Identification of Burrowing Worms and Bacteria Associated with Shell Injuries on Red Abalone Cultured in Baja California, Mexico

Jorge Cáceres-Martínez, Yessica Hernández-Mercado, Roberto Cruz-Flores, Montserrat Muñoz-Flores and Rebeca Vásquez-Yeomans

Burrowing worms are a group of spionid polychaetes that may settle on the shell of bivalve mollusks and gastropods such as abalone. The species that cause the greatest damage to their host belong to the genera *Polydora* and *Boccardia*. These worms are commonly referred to as mud worms from the great quantity of organic material and detritus that accumulates in the holes carved in the shell of their mollusk hosts. The worms settle on the shell as larvae or crawl in as juveniles and start to burrow into the shell to create a living space. Additionally, some species build a chimney with organic matter and detritus that protrudes from the shell that the worm uses to emerge and feed by capturing microorganisms, microalgae and organic matter in the water surrounding the mollusk shell.

FIGURE 1. *A juvenile red abalone affected by burrowing worms. Note the open channel over the shell. The soft body was easily detached from the shell.*

If the number of mud worms per shell is high or if the shell is thin, the damage can be considerable and can surpass the capacity of host cells to repair the damage. The shell becomes fragile and more vulnerable to predators. If the damage caused by the worm affects the muscle that supports the shell, the muscle can be easily detached, condemning the mollusk to death.

Mud worms are not considered true parasites because they do not feed on tissues of their host, but are considered epibionts, which means that they are in a type of biological relationship in which one animal lives over the other with no or minor negative effects. In general, this relationship can be beneficial for the epibiont because it acquires a place to live without harming the host.

However, under certain circumstances this relationship can be life threatening for the host. When a worm reaches the inner surface of the mollusk shell and comes in contact with soft tissues such as the mantle or muscle, the host reacts immediately to try to cover the hole and to avoid damage to its soft tissue. Specialized cells in the mantle called amebocytes migrate to the affected area and produce a chitinous protein called conchiolin to repair damage to the shell and cover up the hole. This reaction results in an inner, soft, dark, chitinous blister. Over time soft blisters are covered by layers of calcite and once again the mollusk produces a hard shell surface. This phenomenon constantly takes place in nature and, as a result of this relationship, on some occasions half pearls are produced by this kind of shell repair.

If the number of mud worms per shell is high or if the shell is thin, the damage can be considerable and can surpass the capacity of host cells to repair the damage. The shell becomes fragile and more vulnerable to predators. Furthermore, open holes allow the mud worm to contact the soft tissues that are then mechanically disrupted, resulting in an injury that can become infected by microorganisms such as opportunistic bacteria that are abundant in the organic material and detritus that accumulates in the blisters. If the damage caused by the worm affects the muscle that supports the shell, the muscle can be easily detached, condemning the mollusk to death.

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FIGURE 2. *The most frequent affected area of the shell was close to the spire and in severe cases the perforation may form a channel or rupture that reaches the shell border.*

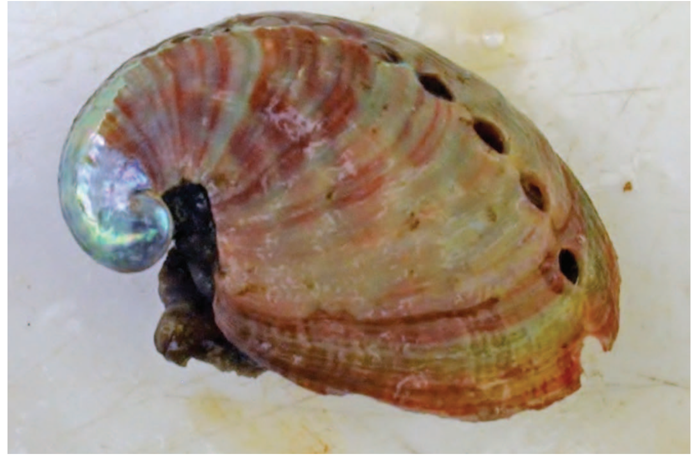

FIGURE 4. *Severe damage in the shell of a juvenile red abalone caused by burrowing worms. Note the wide rupture of the shell reaching the border and the chitinolytic brown deposit.*

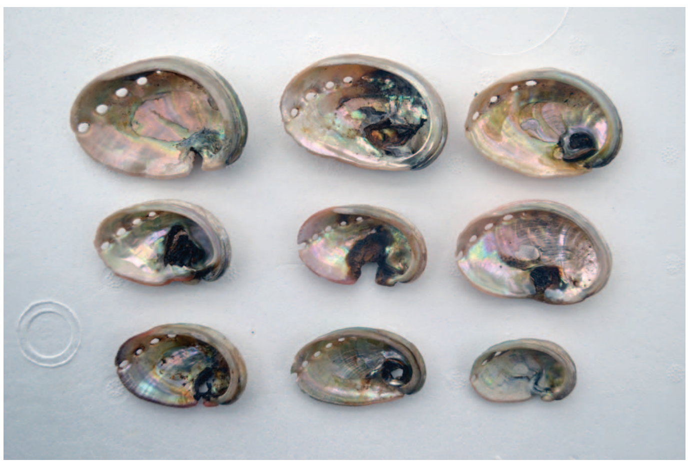

FIGURE 3. *The extent of the damage is more clearly observed on the inner surface of the shell. Note that the lesion in the last shell is covered by calcite.*

FIGURE 5. *Reduction of the mantle and foot muscle, darkened soft body and the organic brown deposit is evident in a juvenile red abalone severely affected by the burrowing worm.*

Mud Worms in Abalone Culture

In high-density abalone aquaculture, infestations by burrowing worms can reach lethal levels. There are examples of high mortalities of abalone associated with burrowing worms in Australia and South Africa. Between 1995 and 1997 producers of Tasmanian blacklip abalone *Haliotis rubra* reported cumulative mortality of more than 50 percent related to the burrowing worms *Boccardia knoxi* and *Polydora hoplura* (Lleonart *et al*. 2003). In South Africa, infestations of the burrowing worms *Polydora hoplura* and *Boccardia* sp. have had a negative impact on the culture of abalone *Haliotis midae* (Simon *et al*. 2006).

To date, no records of burrowing worms in cultured red abalone *Haliotis rufescens* in Mexico have been published. However, in recent years, producers have occasionally noted that some juvenile abalones show unusual perforations and scars over the shell and some organisms easily lose their shell (Fig. 1). Perforations and scars can be observed all over the shell but most are located close to the spire (Fig. 2). Extensions of the damage are more clearly observed on the inner surface of the shell (Fig. 3). In severe cases, perforations reach the border of the shell (Fig. 4). These animals showed a reduction and darkening of the mantle, foot muscle and accumulation of dark organic material under the shell with an unpleasant aspect (Fig. 5), signs that usually suggest the presence of burrowing worms and opportunistic bacteria. To identify these worms and bacteria, conventional taxonomy and DNA analyses were carried out.

Study Methods

Thirty-three red abalone with external shell lesions were collected from an aquaculture facility in spring 2015. Soft tissues were detached from the shell and the conchioline layer was carefully removed with a needle (Fig. 6). Dark organic material and detritus behind this layer was observed and several worms remained among this material (Fig. 7). Worms were carefully collected with dissection clips and a needle (Fig. 8). Twelve of the best preserved worms were identified with specialized taxonomic keys. A fraction of worm tissue was collected for DNA analysis using the 18S rDNA gene sequence. In parallel, under aseptic conditions, samples of organic deposits were collected and transferred to culture media for bacteria growth (Marine Agar and Thiosulfate Citrate Bile Salts Sucrose [TCBS]). Bacteria were cultured and isolated for identification using conventional bacteriological procedures and analysis of the 16S rDNA gene sequence.

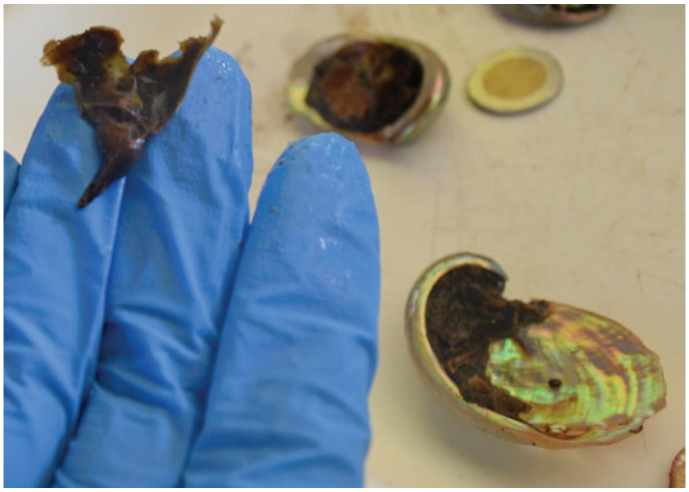

FIGURE 6. *A piece of the chitinolytic layer that covered the perforation extracted from the inner shell.*

FIGURE 7. *Organic material and detritus found under the chitinolytic layer where small pieces of several worms were observed.*

FIGURE 8. *The procedure to extract burrowing worms from organic deposits.*

Results and Discussion

The polychaete spionids were identified as *Boccardia proboscidea* by a modification of the fifth setiger, a type of filament in the external body that aids in locomotion and excavation, and also by the presence of branchiae before this structure. Furthermore the 18S rDNA sequences indicated 99 percent similitude and 99 percent identity with sequences of these species deposited in GenBank. This worm is a common polychaete that is distributed along the western coast of North America. It is a species closely related to those mentioned previously associated with similar lesions in cultured abalone in Australia and South Africa.

These burrowing worms have several biological characteristics that make their control difficult. The reproductive strategies of these species make them powerful invaders that are difficult to eradicate if they are allowed to spread in aquaculture facilities. *B. proboscidea* is poecilogonous, a term that refers to a type of development in which a female may produce planktotrophic larvae as well as bottom-crawling juveniles within a single egg capsule. This means that infestation can occur by settlement of planktonic larvae, which easily travel with water currents throughout the aquaculture facility, or by crawling along the bottom into the shell of the host abalone. Additionally, members of this family of worms have the ability to regenerate from severely damaged limbs. An adult *Polydora* sp. cut into three pieces – head, middle and posterior – can regenerate an entire functional worm from each body piece in 40 days under laboratory conditions (Tinoco-Orta and Cáceres-Martínez 2003). This regeneration phenomenon, called architomy, increases the potential for infestation by these worms.

Conventional bacteriological analyses such as Gram stain,

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TABLE 1. Comparison of sequences in GenBank by BLAST (Basic Local Aligment Search Tool) obtained from bacterial isolates of chitinous and organic matter of blisters formed by *Boccardia proboscidea* using 16S rDNA recovered from *Haliotis rufescens*.

GenBank access number	Species	Similitude (%)	Identity (%)
MNR_117891.1	*Vibrio chagasii*	100	99
NR_074953.1	*Vibrio splendidus*	100	99
NR_044078.1	*Vibrio crassostreae*	99	98
NR_115806.1	*Vibrio cyclitrophicus*	100	99
NR_116067.1	*Vibrio atlanticus*	100	99
NR_036929.1	*Vibrio tasmaniensis*	100	99

KOH, oxidase, catalase, motility, growth in NaCl and sensitivity to the vibriostatic agent 0/129 showed that they were Gram-negative colonies, all short bacilli that were sensitive to the vibriostatic agent 0/129 and all characteristics that place them among *Vibrio* bacteria. DNA analysis of the 16S rDNA gene sequence corroborated that the bacteria present in the dark blisters and organic deposits belong to the genus *Vibrio*; and that the majority of them are considered to be part of the clade or group *Splendidus* (Table 1); within this clade, bacteria such as *V. splendidus* is able to cause secondary infections in soft tissue. Members of the genus *Vibrio* have been described as opportunistic pathogens affecting all life-cycle stages of bivalve mollusks from larvae to adult (Allam *et al*. 2002). *Vibrio tasmaniensis, V. splendidus* and *V. neptunius* are species that are distributed throughout the world, associated with aquaculture, and are causative agents of diseases in aquatic organisms when environmental conditions are favorable (Thompson *et al*. 2003, Guisande 2004). In particular, strains of *V. splendidus* and related species *V. harveyi* and *V. tapetis* have been related to "shell disease" in bivalve mollusks (Paillard *et al*. 2004). These diseases are characterized by the abnormal production of deposits of organic matter on the inner surface of the valves and a brown coloration of the mantle tissue that comes in contact with deposits of organic matter.

This study revealed a typical case of the presence of boring polychaetes and associated opportunistic bacteria in red abalone cultured in Mexico. To contribute to their control, the correct management practices at appropriate times need to be carried out, including maintaining a visual surveillance program for early detection of infested abalones. This can be done during feeding and/or during clearing of the juvenile population and growth monitoring. All infested abalones must be removed from culture tanks and disposed inland, where they must be sacrificed, dried and ideally buried or incinerated. A good filtration system is necessary to avoid dispersion of planktonic larvae during spawning periods as well as for removing small crawling stages or fractionated worms dispersed by currents or handling. The removal of solid wastes from the bottom of culture tanks is essential. The removal of empty shells from the system may aid in preventing infestation by these worms. Moreover, high water quality standards and a regular bacteriological analysis may aid in guaranteeing low levels of opportunistic bacteria. Training on good sanitary practices of all workers involved in aquaculture production is an essential step for avoiding these type of disease outbreaks.

Acknowledgments

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Notes

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