Buried Alive? The Effect of Sediments on White Abalone Larvae Settlement

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ABSTRACT

Along the coast of California, the negative impacts of sediments input from the post-mining legacies of the Gold Rush era, agricultural runoffs, coastal development projects and erosions from the loss of ground cover have yet to capture the attention of scientists. Many studies have looked at the responses of corals and sea urchins in the presence of sediments. Yet, there are only a few that focused on abalones, moreover an endangered species, the white abalone (*Haliotis sorenseni*). For many abalone species, crustose coralline algae (CCA) play a major role in cueing the planktonic stage larvae into settling onto the substrate. When sedimentation occurs, it interrupts the abalone's lifecycle by trapping larvae in sediments and also reducing the available crustose coralline algae (CCA) surface. In order to investigate the effect of sediment on white abalone larvae settlement, an experiment, using different thickness of silt and ground oyster shell treatment, was conducted. It was found that there was a significant decrease in larvae settlement rate when either types of sediment were present. Besides, many larvae were observed to be trapped in the thick silt treatment, whereas in the other sediment treatments, larvae were observed settling on the side of the culture wells and also on the sides of the CCA plate.

KEYWORDS

Benthic, marine, substrate, captive breeding, silt

INTRODUCTION

With much of the world's population rapidly growing close to the 11 billion marks by the end of the century, the demand for more resources ultimately places tremendous pressure on the natural world to produce even more to feed the world. As a result, the environment has taken a toll, and many of these causes can be traced back to anthropogenic factors. One of the collateral damages seen is the decimation of the abalone populations throughout the coast of California. This is the result of various factors, some are natural, some are human-induced. However, the defining lines between the natural and anthropogenic causes are blurry.

Warming ocean waters, as a result of climate change, has created several problems for the abalone populations. First, warm seawater is nutrient-poor and does not support the growth and recruitment of kelp over long periods, and this is significant for the survival of all the abalone species. In 1963, Leighton & Boolootian found that less kelp meant fewer and less quality food sources for the abalones to develop. Also, the recent sudden rise in urchin population further lowers the abundance of kelp along the coast (Vilchis et al., 2005).On the other hand, existing kelp forests are also threatened by sediment deposition along rocky seafloors. These sediments originate from the post-mining legacies of the Gold Rush era, agricultural runoffs, coastal development projects and erosions from the loss of ground cover. Sediments prevent the kelp from anchoring itself to the seafloor and coming loose during a storm event (Harrold & Reed, 1985). This combination furthers the abundance of kelp along the coast and puts more pressure on the existing white abalone population. The higher seawater temperature does also contribute to the rapid spread of Withering Syndrome (WS), a deadly bacterial infection, among the abalone species (Friedman & Finley, 2003). On the other hand, oil spills and chemical runoffs from coastal regions, especially in the waters of Southern California, have profoundly impacted the recruitment and survival rate, mature individuals. Besides, the high value of abalones on the international market encourages illegal poaching, which will further dampen the any abalone conservation efforts (Rogers-Bennett et al., 2016).

The white abalone (*Haliotis sorenseni*) is the subject of focus as it was the first marine invertebrate to be listed on the U.S. federal endangered species list [National Oceanic and Atmospheric Administration (NOAA 2001); Federal Register 65 FR 2616, and 66 FR 29046]. Since the life history of white abalones is not well understood, captive breeding programs have only seen success in the recent decade (Rogers-Bennett et al., 2016). Japanese abalone enhancement programs in the fishery industry demonstrated the magnitude of a national scale of abalone release is not large enough to sustain a minimum viable population (MVP), not to mention a harvestable abalone population (Hamasaki & Kitada, 2008). However, their data suggests that a local scale release of abalones, based on intensive studies on carrying capacity and habitat parameter variations, is the best way to maximize the chances of success. Furthermore, isolation of individual mature white abalones has an adverse impact on the recruitment rate (Davis, Haaker, & Richards, 1998; Hobday & Tegner, 2000; Vilchis et al., 2005). Based on experimental work, proximity and high density of sexually matured individuals are the key factors in facilitating an optimal reproductive environment for the wild white abalones (Babcock & Keesing, 1999). Another factor that has been largely overlooked is the impact of sediments on the abalone lifecycle itself. For many abalone species, crustose coralline algae (CCA) play a major role in cueing the planktonic stage larvae into settling onto the substrate (Morse, Froyd, & Morse, 1984). Studies have showed high larvae mortality rates are associated with delayed settlement and also the inability to settle on appropriate substrates (Onitsuka et al., 2010; Roberts & Lapworth, 2001). Sediment has the ability to prevent abalone larvae from anchoring itself to a (CCA) surface and undergo metamorphism (Raimondi, Barnett, & Krause, 1997). Additionally, sedimentation on CCA surfaces resulted in bleaching and a reduction in CCA surfaces (Chew, Hepburn, & Stephenson, 2013). This will ultimately result in high mortality rates of abalone larvae and creates a challenge to increasing recruitment rates along the coast.

The primary objective of this research is to answer the critical questions with regards to whether the grain size of sediment and the thickness of sediments have an impact on the settlement stage of white abalone larvae.

METHODS

To understand the impacts of sediments on white abalone larvae, a laboratory experiment was conducted at the Bodega Marine Laboratory (BML) located in Bodega Bay, California. A thin layer of crustose coraline algae (CCA) and a layer of tree-liked upright CCA were cultivated on black aquarium sheets until it was covered. The CCA layer was then divided into 1cm by 1cm squares (CCA plates) and then carefully rinsed with filtered seawater. *H.sorenseni* larvae were obtained from induced spawning of sexually matured adults that were cultivated at the laboratory under optimal seawater conditions. Fertilized eggs were gathered from several adults and hatched into larvae, which were then kept in a culture tank with running filtered seawater at 14°C.

In order to investigate the effects of the physical properties of sediments on larval settlement, silt and ground oyster shell powder were used in the experiment. When exposed to water, silt becomes sticky due to its fine characteristics, while oyster shell powder is less sticky and loose due to the larger grain size. Thirty 8-day old larvae were exposed to seven treatments in total, each with five replicates:

(1) a culture well with filtered seawater and without a CCA plate (negative control)
(2) a culture well with filtered seawater and a CCA plate (positive control)
(3) a culture well with filtered seawater and a plate with upright CCA (positive up control)
(4) a culture well with filtered seawater, a CCA plate and a 20μm layer silt (thin silt treatment)
(5) a culture well with filtered seawater, a CCA plate and a 100μm layer silt (thick silt treatment)
(6) a culture well with filtered seawater, a CCA plate and a 20μm layer ground oyster shell powder coating (thin oyster shell powder treatment)
(7) a culture well with filtered seawater, a CCA plate and a 100μm layer ground oyster shell powder coating (thick oyster shell powder treatment)

Wet silt was collected from the BML's raw seawater treatment system and then placed into a glass crucible and heat treated in an oven at 85°C for 12 hours. The dried silt is then grinded with a pestle and mortar and sifted through a 63-micron sieve. It is then placed in a 100-mL beaker and rehydrated with 50ml of filtered seawater. The silt was allowed to settle for 5 hours and the excess seawater was discarded. As for ground oyster shells, dried Pacific Oysters (*Crassostrea gigas*) shells were grinded with a pestle and mortar. It is sifted using a 150-micro sieve and placed into a 100-mL beaker. Then, 50mL of filtered seawater is added and the shell powder was allowed to settle for 5 hours before excess seawater was discarded. A 6-well culture plate was used for each treatment and the replicates were prepared in 5 individual culture well (radius = 1.75cm ; volume = 15.5cm^3). For each treatment, one CCA plate is placed at the bottom of each well, except for the negative control, and filled with 5mL of filtered seawater using a micropipette. Sediments were then evenly deposited using a micropipette into each well and swirled gently to allow the sediments to settle evenly. The average of ten aliquots of 2mL volume were taken from a pitcher containing 400mL of filtered seawater and larvae to determine the volume needed to obtain thirty individual larvae. Using the equation:

$$volume\ required\ per\ replicate\ (x) = \frac{target\ larva\ count}{average\ aliquot\ larva\ count} \times (volume\ per\ aliquot)$$

it was determined that the volume required per replicate was 7.02mL. Subsequently, the required volume was pipetted into the wells using pipet tips with larger openings to prevent damage inflicted onto the larvae. Lastly, the culture plates were placed in a cold room that was maintained at 14°C throughout the duration of the experiment.

At 18.5 hours after introduction, the number of abalone larvae in each replicate were counted under a dissecting microscope. The abalone larvae were classified under two main categories: (1) Settled and (2) Unsettled. Within category (1), the larvae were subcategorized into (a) settled on CCA plate surface and (b) settled on the side of CCA plate, side of the well, and bottom of well. As for category (2), the larvae were subcategorized into (a)swimming or laying on its side, (b)dead and (c) deformed. The rate of metamorphosis for each treatment was determined using the given formula: metamorphosis rate (%) = $\frac{N_m}{(N_t)} \times 100\%$, where $N_m\ and\ N_t$ represent the number of metamorphosed individuals, and total number of individual larvae respectively. The metamorphosis rates across the different treatments were analyzed using Pearson's Chi-squared test. In addition, the effect size of each treatment was determined using the odds ratio under the Fisher's exact test. Both of these statistical analyses were carried out using a statistical computer software called R-Studio.

RESULTS

The percentage of settled larvae for each treatment group was calculated and tabulated in table (Table1). The low settlement rate of 19% in the negative treatment had shown that the majority of the larvae did not settle due to the absence of CCA.

Positive and Positive Upright Treatments

It was confirmed the settlement of *H.sorenseni larvae* in both the positive and positive (upright) treatments was induced by the presence of CCA, where settlement rate was at 72.1% and 80.3% respectively.

Silt Treatments

The thin silt treatment (SA) had a larvae settlement rate of 73.1% whereas larvae in the thick silt treatment only experienced a 58.3% settlement rate.

Ground shell Treatments

As for the thin ground shell treatment, only 39.8% of the larvae settled whereas larvae in the thick ground shell treatment settled at a higher rate of 57.4%.

Statistical Analysis

A Chi-squared test and Fisher's Exact test were performed to compare the significant differences of each treatment to the positive treatment. The positive and negative treatment was significantly different ($p = 2.174623e-10$; odds ratio = 10.80333). Both the positive and positive upright treatments were not significantly different ($p=0.3026$; odds ratio = 0.6371521). When examining the silt treatments, the thin silt treatment (SA) treatment showed no significant difference ($p= 1$; odds ratio = 0.9511268), whereas the thick silt treatment (SB) was borderline significantly different ($p= 0.0644$; odds ratio = 1.84768). On the other hand, the thin ground shell treatment (GA) was significantly different ($p= 0.0001291$; odds ratio = 3.880786), whereas the thick ground shell treatment (GB) was not significantly different ($p= 0.1183$; odds ratio = 1.909258) from the positive treatment.

Table 1: Percent settled of *H.sorenseni* larvae in all treatment groups.

Treatment	Number of settled larvae	Number of unsettled larvae	Total number of larvae	Percent of settled larvae
Positive	44	17	61	72.1%
Positive (Upright)	53	13	66	80.3%
Negative	15	64	79	19.0%
Thin Silt (SA)	49	18	67	73.1%
Thick Silt (SB)	95	68	163	58.3%
Thin Ground Shell (GA)	37	56	93	39.8%
Thick Ground Shell (GB)	31	23	54	57.4%

DISCUSSION

Based on the experiment results, it was observed that there were insignificant differences in the settlement rate between the positive treatment and the positive (upright) treatment. White abalone larvae settled regardless of the shape and form of the CCA and proves to demonstrate that the presence of CCA is important for the larvae settlement stage (Morse et al., 1984). The data also strongly suggests that smaller grain-sized sediments and greater thickness of sediments will impede the settlement of white abalone larvae.

In heavy the silt treatment, in particular, larvae were trapped and prevented from swimming, which resulted in a high mortality rate. Such similar observation was stated in other sediment experiments with two other abalone species and small coral organism *(Babcock & Davies, 1991; Chew et al., 2013; Fabricius & Wolanski, 2000; Onitsuka et al., 2008; Walker, 2007).* However, in the thin silt treatment, it was rather surprising to observe a high settlement rate throughout the replicates. Larvae was observed to have settled on the side of the culture wells, as well as on the sides of the raised CCA plates, which prevented the larvae from getting trapped in silt. This may be an indication that the different formations of small rocky intertidal structures, combined with wave action and storm surges, may not be conducive for sedimentation to occur in natural settings (Onitsuka et al., 2008). In addition, some abalone larvae might find non-sedimented CCA surfaces, which is sometimes the underside of rocks or a moderate splash zone with high sunlight availability.

Given that such formation is difficult and rare in natural settings, the survival rate of abalone larvae may not be considered high. Nevertheless, storm systems that pass through the coast of California may reduce sedimentation impacts on abalone habitat and benefit recruitment rate. A study in Japan has documented that abalones spawn immediately a typhoon that went through the island and Japanese researches hypothesized that the coinciding spawning might be beneficial for the abalone larvae as it helps reduce sedimentation on CCA surfaces and triggers the metamorphosis processes (Kiyomoto et al., 2013). All of these variables may affect larvae anchoring and the settlement processes.

Conversely, the settlement rates in the ground shell treatment were similar to the ones in the silt treatment. Based on the work that Onitsuka and his colleagues produced, it was hypothesized settlement rate should be higher with a ground shell treatment. Even though more experiments should be conducted to obtain evidence to support the claim that the larvae settlement rate is higher in ground shell treatments, there are greater needs to investigate the effects of fine sediments on abalones. In 2000, Fabricius & Wolanski have demonstrated the fine sediments significantly impacted corals by suffocating them within a short time frame, which justifies the need for further investigation.

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