 °C, larvae were ready to settle in five days. These settlement periods are significantly shorter than reported in previous studies, but are similar to rates for sympatric species such as red, green and pink abalone.

In general, juvenile and early adult growth was maximized at 15 °C. Rapid growth could be achieved at 18 °C on a mixed diet of macroalgae; however, in general, mortality rates were high in these abalone and they were prone to withering syndrome (WS).

WS is a chronic, progressive wasting disease, caused by *Candidatus Xenohaliotis californiensis*, an intracellular Rickettsiales-like prokaryote that infects an abalone's gastrointestinal tract.

The researcher wrote in his final report to Sea Grant that overall, white abalone grow and survive best at temperatures of 15  $^{\circ}{\rm C}$  or

less on a mixed diet of giant kelp (*Macro-cystis pyrifera*) and Pacific dulse (*Palmaria mollis*). At this temperature, there was also little or no expression of WS.

### DISPERSAL

In terms of basic science, one of the most exciting discoveries of this project was the "standing" behavior of juvenile and young adult white abalone in response to the presence of drifting substrate. Abalone were observed to

raise themselves onto their shell edge and "climb" onto drifting pieces of kelp. This "rafting" behavior, which was studied in a series of experiments with 1- to 3-year-old abalone and which has never been described for any other abalone species, may explain the wide distribution of white abalone throughout the Southern California Bight.

To test the frequency and duration of rafting, fragments of macroalgae typically found in white abalone habitat were passed down a flume stocked with abalone. More than six percent "climbed" onto giant kelp during the short 20-second transit time of the algae in the flume. Significantly more abalone (p<0.01) "climbed" onto giant kelp than any other test substrate, including two other macroalgae and a piece of rubber "substrate." Trials with red abalone resulted in no instances of "standing" or "climbing" behavior.

In separate experiments to measure the duration of rafting, white abalone stayed attached to the kelp blades for up to 51 days. The scientist wrote that algal rafting could potentially transport benthic life stages or groups of small abalone far beyond the range of larval dispersal.

## **SEARCHING FOR WILD ABALONE**

Submarine and diver surveys of white abalone habitat in the 1990s showed that populations were too low to sustain reproduction. Divers from the California Department of Fish and Game, Channel Islands National Park, and the Channel Islands National Marine Sanctuary searched in vain several times for white abalone in its historic range. During the course of the surveys, only six *red* abalone were observed. Dives in the same areas in the 1980s would have detected hundreds of white and red abalone. The divers noted that brittle stars have taken over many of the former abalone habitat areas.

### **GENETIC ANALYSES**

Prior to its federal listing, 20 white abalone were collected as potential broodstock for a captive rearing program. Using DNA

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from these animals, researchers developed white abalone genetic markers, including five nuclear microsatellite loci and partial sequences of one nuclear (VERL) and two mitochondrial (COI and CytB) genes, to assess genetic variability in the species, aid in species identification and potentially track the success of future outplanting efforts. All five microsatellite loci were polymorphic and followed expectations of simple Mendelian inheritance in laboratory crosses. Each of the wild-caught specimens exhibited a unique composite microsatellite genotype, suggesting that significant genetic variation remains in natural populations. A combination of nuclear and mitochondrial gene sequencing demonstrated that one of the 20 wild-caught animals was not a white but a pinto abalone, Haliotis kamtschatkana. Similarly, another animal of uncertain identity, accidentally collected during dredging, was also a pinto abalone. Had these two animals been included as broodstock, white abalone could have been unintentionally hybridized. Molecular genetic identifications will be useful in preventing broodstock contamination and monitoring future restocking operations.

## TREATING WITHERING SYNDROME

In this project, studies were conducted to further investigate the effects of WS on white abalone and to determine whether oxytetracycline (OTC), which does not kill the WS pathogen but stops its ability to proliferate, could be administered as a cure.

White abalone were medicated at 90.82 mg/kg of OTC daily for 20 days and the efficacy, elimination and potential to protect against exposure to the WS pathogen were examined. The experiments demonstrated that OTC eliminates WS-bacteria. High concentrations of antibiotic (1089 ppm) were observed in the digestive gland after medication, a pattern similar to that observed for red abalone. Depletion occurred over a prolonged period (many months).

To examine the degree to which the accumulated OTC protects animals from WS, white abalone were medicated with OTC; a subset was then "challenged" with the WS pathogen at five times during the antibiotic-depletion period. The experiments showed that abalone require more than 24 days to clear the pathogen. Medicated abalone were protected from re-infection on days 24, 40 and 67 after medication, but by day 146, they were again susceptible to infection.

Single oral doses of OTC led to antibiotic levels greater than 200 ppm in the digestive gland, which the scientist reports is sufficient to treat and prevent WS infections.

It takes a prolonged treatment regimen, however, to completely eliminate the WS pathogen from white abalone, even in relatively cold water (13.3  $^{\circ}$ C).

OTC doses that were sufficient to control WS-related mortality did not completely eliminate the causative pathogen from white abalone. It took multiple doses of 0.206 mg OTC per kilogram of abalone, administered in feed over several months, to create groups of abalone that were completely free of the pathogen. Their pathogen-free status was confirmed by subjecting animals to water temperatures that would otherwise trigger WS expression (greater than 18 °C): The animals did not develop WS.

Abalone were discovered to be capable of absorbing antibiotics from the water, either through their skin or because they were drinking the water. The discovery has led to a NOAA Fisheries-funded project to test the efficacy of antibiotic baths in treating WS.

## **COLLABORATING ORGANIZATIONS**

BHP Billiton, California Department of Fish and Game, Channel Islands National Marine Sanctuary, Channel Islands National Park, National Fish and Wildlife Foundation and Reliant Energy.

### **STUDENT**

Kristen Gruenthal, Ph.D.

### **PUBLICATIONS**

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

Gruenthal, K.M. and R.S. Burton. 2005. Genetic diversity and species identification in the endangered white abalone (*Haliotis sorenseni*). *Conservation Genetics*. 6:929-939.

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