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Salinity Tolerance of the Oyster Mudworm Polydora websteri

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SALINITY TOLERANCE OF THE OYSTER MUDWORM

POLYDORA WEBSTERI

by

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ABSTRACT

The marine worm *Polydora websteri* is one of many polychaete species that burrow into the shells of commercially important shellfish. In Maine, local eastern oyster (*Crassostrea virginica*) farmers are struggling with an infestation of this pest. The worm egests muddy wastes in its burrow causing irritation to the oyster. In response, the oyster secretes new shell material over the burrow forming mud blisters. These blisters are unsightly and decrease the market value of infested oysters, especially for oysters sold in the half-shell trade. In addition to the reduction in market value, the worm may cause physiological stress on the oyster. There have been many studies and anecdotal reports published on possible treatments to eradicate the mudworm. The methods used can be expensive, in some cases toxic, and most are unreliable. I investigated the salinity tolerance of *P. websteri* using in-situ and in-vitro experiments. The results from both types of experiments can be used to construct improved treatments for infested oysters. I found that *P. websteri* is not tolerant of extremely low salinities and that a combination of a low salinity exposure followed by a period of dry, cold storage results in 100% worm mortality in as few as 10 days, with minimal host mortality. Future work will focus on scaling up and refining these treatments, as well as looking into possible site-specific management plans for control of *P. websteri*.

ACKNOWLEDGEMENTS

This project would not have been possible without the generous donation of study oysters from Jesse Leach and Eric Moran at Bagaduce River Oyster Company. I would like to thank Paul Rawson, my advisor, for his continual support and advice with my writing and project development. Thanks to my thesis committee for their input on my thesis, especially to Sara Lindsay for helping me learn how to keep worms happy in the lab. I would like to extend my thanks to Nick Brown at the Center for Cooperative Aquaculture Research for sharing the results of his work on *Polydora websteri* infestations in oysters. I would like to thank the original oyster crew, Mick Devin, Brittany Wolfe, Colin Kelsey and Todd Mihal, for their part in developing techniques for extracting and counting worms in oysters. Also thanks to my friends and family for their love and understanding throughout not only my thesis work, but for my time as an undergraduate.

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INTRODUCTION

Polychaetes in the genera *Boccardia*, *Psuedopolydora*, and *Polydora* are known to bore into calcareous substrates, including the shells of commercially important shellfish species (Blake and Evans, 1973). These worms were first described in the late 18th century by naturalists and were documented as pest species in the 1800s following an infestation that destroyed oyster reefs in New Zealand (Blake and Evans, 1973; Nell, 2007; Read 2010). Also known as "mudworms" or "blisterworms", these shell-boring polychaetes can bore through shells of live and dead shellfish, coral, and limestone (Bergman *et. al.*, 1982; Blake and Evans, 1973; Nel *et. al.*, 1996). Mudworm infestations have resulted in commercial loses in abalone, mussel, scallop and oyster fisheries worldwide (Haigler, 1969; Lafferty and Kuris, 1996; Read, 2010; Wargo and Ford, 1993).

The impact of mudworm infestation has been best documented in oyster fisheries (Bergman *et. al.*, 1982; Lunz, 1940; Nell, 2007; Read, 2010). In North America, and New England in particular, *Polydora websteri* is the most common parasite in surface dwelling or shallow burrowing bivalves (Bergman *et. al.*, 1982; Blake and Evans, 1973; Hopkins, 1958; Wargo and Ford, 1993). There are sporadic reports of mudworm infestation in the state of Maine. A heavy infestation of *Polydora websteri* in oysters on the Bagaduce River, ME is a cause for concern due to the loss of production and decline in value as a result of the unsightly blisters formed by the oysters (Jesse Leach, pers. comm., 2011). Although many treatments to rid oysters of the worm parasite have been proposed, the efficacy of most is not well documented. The goal of my study was to examine the

salinity tolerance of *P. websteri*, and determine potential treatments to rid the worm from the shells of eastern oysters.

The oyster mudworm

Polydora websteri is a polychaete in the Spionidae family and the genus *Polydora* (Hopkins, 1958; Loosanoff and Engle, 1943; Read, 2010). The Polydorids, *Polydora*, *Boccardia*, *Pseudopolydora*, all have at least one shell-boring species and are the only spionids that are capable of boring (Blake and Evans, 1973). Species in this complex have a modified fifth segment, or setiger, with specialized setae (Figure 1). Though they share this common characteristic, it may not be involved in shell-boring.

Polydora websteri is a common polydorid in intertidal and subtidal calcareous substrates in Maine (Blake, 1969). Although *P. websteri* was first described by Webster in 1879, the description of the species was refined by Hartman to prevent future misidentification (Loosanoff and Engle, 1943). Adult *P. websteri* have around 100 segments and are approximately 20 mm long (Blake, 1971; Loosanoff and Engle, 1943; Nell, 2007). Like other spionids, *P. websteri* has a pair of long palps that are used to collect particles for feeding or tube building (Blake, 1971). *Polydora websteri* generally have a rounded prostomium and if present, there are three to four eyes arranged in a trapezoidal pattern (Blake, 1971; Pollock, 1998). The modified fifth setiger has heavy flattened setae and is two times larger than surrounding segments (Blake, 1971; Pollock, 1998). The pygidium, or posterior end, is cup shaped with a dorsal notch (Figure 1; Blake, 1971).

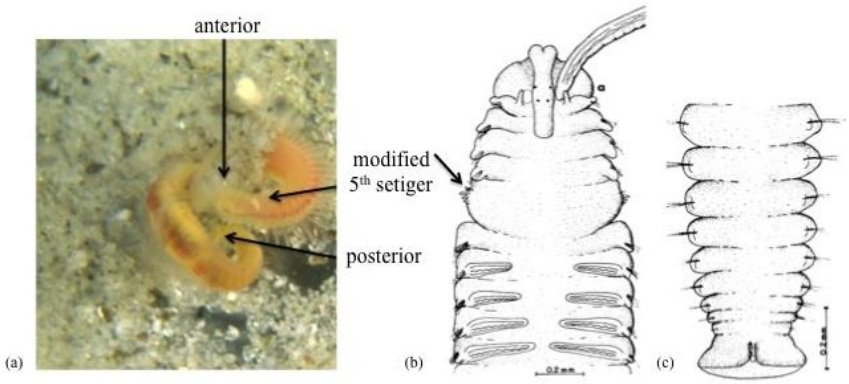

Figure 1. Adult *Polydora websteri*. Image of *P. websteri* individual from study oysters, 2.5x magnification (a) and illustration of anterior (b) and posterior (c) from Blake (1971; Figure 3 a, k).

Polydorid larvae in general are common in northeastern American waters and have received significant attention. The larvae of *P. websteri*, however, have not been studied as extensively, with the exception of the studies of Hopkins (1958) and Blake (1969). *Polydora websteri* have egg capsules that are arranged as "beads on a string" attached to the inner wall of a sediment tube (Blake, 1969; pers. observation, 2011). Larvae are capable swimmers at the three-setiger stage, measuring 0.35 to 0.4 mm in length with visible pigmented eyespots (Blake, 1969; Hopkins, 1958). Larvae reach a 14 segment stage in 42 days and metamorphose at the 17 setiger stage, after which they settle on calcareous material (Blake, 1969; Hopkins, 1958). Hopkins (1958) observed that *P. websteri* were present year round in coastal waters of Lousiaina. He noted, however, that larvae were more abundant in warmer months. In Maine, Blake (1969) observed larvae to be present from April until August with larvae being most numerous in May and June. Blake (1969) also noted that no species of *Polydora* larvae were present in the plankton year round and suggested that their reproduction is seasonal due to the water temperature.

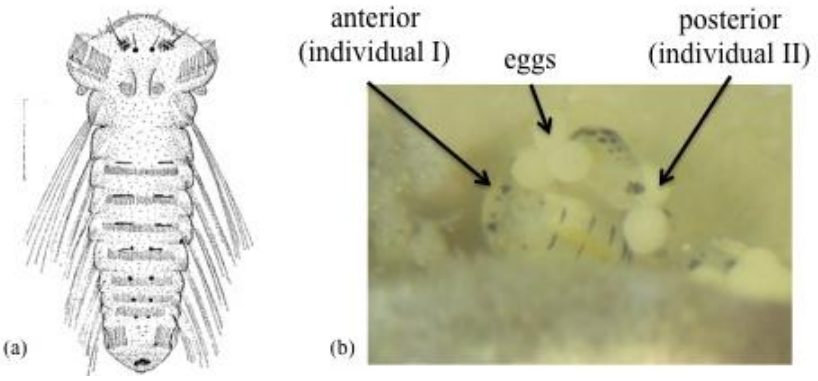

Figure 2. *Polydora websteri* larvae. Illustration of nine-setiger *P. websteri* larvae (a) from Blake (1969; Figure 7b) and photograph of similar stage larvae in study oysters 4x magnification (b). Notice similar eyespots and black banding patterns.

Outside of the host shell, *P. websteri* constructs tubes with any available materials (Haigler, 1969; Loosanoff and Engle, 1943). Shell-boring polychaetes form a variety of burrows in their host (Blake and Evans, 1973). The first type are the surface fouling burrows, where the worm creates a burrow on the surface, but does not penetrate the shell. The second type are U-shaped burrows which are most characteristic of *Polydora*. The worm penetrates the shell and can create either a branched or unbranched U-shaped tube lined with mud and other debris, with the ends exposed to the outer shell surface (Bailey-Brock, 2000; Bergman *et. al.*, 1982; Blake and Evans, 1973; Haigler, 1969; Hopkins, 1958; Nell, 2007; Wargo and Ford, 1993; Zottoli and Carriker, 1974). The most damaging *Polydora* burrow type in bivalve shells are mudblisters where the burrow extends to the nacreous (inner) layer of the host shell. The worm accumulates mud and other debris inside the shell, which causes the host to secrete shell material forming an unsightly blister.

There are several hypotheses regarding when shell-boring polydorids infest their host (Blake and Evans, 1973). Most authors believe that the larvae settle on the outside of the shell and excavate a burrow (Blake and Evans, 1973). Mudworms typically invade

the shells of oysters and other molluscs when the worms are at a late larval or early juvenile stage (Blake, 1969; Haigler, 1969; Hopkins, 1958; Loosanoff and Engle, 1943). The worm initially settles into the lip crevice or other groove on the outside of the host shell (Hopkins, 1958; Read, 2010; Zottoli and Carriker, 1974). Once on the shell surface *P. websteri* bores into the shell. The boring mechanism of *P. websteri* has been described in detail by Haigler (1969), who determined that it is predominately a chemical process whereby the worm secretes an acid that dissolves the shell material (Haigler, 1969; Hopkins, 1958; Nell, 2007; Zottoli and Carriker, 1974). The giant setae on the modified fifth setiger do not play a principal role in burrowing (Blake and Evans, 1973). These modified setae may be used for anchoring the worm during borrowing or as an abrasion tool once a burrow has been initiated (Blake and Evans, 1973). Worms will burrow through all layers of the shell, and in some cases, blisters in heavily infested oysters will often be on top of other blisters (Haigler, 1969; Read, 2010). In cupped oysters, such as *C. virginica*, blisters are more common in the cup valve than in the flat valve, but the cause of this bias is unknown (Loosanoff and Engle, 1943).

The Eastern Oyster, Crassostrea virginica

Oysters react to the accumulation of silt from the worm by secreting the protein conchiolin and shell calcite to cover and confine the burrows (Bergman *et. al.*, 1982; Haigler 1969; Lafferty and Kuris, 1996; Loosanoff and Engle, 1943; Nell, 2007; Wargo and Ford, 1993). Later the oyster secretes a layer of shell nacre, closing off the burrow and forming the mud blister that contains the worm burrow and muddy deposits (see Figure 3; Bailey-Brock, 2000; Gallo-Garcia *et. al.*, 2004; Lunz, 1940; Wargo and Ford,

1993). In some oysters there can be six to seven layers of mudblisters and in cases of very heavy infestations, this can reduce the space the oyster has to grow (Dunphy *et. al.*, 2005, Lafferty and Kuris, 1996; Loosanoff and Engle, 1943; Lunz, 1940). Although some reports have suggested that the oyster meat is unaffected by mudworm infestation, other reports indicate that heavy infestations result in lowered meat quality (condition), and yellow abscesses can form if the worm comes in contact with the adductor muscle (Bower, 2004; Haigler, 1969; Hooper, 2001; Hopkins, 1958; Loosanoff and Engle, 1943; Nel *et. al.*, 1996; Read, 2010; Wargo and Ford; 1993). Infestations can alter the growth patterns of the oyster host, resulting in a distorted shell shape. Often the burrows considerably weaken the shells, making the oysters more susceptible to predators (Bergman *et. al.*, 1982; Haigler, 1969; Hopkins, 1958; Lafferty and Kuris, 1996; Lunz, 1940; Read, 2010; Zottoli and Carriker, 1974).

Figure 3. Oyster heavily infested with mudworm. The gray and brown areas are old and new mudblisters formed by the oyster in response to the mudworm infestation.

Polydora websteri infestations can also have considerable physiological impacts on the oyster host. Energy costs of shell formation in mollusks are generally considered to be a minor component of an individual's energy budget (Beniash *et. al.*, 2010; Day *et. al.*, 2000; Palmer, 1992). Increased shell deposition in mudworm infested oysters may divert energy from growth and reproduction (Hooper, 2001; Lafferty and Kuris, 1996;

Loosanoff and Engle, 1943; Lunz, 1940; Nel *et. al.*, 1996; Wargo and Ford, 1993). Dunphy *et. al.* (2005) and Wargo and Ford (1993) have suggested that infested oysters may be impacted nutritionally if *P. websteri* is a suspension feeder and competes for food with its host or if large mudblisters disrupt the host's feeding currents. Such impacts could decrease the physiological condition of the oyster making it more susceptible to illness and environmental stress, which Hopkins (1958) and others (Gallo-Garcia *et. al.*, 2004; Lafferty and Kuris, 1996) suggest is the cause of mortality of mudworm-infested oysters.

Decreased oyster condition, characterized by factors such as decreased shell integrity and presence of mudblisters, as a result of mudworm infestation impacts the commercial value of the oyster. *Polydora websteri* is the most damaging of the shell-boring polychaetes and these worms may have played a role in the disappearance of oyster beds throughout the world (Loosanoff and Engle, 1943; Nell, 2007). Although infested oysters are fit for human consumption, they are not easily sold due to their appearance and weakened shell integrity. Most notably, the unsightly blisters decrease the commercial market value of oysters sold in the half-shell trade (Bower, 2004; Dunphy *et. al.*, 2005; Gallo-Garcia *et. al.*, 2004; Hooper, 2001; Lafferty and Kuris, 1996). Additionally the weakened shell makes shucking the oyster difficult (Haigler, 1969; personal observation) and oyster farmers for years have suffered considerable financial loss due to infestations (Lunz, 1940; Nell, 2007).

Proposed treatment methods for mudworm infestations

There are a multitude of proposed preventive and control measures for mudworm infestations. These treatments, both published and anecdotal, come from researchers and farmers throughout the world. The goal of all such treatments has been to develop a cost-effective treatment that kills the mudworm without greatly impacting oyster survival or growth.

One group of potential control and preventive measures involves identifying culture sites that are worm free. This can include altering the position of oysters in the water column at sites where infestations are common. For example, raising the cage height and growing oysters away from the bottom substrate may reduce mudworm infestation while increasing oyster growth (Bower, 2004; Loosanoff and Engle, 1943; Nell, 2007; Nel *et. al.*, 1996). In contrast, some Maine farmers have reported that growing oysters on the bottom where they are at least partially covered by sediment reduces the access of mudworm to the oysters and limits infestations (Dana Morse, pers. comm.). Other reports suggest that growing oysters intertidally reduces mudworm infestation; the periodic exposure of oysters to the air and associated drying of the shell surface reduces successful settlement of worm larvae and survival of the adult worms in burrows (Nel *et. al.*, 1996). While this may reduce mudworm abundance, it may also reduce the growth rate of the host and make it more susceptible to predation. The same drying effect can be achieved for oysters grown in surface cages by periodically lifting the cage and oysters from the water and allowing the shell surfaces to dry. The Oyster-Gro system was designed with such exposure in mind to help control fouling organisms that impact cage culture because cages can be raised out of the water for a period of time

(Dana Morse, pers. comm.). This design may have the added benefit of increasing mudworm mortality due to the periods of air exposure.

Chemical treatments have also been used to control mudworm. Some of the proposed chemical treatments are toxic, require careful handling, and permitting, all of which has limited their commercial use (Dunphy *et. al.*, 2005; Nel *et. al.*, 1996). Some examples of chemical treatments that have been applied include, soaking oysters in copper sulfate ($CuSO_4$), chlorine, 2% formaldehyde, marine dipterenes from algae, phenol, 0.2% calcium hydroxide (lime) or tetrachorethylene (Dunphy *et. al.*, 2005; Gallo-Garcia *et. al.*, 2004). While these chemicals may kill most of the mudworm infesting an oyster, Lafferty and Kuris (1996) found that there was rapid re-infestation once oysters were placed back in the water. In addition, these chemicals may affect whether the oysters can be sold because oysters treated chemically may not be safe for consumption.

Non-chemical treatments have also been used with varying success. The New South Wales (Australia) Government outlined several non-chemical approaches to controlling mudworm in Sydney Rock and Pacific oyster culture operations (Nell, 2007). Preventive measures they recommended include washing oysters every two to three weeks to remove mud, culturing oysters away from the bottom substrate, and ensuring that the farm site has adequate tidal flushing. Control measures included either air-drying or bathing oysters in freshwater, brine (saturated salt) or iodine solutions.

Other reports provide more detail on potential treatments and their relative effects. For example, Nel *et. al.* (1996) found that when *C. gigas* oysters infested with *P. hoplura* were treated by a twelve-hour freshwater soak or a heated (70°C) saltwater soak for 40 seconds, there was reduced worm infestation without significantly affecting oyster

survival. Dunphy *et. al.* (2005) reported on the treatment of *Tiostrea chilensis* oysters infested with *Boccardia acus*, another shell-boring polychaete, by hyposaline (freshwater) and hypersaline (brine) treatments for three to five hours. They found that the hyposaline baths were more effective than hypersaline baths (Dunphy *et. al.*, 2005). Finally, Hooper (2001) described a complex treatment that was applied on oyster farms in North Carolina, wherein infested oysters were treated by air-drying for 48 hours, followed by a 15-minute saturated salt immersion, which was then followed by another 2 hour air-drying before oysters were placed back into bottom culture. He noted that air-drying substantially increased worm mortality (Hooper, 2001).

Recently, experiments in Maine suggest that dry, cold (38°F/3°C) storage of oysters for two months significantly reduced infestation by *P. websteri* with little host mortality (Jesse Leach and Nick Brown, unpublished results). The study by Leach and Brown was conducted during the winter months when low temperature limits oyster growth, so the overall "cost" of the treatment on farm productivity was minimized. A follow-up study conducted in late summer and early fall showed that dry, cold storage for as little as three weeks could rid oysters of mudworm, but there was higher oyster mortality, perhaps because of the metabolic state of the host. In addition, the extended period necessary for dry, cold storage to be effective and the additional handling of large numbers of oysters during heavy infestations may make this approach cost-prohibitive.

The foregoing suggests that proposed treatments for mudworm infestation are either ineffective, costly, or can be logistically complex. At the same time, there has been little work investigating the basic biology and ecology of the parasite, *P. websteri*. Such information will be highly useful in designing consistently effective treatments for

mudworm. In this study, I explored the salinity tolerance of *P. websteri* in in-situ experiments where worms were maintained within burrows and in-vitro experiments where they were removed from burrows. In addition, I examined possible treatment methods involving dry, cold storage combined with hyposaline exposures. I hypothesized that worms would be more tolerant to reduced environmental salinity when inside of the oyster shell because of the protective microenvironment afforded by their burrow. I expected that a combination treatment of a freshwater soak followed by dry, cold storage would be most effective in killing worms without significantly affecting oyster mortality.

MATERIALS AND METHODS

Obtaining and Maintaining Oysters

Eastern oysters (*C. virginica*) used in this study were collected from the Bagaduce River Oyster Company in Penobscot, ME on September 9, 2011. At the time of collection the water temperature was 18°C and salinity was 30 ppt in the Bagaduce River. Oysters were transported on ice to the University of Maine in Orono where they were held in a recirculating seawater system at 18°C and 30 ppt. During this time oysters were fed a diet of prepared (Shellfish Diet 1800) or cultured algae *Isochrysis galbana*. Oysters and associated *P. websteri* worms were used in three separate experiments investigating salinity tolerance of the worm in burrows within intact shells (in-situ) and when removed from their burrows (in-vitro).

Experiment I- Salinity and dry, cold storage tolerance of the mudworm, in-situ

The first experiment exposed oysters to a broad range of salinity treatments combined with a period of dry, cold storage and examined survival of both hosts and

parasites (Figure 4). For this experiment, oysters were held in individual (n=24), one-liter, plastic beakers. Seawater was prepared using Instant Ocean for a stock solution of approximately 30 ppt (full strength seawater or FSS). Reverse Osmosis (R.O.) water was used to dilute 30 ppt FSS for the 20 ppt and 10 ppt treatments. Reverse Osmosis water alone was used for the 0 ppt treatment. Approximately 400 mL of water of each salinity was added to six 1 L beakers (n=6 beakers per treatment).

The beakers, along with experimental oysters were placed into an environmental chamber held at 18°C. At the end of three days (~72 hours), three oysters from each salinity treatment were transferred to new one-liter beakers without water and held in a refrigerator at 3°C, representing dry, cold storage. The other three oysters from each treatment were placed into new beakers with 30 ppt seawater to which a dense suspension of cultured algae were added (recovery). Oysters were assumed to have survived the salinity treatments if they cleared the algae overnight. After this check for survival, oysters were shucked and their meats discarded. Using a metal probe, I picked through the blisters on the inside of each oyster shell and counted the number of live and dead worms. Once all of the blisters were examined, the shell was labeled, packaged and frozen at -20°C. Before discarding the seawater the oysters had been held in, I counted and assessed any worms that had crawled out of the oyster while still in the beaker.

At the end of two weeks, I removed the oysters from the dry, cold storage treatment and checked for oyster mortality using the recovery procedure described above. The following day, the blisters on the inside of these oysters were examined, and mortality among the treatments was compared.

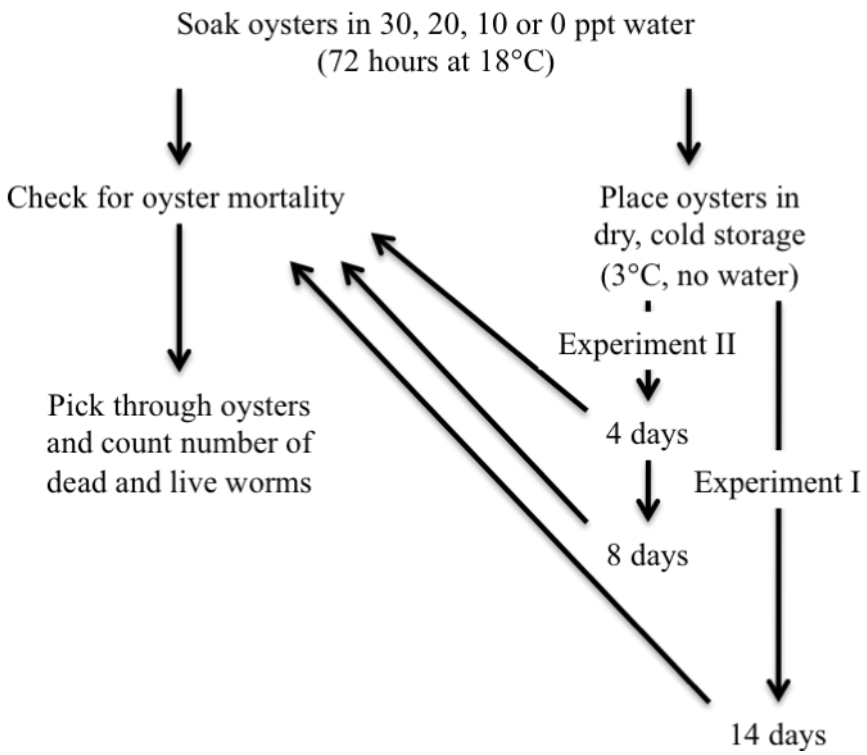

Figure 4. Methods for Experiment I and Experiment II. Flow chart summarizing the methods that were used in Experiment I- Salinity and dry, cold storage tolerance of the mudworm, in-situ and Experiment II- Salinity and dry, cold storage tolerance of the mudworm, in-situ.

Experiment II- Salinity and dry, cold storage tolerance of the mudworm, in-situ

A second experiment was conducted to examine survival of host and parasites after salinity treatments and after salinity treatment plus four or eight days in dry, cold cold storage. Nine oysters were placed in individual 1 L plastic beakers with 30 ppt seawater (control) and nine oysters were placed in R.O. water (0 ppt treatment). The beakers were placed in an environmental chamber at 18°C for three days. After three days, six oysters from each treatment were placed in dry, cold storage and the remaining three oysters were checked for mortality (as described in Experiment I). The next day, I shucked the latter set of oysters and counted the number of live and dead worms within

blisters (day 0). Three days later I removed an additional three oysters from each treatment in dry, cold storage, checked for oyster mortality, then examined worm mortality in the blisters of each oyster the next day (day 4). After another three days, I removed the remaining oysters from each treatment from dry, cold storage, checked for mortality and examined blisters for worm mortality (day 8).

Experiment III- Salinity tolerance of the mudworm, in-vitro

In a third experiment, I removed *P. websteri* individuals from their burrows in the oyster shell and exposed them to a range of salinity treatments to determine their in-vitro salinity tolerance (Figure 5). For this experiment, I extracted 72 uninjured worms from burrows within recently sacrificed oysters. To obtain undamaged worms, I placed the oysters in a 3.5% $MgCl_2$ solution for twenty minutes. At this concentration $MgCl_2$ acts as sedative, relaxing the worms and making it easier to remove them from burrows. Once worms were removed from the oyster, I placed them into individual cells in six-well plates. Each well had approximately 0.5 g dried mud and was filled with 10 mL of 30 ppt seawater. Over the next two days, I used a series of water changes to gradually decrease the salinity in the non-control salinity treatment wells (Figure 6). The result was three six-well plates (n=18 worms) in each of four salinity treatments, 30 ppt (control), 20 ppt, 10 ppt and 0 ppt. Salinity was measured with a refractometer. I added approximately 5 mg of powdered baby food to each well to serve as a food source for the worms. Each day, I checked to see if the worms were alive or dead and whether or not they had built and occupied a sediment tube. After checking for mortality and activity, 5 mL of water

was changed from each well and replaced with the appropriate strength water. The experiment was continued for 16 days.

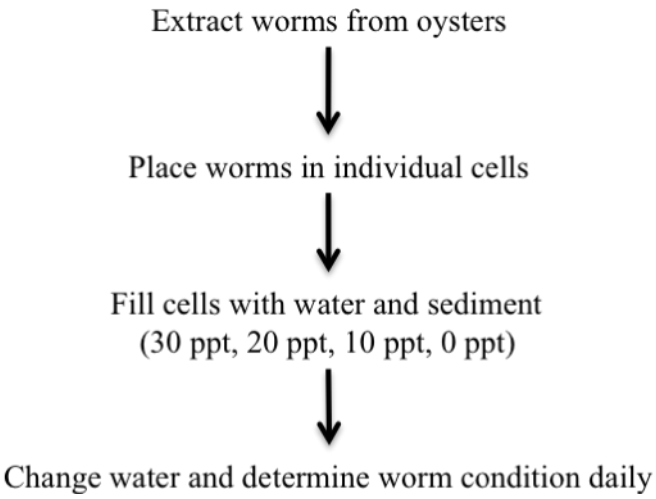

Figure 5. Methods for Experiment III. Flow chart summarizing methods used in Experiment III on salinity tolerance of the mudworm, in-vitro.

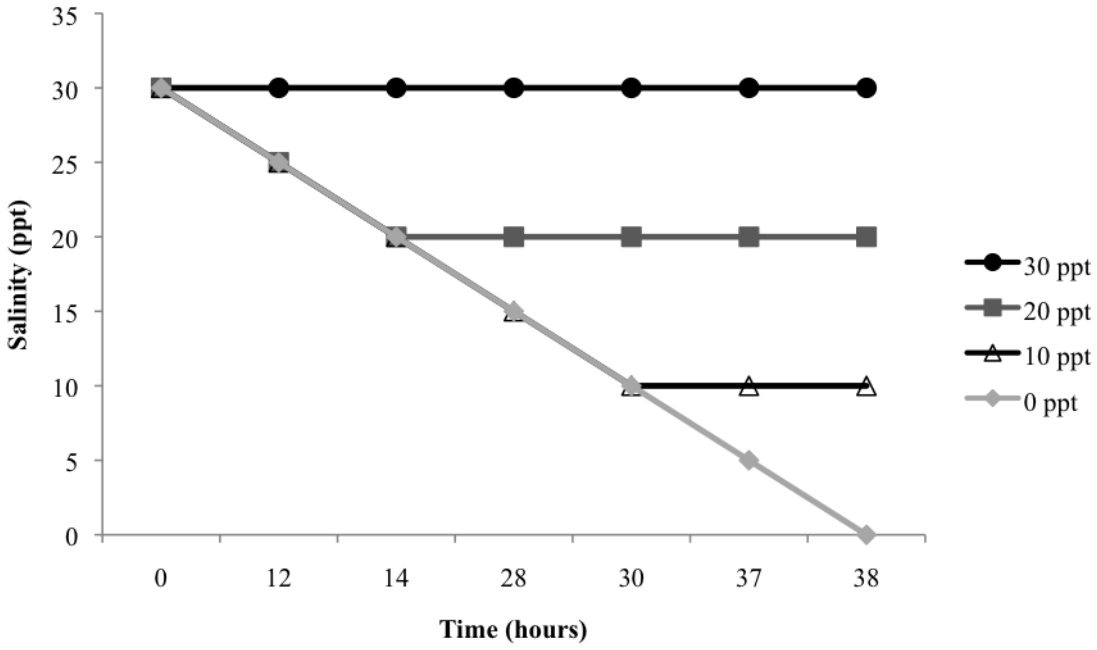

Figure 6. Salinity adjustments in Experiment III. Salinity in each treatment (30 ppt, 20ppt, 10 ppt, 0 ppt) over the time during initial stages of Experiment III. Observations began at 38 hours once each treatment was at the desired salinity.

Statistical Analysis

In Experiments I and II, I used two-way ANOVA to analyze worm mortality among oysters exposed to salinity stress and varying times of dry, cold storage, post-salinity stress exposure. The proportion of worms killed by each treatment was arcsine transformed (y=arcsin(sqrt(x))) prior to analysis. The ANOVA models included salinity treatment and time of exposure to dry, cold storage as main effects and a salinity-by-time interaction term. Statistical significance for each term was determined by comparing the mean square for each term to the model mean square for error with a p-value <0.05 considered as significant. I used SYSTAT ver12 for each ANOVA.

For Experiment III, a goodness of fit test via an RxC contingency table was used to ask whether the mortality of worms was associated with salinity treatment. In addition, we used an RxC test to determine if worm behavior (number forming tubes) was associated with salinity treatment. In each case, the test used a chi-square statistic that was compared to a chi-square table with the critical value determined at α of 0.05 and degrees of freedom of 3.

RESULTS

In both in-situ experiments, oyster hosts were placed in 30 ppt seawater with algae after each experimental treatment and prior to processing their shells. All of the oysters in Experiment I, and all but one oyster in Experiment II cleared the algae within 24 hours suggesting that short-term mortality of the oysters due to the reduced salinity was negligible.

The mortality of *P. websteri* in intact burrows was dramatically influenced by both salinity and dry, cold storage treatments (Figure 7). After the initial 72-hour exposure to four different salinities, there was increased mortality for worms in oysters held in 0 ppt compared to other treatments. Among the oysters in the 0 ppt treatment (n=3), the mortality of worms averaged 60%. In contrast, there was virtually no difference in initial mortality for worms exposed to the 30, 20, and 10 ppt treatments. Two weeks in dry, cold storage resulted in 100% worm mortality, regardless of initial salinity treatment. The dramatic change in the relative mortality among salinity treatments after two weeks of dry, cold storage resulted in a highly significant salinity-by-time interaction term in the two-way ANOVA for this experiment (Table 1). Overall, the ANOVA model explained 99% of the variance in worm mortality (R^2= 0.99). Despite the magnitude and significance of the interaction term in this analysis, it is clear that initial salinity treatment had a dramatic impact on initial worm mortality (Figure 7).

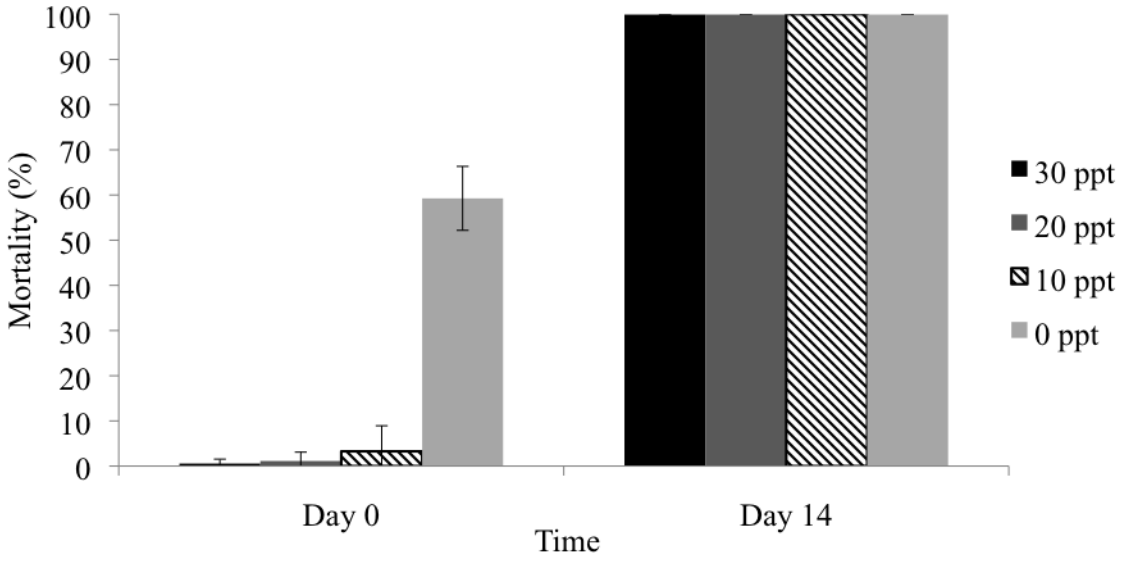

Figure 7. Worm mortality in Experiment I. The average percent mortality for worms in intact burrows after an initial 72-hour exposure to decreased salinity (day 0), and exposure to decreased salinity combined with 14 days in dry, cold storage (day 14). An average of 41 worms were counted per oyster (range 16 to 66). Error bars indicate mean percent mortality ± one standard deviation.

Table 1. ANOVA, Experiment I. Two-way analysis of variance examining the importance of initial salinity exposure (0, 10, 20, or 30 ppt) and length of dry, cold storage (time) on the variance in worm mortality in intact burrows. The columns d.f., MS, and F indicate the degrees of freedom, mean square and F ratio for each effect, respectively. Significance values are indicated by asterisks (*p<0.05, **p<0.01, ***p<0.001).

Effect	d.f.	MS	F
Salinity	3	0.244	34.4***
Time	1	10.101	1421.8***
Salinity x Time	3	0.244	34.4***
Error	16	0.007	

Given the patterns of mortality observed in the first experiment, I conducted a second experiment investigating the effect of a 72-hour exposure to 0 ppt water combined

with shorter periods of dry, cold storage on worm mortality in intact burrows (Figure 8). During the initial exposure, worm mortality reached ~20% in the 0 ppt treatment while no mortality was observed in the control (30 ppt) treatment. When oyster hosts were then held in dry, cold storage for four days, worm mortality was nearly 100% in the 0 ppt treatment and approached 60% in the control treatment. However, by day eight of dry, cold storage, there was 100% worm mortality in both salinity treatments. As in the first experiment, the salinity-by-time interaction term in the two-way ANOVA was statistically significant and the model explained 97% of the variation in mortality (R^2= 0.97). Even so, it is evident that as little as eight days of dry, cold storage results in substantial worm mortality, and that the initial exposure to R.O. water can dramatically increase worm mortality when combined with shorter periods of dry, cold storage. In this experiment, one oyster did not survive. This animal was in the control (30 ppt) treatment at day 0. Oysters were not assessed prior to being treated so this oyster could have been dead before the start of the experiment.

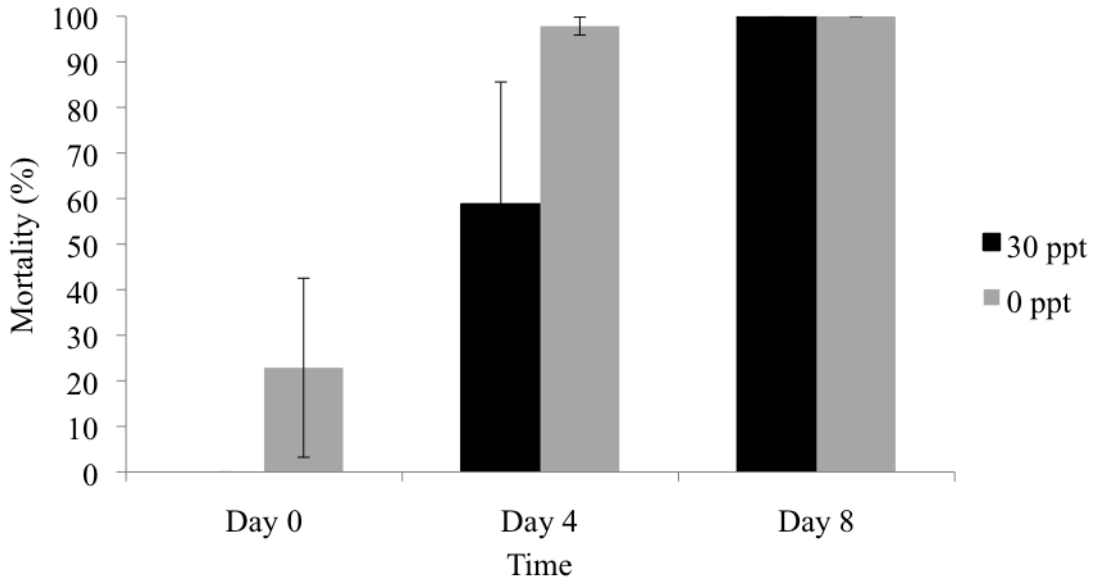

Figure 8. Worm mortality in Experiment II. The average percent mortality of worms in intact burrows after three days of exposure to 0 and 30 ppt treatments (Day 0) and after an initial exposure combined with either four (Day 4) or eight days (Day 8) in dry, cold storage. The worms in each of three oysters were counted per treatment at each time point. An average of 38 worms were counted per oyster (range 21 to 72). Error bars indicate mean percent mortality ± one standard deviation.

Table 2. ANOVA, Experiment II. Two-way analysis of variance examining the importance of initial salinity exposure (30 or 0 ppt) and length of dry, cold storage (time) on the variance in worm mortality in Experiment II in intact burrows. The columns d.f., MS, and F indicate the degrees of freedom, mean square and F ratio for each effect, respectively. Significance values are indicated by asterisks (*p<0.05, **p<0.01, ***p<0.001).

Effect	d.f.	MS	F
Salinity	1	0.534	19.8***
Time	2	2.826	104.7***
Salinity x Time	2	0.138	5.1*
Error	12	0.027	

A third experiment investigated the salinity tolerance of *P. websteri* outside of burrows. Decreasing salinity had a substantial impact on the survival and condition of

free-living worms. There was a strong association between salinity treatment and the proportion of dead worms by day 16 (R x C test of independence; $\chi^2 = 64.9$, df=3, $p<0.001$). Mortality reached 100% within three days in the 0 ppt treatment and in the 10 ppt treatment had reached 11% by 12 days (Figure 9). In contrast, all of the worms in the 20 and 30 ppt treatments survived to the end of the experiment.

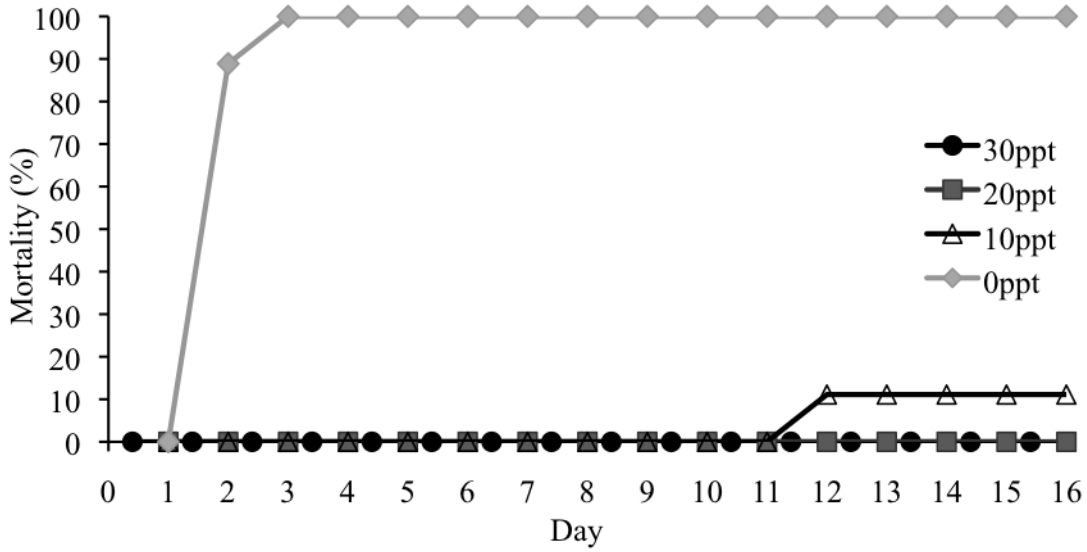

Figure 9. Worm mortality in Experiment III. Percent mortality of worms outside of burrows when exposed to four salinity treatments (n=18 worms per treatment. The data points for the 30 ppt treatment were offset for clarity.

The sublethal effects of decreased salinity on *P. websteri* were also evident, particularly for worms held in the 10 ppt treatment. By day seven of the experiment, a number of worms exhibited substantial degradation of the terminal posterior segments (Figure 10). The proportion of worms with visible degradation (poor condition) increased to nearly 80% by the end of the 16-day experimental period (Figure 11). The propensity of worms to build sediment tubes also decreased with decreasing salinity (Figure 12). There was a strong association between percentage of worms in tubes and treatment by day 16 (R x C; $\chi^2 = 39.4$; df=3; $p<0.001$). The proportion of worms in tubes was

consistently the highest in the 30 ppt treatment. Although the proportion of worms in tubes in the 20 ppt treatment was lower and more variable, the majority of worms in this treatment were in tubes throughout the experiment. In contrast, the percentage of worms in tubes in the 10 ppt treatment decreased over time until there were no worms in tubes by day eight.

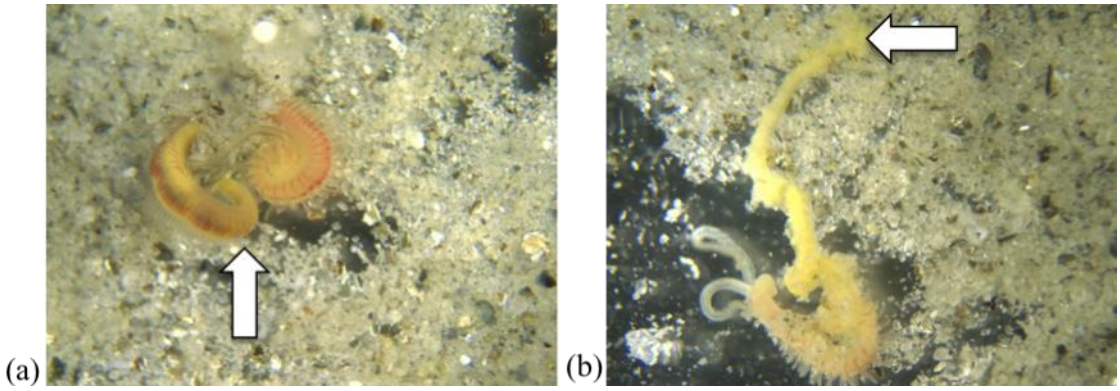

Figure 10. Normal and degraded *Polydora websteri*. Normal worm (a) in 30 ppt (control) treatment at day eight in the experiment. Worm in 10 ppt (b) at day eight. Unlike the control, this worm is beginning to degrade at its posterior end (indicated by arrows).

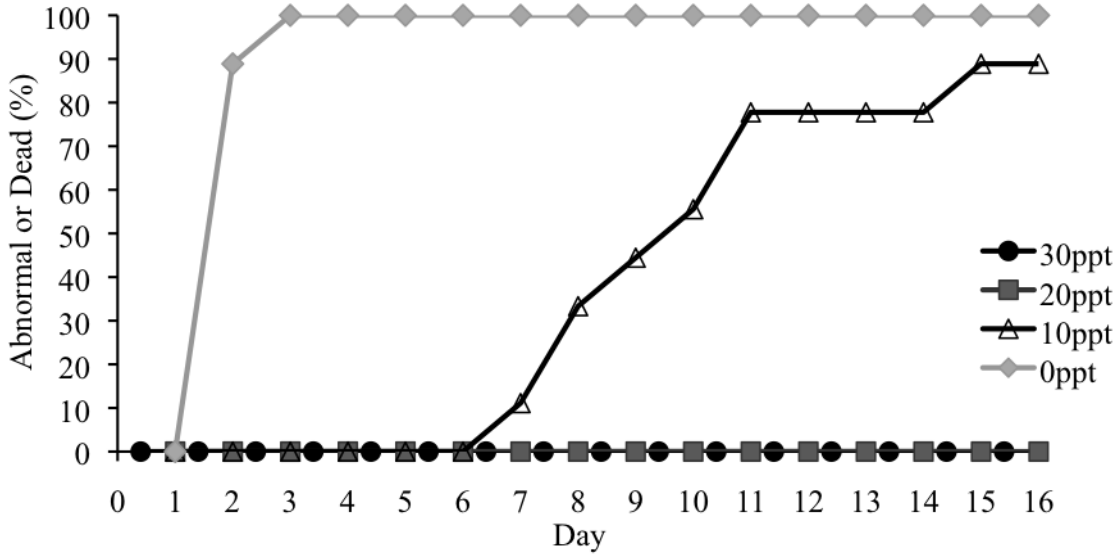

Figure 11. Worm condition in Experiment III. The percentage of worms that were dead or in poor condition in each of four salinity treatments (n=18 worms per treatment). Poor condition was characterized by worms that were beginning to degrade at their posterior ends (see Figure 10). The data points for the 30 ppt treatment were offset for clarity.

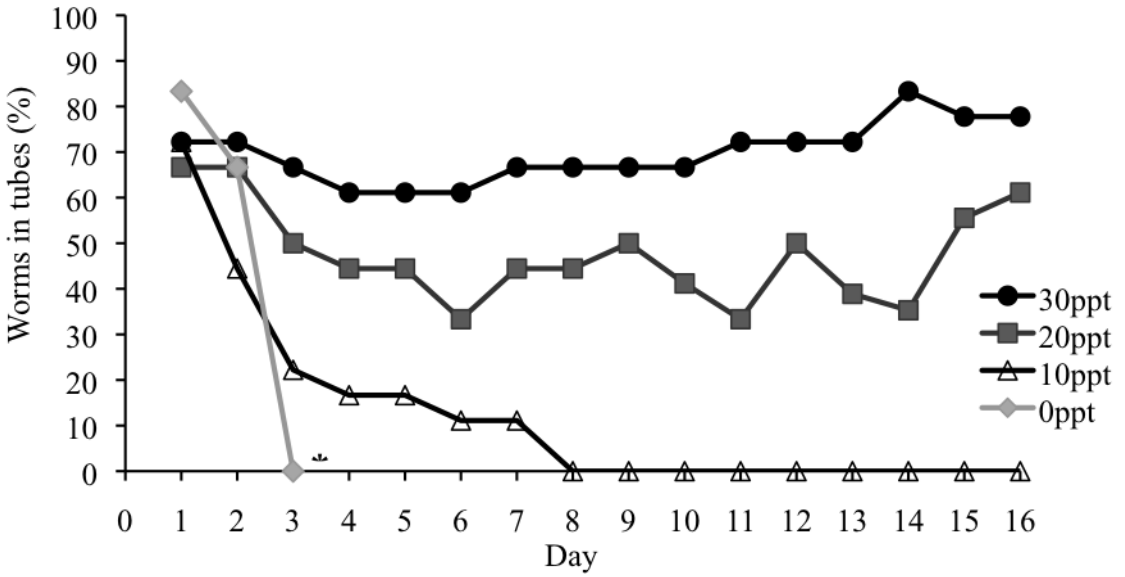

Figure 12. Tube building in Experiment III. The percentage of worms in sediment tubes in each of four salinity treatments (n=18 worms per treatment). All of the worms in the 0 ppt treatment were dead by day 3.

DISCUSSION

Diverse treatments have been proposed for ridding oysters of mudworm infestations (e.g. Dunphy *et. al.*, 2005; Gallo-Garcia *et. al.*, 2004; Lafferty and Kuris, 1996; Loosanoff and Engle, 1943; Nel *et. al.*, 1996; Nell, 2007). Those that involve chemical treatments, such as iodine baths, are not suitable because they can reduce the marketability of treated oysters, whereas those that involve multiple, complex steps will not be as practical for oyster farmers to implement in a cost-effective manner. On the other hand, exposure to increased and decreased salinity is a tractable alternative for killing the mudworm. For example, Dunphy *et. al.* (2005) and Nel *et. al.* (1996) suggest that short-term exposure to freshwater can reduce mudworm infestation while the oyster host is relatively unaffected. In the present study, I used R.O. water as a proxy for freshwater and I too found that survival of the oyster was unaffected by short-term exposure of up to three days in freshwater. The oyster is able to close its valves and

protect itself from the low-salinity water. In contrast, the worm burrows are exposed to the outside environment and lower salinity water will enter the burrows, creating a physiological challenge for the worm.

Freshwater treatments are simple and low cost and some authors have considered them to be highly effective. For example, Dunphy *et. al.* (2005) suggested that treatments resulting in greater than 50% worm mortality are "commercially effective". I observed substantial worm mortality (25-60%) after intact oysters were exposed to freshwater for 72 hours. However, I believe 100% worm mortality, or close to 100% worm mortality, is necessary to recover the marketability of oysters. I observed that even though a freshwater exposure kills a significant proportion of worms inside of the oyster, there were still many living worms in the shell. As previously mentioned, there can be as many as six or seven layers of burrows in heavily infested oysters (Loosanoff and Engle, 1943; personal observation, 2011). I also observed that worms that were deep inside the shell, under many layers of burrows, were seemingly unaffected even after three days in freshwater. Although burrows are exposed to water, it takes time for the burrow to come into equilibrium with the outside environment. The deeper burrows are probably less susceptible to changes in the environment, therefore worms in these burrows do not experience the same stress as those in outer burrows. Thus, in heavily infested oysters where there may be upwards of 100 worms, if 50% the worms are left alive inside the shell they can still cause major damage. They will continue to extend their burrows, and accumulate mud and debris inside the shell, thereby decreasing the value of the oyster.

An understanding of the basic biology of a pest species is necessary in order to effectively control and mitigate the impact of the species (Simon and Booth, 2007). Most

of what is known about *P. websteri*, and other shell-boring polychaetes, are chance observations of larvae (Hopkins, 1958), small investigations of their tolerance to select environmental conditions (Nel *et. al.*, 1996), and one in-depth study on the mechanism by which they bore into shells (Haigler, 1969). Although hyposaline and hypersaline treatments have been proposed as viable treatments for the control of mudworm, no study prior to mine systematically investigated the salinity tolerance of the worm outside of the oyster shell. In this study, I investigated the salinity tolerance of the worm and observed that salinity tolerance inside the shell differed substantially from salinity tolerance of the worm outside of the shell.

There were no discernable differences between worms placed in 30 ppt (control) and 20 ppt treatments. In these treatments, worms had normal, red-orange, coloration (Blake, 1971), were actively moving, and a high proportion of the worms continued to build tubes throughout the experiment, which is typical behavior for individuals of *P. websteri* that are removed from their burrows (Haigler, 1969; Loosanoff and Engle, 1943). The slightly lower frequency of worms in tubes in the 20 ppt treatment was likely due to stress related to the lower salinity even though this stress did not reduce worm survival over the two-week study. I also observed that proportion of worms in tubes increased over time in the 30 ppt treatment, which suggests that they were acclimating to the test container. These results were expected considering the oysters sampled for this project experience a relatively constant salinity of 26 to 30 ppt on the Bagaduce River. The river is tidally influenced and well mixed which means that the organisms in the river are subjected to a constant salinity, although with certain events they could experience salinities as low as 20 ppt.

The 10 ppt salinity treatment imparted substantially higher stress on worms that had been removed from their burrows. Although most of the worms in the treatment survived well over a week, the color of the worms had changed from red-orange to yellow and then white by day two. This discoloration is indicative of worms in poor condition. By day seven, I observed that worms had begun degenerating, starting with the posterior segments. Although some spionids autotomize, or lose segments, under stressful conditions (Stock, 1965), in this case the degredation is likely due to the worms' inability to osmoregulate. Polychaetes have nephridia, which can function to excrete excess water during initial exposure to low salinity environments. For longer periods of immersion in lower salinity, the nephrons may not be able to keep up with the amount of water that needs to be excreted and the worm's tissues begin to degrade.

Stress is also indicated by altered tube building behavior. As mentioned above, building tubes when outside of burrows is normal behavior for *P. websteri*. Over time, the proportion of worms in tubes eventually decreased to zero in the 10 ppt treatment. Because tube building takes energy, my results suggest that in the 10 ppt treatment, the condition of worms decreased to the point where the worms may not have had energy, or had compromised nervous systems, which prohibited tube building. This finding illustrates the physiological impact that a large decrease in salinity had on the worms, even though survival was relatively high up until the last two days of the experiment.

The 0 ppt treatment, which was intended to mimic a freshwater treatment, resulted in 100% mortality in just three days. Nel *et al.* (1996) reported that when "polydorids" were removed from burrows and placed in freshwater they died within 10 minutes. The *P. websteri* worms used in this study took three days to die in the 0 ppt treatment. The

difference between my results and those reported by Nel *et al.* (1996) could be due to time (approximately two days) it took for salinity to be reduced to 0 ppt. My methods were intended to mimic the change in salinity that worms might experience inside of burrows where they are likely buffered from abrupt changes. My observations of high mortality and poor condition among free-living worms held at 0 ppt are consistent with Nel *et. al.* (1996) and indicates that *P. websteri* is not tolerant to freshwater. However, my results also illustrate the protection afforded by burrows. When oysters were exposed to 0 ppt for three days, I observed a maximum of 60% worm mortality. Therefore, shell protection needs to be considered when creating effective control methods. Further, my results suggest that an increased duration of exposure to freshwater, perhaps four to five days, may prove effective at ridding oysters of 100% of the mudworm infestation, as long as the oyster host does not experience significant mortality from such treatments.

My study also examined whether a 72-hour exposure to low salinity combined with a period of dry, cold storage could increase mudworm mortality while the worms are still in burrows. Nick Brown (Center for Cooperative Aquaculture Research) and Jesse Leach (Bagaduce River Oyster Company) found that worm mortality was near 100% when oysters were held in dry, cold storage for two months. They found little host mortality when the treatment was applied to oysters placed in storage during the winter when oyster metabolism was at an annual low. More recently, Brown and Leach (pers. comm.) found that dry, cold storage for as little as three weeks could rid oysters of worms. However, their second experiment was conducted in early fall and they observed increased host mortality. My results indicate that the duration of dry, cold storage can perhaps be reduced to eight days by treating oysters first with an exposure to freshwater.

When oysters were left in dry, cold storage for four days after an initial three-day 0 ppt soak, I observed nearly 98% worm mortality. This indicates that combining a desiccation stress to a salinity stress increases worm mortality.

The goals for any treatment are to reduce the complexity of the procedure, to have an effective method, and to reduce the amount of time the oysters are out of the water. A combination treatment of 0 ppt, or freshwater, soak and dry, cold storage is a simple method of treating oysters. Freshwater is readily available and farmers can rent portable, industrial-size refrigerators. The refrigerators are currently rented for the longer storage treatments so this new method would reduce the amount of time the unit will need to be rented, saving more money. Although oysters are able to survive periods of time out of water, they are not growing and this ultimately costs the farmer money because oysters will have to spend more time in the water post-treatment to reach market size. Reducing treatment time to around 10 days means that oysters will be worm-free and ready to go back into seawater for growth and recovery. Once the worms are dead, the oyster will secrete shell material over the burrows, restoring appearance and market value.

Future work will be required before this treatment can be used commercially. The first objective would be to scale up my experiments. In my experiment, oysters were held in individual containers, which is not practical for a commercial operation. A farmer would most likely put oysters in mesh bags that would have hundreds of oysters in each bag. The amount of oysters will likely influence the amount of time needed for dry, cold storage to result in 100% worm mortality because the density of oysters may create a more humid environment during the dry, cold storage period.

Ideally, farmers would benefit from site-specific pest management plans that limit infestation in the first place, particularly given the variability in effectiveness of treatments based on location, host shellfish species and pest species (Gallo-Garcia *et. al.* 2004; Loosanoff and Engle, 1943; Nell, 2007). In my experiment, eastern oysters from the Bagaduce River, ME infested with *P. websteri* were treated by a soak in 0 ppt water followed by dry, cold storage. Such a treatment may not be applicable to oysters grown in lower salinity water, or oysters grown intertidally where worms, as well as oysters, are acclimatized to periods of dry or fresh conditions. It would be more beneficial to document the life cycle and ecology of the worm to aid the design of measures preventing infestation in the first place. At what life stage do worms enter the oyster shell? At what time of year are they most likely to infect oysters? Will *P. websteri* preferentially settle on something other than oyster shell? These are just some of the questions that should be answered to construct successful preventive measures.

While studying oysters in Louisiana waters, Hopkins (1958) observed the planktonic larvae of *Polydora websteri* leaving an oyster shell. Through the use of plankton tows and observations of infested oysters over a year, he suggested that *P. websteri* reproduces year round at temperatures ranging from 10°C to 30°C. He hypothesized that larvae develop within egg cases throughout the year but development is more rapid during warm months. Although this provides some information on the life cycle of *P. websteri*, it may not apply to farmers in Maine. Blake (1969) observed polydorid larvae in Maine waters from April to August, with more larvae in May and June. While excavating burrows in the oysters from the Bagaduce River, I observed eggs in burrows with adult worms. In other blisters there appeared to be a "nest" of larvae that

I believe belonged to *P. websteri*. These observations were made in October and the oysters were held at 18°C and 30 ppt. Though Blake (1969) suggested that reproduction is seasonal, future experiments should look into the presence or absence of larvae in the water column as well as severity of infestation each month. If time, stage of settlement and water temperature at time of settlement can be determined, farmers may be able to prevent or lessen *P. websteri* infestation.

In summary, my experiments indicate that a combination treatment of a 0 ppt exposure with an eight-day dry, cold storage period results in 100% worm mortality. My method is one that improves existing treatments so that there is increased worm mortality, a decrease in treatment time and minimal impact on the oyster host.

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Shannon Whitney Brown was born on December 16, 1989 in Putnam Valley, New York. She graduated salutatorian of her class at Putnam Valley High School in 2008. At the University of Maine, she majored in Marine Science with a concentration in Marine Biology and was recently inducted into Phi Beta Kappa. She spent the fall of her junior year at the University of Maine's Darling Marine Center for the Semester by the Sea program. In the summer of 2011, she participated in a NSF funded Research Experience for Undergraduates at the University of Delaware where she studied horseshoe crabs. She gratefully accepts being a "worm person" after working in Dr. Sara Lindsay's lab for the past three years.

Upon graduation, Shannon plans to attend graduate school to obtain her master's degree in Marine Science. She hopes to some day work for an environmental agency where she can conduct marine research and science outreach to the community.