Western Washington University
Western CEDAR

WWU Graduate School Collection   WWU Graduate and Undergraduate Scholarship

Fall 2015

Physiological and Behavioral Responses to Changes in Salinity in the Invasive Bivalve Nuttallia obscurata Compared to the Native Bivalve Leukoma staminea

Tori B. Talkington
Western Washington University, tori.talkington@wwu.edu

Follow this and additional works at: https://cedar.wwu.edu/wwuet

Part of the Biology Commons

PHYSIOLOGICAL AND BEHAVIORAL RESPONSES TO CHANGES IN SALINITY

IN THE INVASIVE BIVALVE *NUTTALLIA OBSCURATA* COMPARED TO THE

NATIVE BIVALVE *LEUKOMA STAMINEA*

by

Tori B. Talkington

Kathleen L. Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

Chair, Dr. Deborah Donovan

Dr. Brian Bingham

Dr. Jose Serrano-Moreno

MASTER'S THESIS

PHYSIOLOGICAL AND BEHAVIORAL RESPONSES TO CHANGES IN SALINITY

IN THE INVASIVE BIVALVE *NUTTALLIA OBSCURATA* COMPARED TO THE

NATIVE BIVALVE *LEUKOMA STAMINEA*

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

by
Tori Talkington

2015

ABSTRACT

Nuttallia obscurata is a euryhaline invasive clam with populations that are rapidly spreading along the northeast Pacific coast. It inhabits areas of changing salinity such as the high intertidal and areas of freshwater seepages, areas uninhabited by local clam species. *N. obscurata*'s euryhalinity, salinity tolerance, and ability to survive in uniquely stressful areas likely facilitated its settlement and rapid spread into these distinctive microclimates. Previous research on *N. obscurata* is minimal, however favorable physiological and behavioral responses likely allow *N. obscurata* to live in these environments. The physiological response of osmolyte production and the behavioral responses of filtration rate have yet to be studied in *N. obscurata*, but likely play in important role in their tolerance and therefore invasiveness. I compared these responses to those of *Leukoma staminea,* a local stenohaline clam that inhabits the mid-intertidal. By comparing the physiology and behavior of *N. obscurata* and *L. staminea,* I may be able to determine if euryhalinity and inhabitation of open niche areas helps *N. obscurata* invade.

The important osmolytes previously noted in bivalves, betaine and sorbitol, were examined using High Performance Liquid Chromatography. Osmolyte concentration in clams under different salinities (1 ppt, 20 ppt, 30 ppt, 40 ppt and 60 ppt), under short-term (5 to 90 minutes) exposure, and under long-term (24 hr) exposure was examined. Betaine was a cellular component in both *N. obscurata* and *L. staminea*. Large stores were found in *N. obscurata* under 30 ppt salinity with concentrations decreasing under hypoosmotic salinities (1 ppt and 20ppt). This suggests that betaine is produced in large amounts in *N. obscurata* tissue and possibly utilized for osmotic compensation under decreased salinities. Betaine concentration in *N. obscurata* decreased under hyperosmotic conditions, likely because *N. obscurata* was utilizing energy stores to survive and not regenerating betaine. Other osmolytes, such as proline, glycine

or taurine, may also be utilized in *N. obscurata* to cope with osmotic stress. There was evidence that taurine was a cellular component in some samples, but the concentration of taurine could not be determined. *L. staminea* produced betaine at a very low concentration under 30 ppt salinity and increased betaine under hypoosmotic and hyperosmotic conditions, helping the species cope with osmotic stress. Betaine increased significantly over time in *N. obscurata* gill tissue, showing that *N. obscurata* accumulates betaine quickly to compensate with fast environmental changes. Whether this accumulation is part of *N. obscurata's* normal cell function or a response to stress is unknown. Sorbitol was not a major cellular component in *N. obscurata* or *L. staminea*.

Filtration rates of *N. obscurata* and *L. staminea* were tested using fluorometry over multiple salinity levels (1 ppt, 20 ppt, 30 ppt, 40 ppt and 60 ppt) for 340 minutes. *N. obscurata* filtered under a wider range of salinities than did *L. staminea*. Salinity significantly affected filtration rate and neither species filtered under extreme hypoosmotic or hyperosmotic conditions (1 ppt and 60 ppt), implying that although *N. obscurata* inhabits some hypoosmotic areas, it is unlikely to expand populations to freshwater. Both species filtered at higher rates compared to previously studied bivalves. This is of special concern for the already invasive and expanding populations of *N. obscurata,* which already outcompete some local clam species in disturbed areas.

While physiological and behavioral responses were examined separately in this experiment, they likely interact as part of a complex system. Filtration rate altered the exposure level of clam tissue to the external environment, thereby affecting osmotic response and osmolyte concentration. Future studies should continue to examine physiology and behavior in *N. obscurata*.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Invasions of non-indigenous species (NIS) have reshaped the structure and dynamics of ecosystems around the world (Carlton and Geller, 1993; Lodge, 1993; Mack *et al.*, 2000; Verling *et al.,* 2005). There are an estimated 50,000 NIS present in the United States, and the rate of NIS invasions in the Pacific Northwest have increased dramatically over the last 200 years (Carlton and Geller, 1993; Pimentel and Lach, 2000; Ruiz *et al.*, 2000). Over 59% of NIS cause major ecological and biological diversity damage and have high economic costs (Office of Technology Assessment, 1993; Bax *et al.,* 2003; Byers, 2005; Grosholz, 2005). Forty-two percent of threatened and endangered native species are at risk because of competition with or predation by NIS (Pimentel and Zuniga, 2005; Mooney *et al.,* 2005). Marine species introductions have increased dramatically in the United States over the past 40 years, accounting for more than 500 NIS invasions, with a majority of invasions occurring on the Pacific coast (Grosholz, 2005). NIS in the phylum Mollusca are some of the most damaging invasive species (Ruiz *et al.*, 1997; Ruiz *et al.,* 2000; Zenetos *et al.,* 2005) and understanding the impacts of molluscan NIS on the Pacific coast is critical as marine introductions increase and expand.

Nuttallia obscurata, commonly known as the purple varnish clam, was first reported on the Pacific coast of North America near Vancouver, BC in 1991 and is now considered an invasive NIS (Mills, 2002). Native to Korea, China, and Japan, *N. obscurata* had spread from Northeastern Vancouver Island, BC to Coos Bay, Oregon by 2002 (Gillespie *et al.,* 1999; Mills, 2002; Byers, 2002). In some studies, *N. obscurata* outcompete the economically important manila clam, *Venerupis philippinarum* (Dudas, 2005). *N. obscurata* may in some cases change food web dynamics, as it is a preferred prey for some crab species and raccoons (Dudas, 2005; Simmons *et al.,* 2014). The bivalve is now found in shellfish markets and is attracting attention from the commercial shellfish industry (Gillespie *et al.*, 2001). *N. obscurata* has sustainable

populations (Dudas, 2005) and in many areas along the Washington and Oregon coasts, these clams are abundant and are attracting recreational shellfish harvesters (Washington Department of Fish and Wildlife, 2013). Little is known about this species' basic biology and the characteristics that led it to succeed as an invader. However, it has been noted that the species is incredibly tolerant to environmental stressors.

N. obscurata is considered an extreme euryhaline, a species which has adapted to great changes in salinity. It is able to survive for 3.75 days at 60 ppt and for up to two weeks at 1 ppt salinity (Siegrist, 2010). This enables the species to live in unique areas such as the high intertidal and freshwater seepage (Byers, 2002, Dudas *et al.,* 2007). These areas are not inhabited by local clams and it is likely that *N. obscurata* was able to inhabit these open niches and spread at such a rapid rate because of its unique euryhalinity.

To survive in such a wide range of salinities, *N. obscurata* likely uses a strong osmotic response. This osmotic response may be uniquely different than local species because they do not inhabit the harsh environments of the high intertidal or areas of freshwater seepages. *N. obscurata* may also have a higher filtration rate than other bivalves leading to its high densities and great rate of population expansion. Because *N. obscurata* lives high in the intertidal, it may also have less time to filter feed and, therefore, need to filter at a higher rate to compensate for its reduced time submerged in water.

N. obscurata's physiological response (osmotic response) and behavioral response (filtration) likely differ from local non-euryhaline bivalves, such as the native littleneck clam *Leukoma staminea*. *L. staminea* lives in the mid to low intertidal zone and dies within 48 hours of hypoosmotic exposure (Byers, 2002; Gillespie et al., 2001; Siegrist, 2010). *L. staminea* is an important species because of its economic and recreational value (Shaw, 1986). Understanding

these responses will help to more fully explain *N. obscurata's* differences from local species, success as an invasive species, species range, effect on the environment, and effect on local species.

It is also important to note that behavioral responses such as filtration rate directly affect the amount of exposure tissues have with the external environment, altering the tissue's physiological osmotic response. Because physiological and behavioral changes are interrelated, studying these changes in tandem will help to more fully understand a clam's reaction to changing salinities.

Osmolyte Concentration

The biological mechanisms *N. obscurata* employs to survive under changing salinity are unknown. Many organisms use physiological mechanisms such as solute and ion regulation to compensate for osmotic stress. Altering the internal ion composition in both osmoconformers and osmoregulators affects ion gradients, osmotic pressures, protein ion interactions, and other cellular functions (Hill *et al.*, 2004). Isolated *N. obscurata* gill tissue survives with no decline in activity for at least two weeks during hypoosmotic exposure (10 ppt to 30 ppt) (Siegrist, 2010). In addition, *N. obscurata* gill tissue survive in extreme hypoosmotic and hyperosmotic salinities for up to 14 days (Wittes and Donovan, unpublished data). All cell membranes are permeable to water, causing cells to shrink in hyperosmotic conditions and swell in hypoosmotic conditions. Changes in cell size and internal water content are undesirable for cells that need a constant cell volume to maintain optimal function. To maintain optimal cell volume, organisms adjust their

cellular ion concentration by increasing their internal solute concentration in hypotonic solutions and decreasing their solute concentration in hypertonic solutions (Yancey, 2001).

Osmolytes, especially betaine, play an important role in osmotic regulation at extreme salinities in bivalves and do not alter the cell's protein structure (Yancey, 2005). Betaine is a chemical that is synthesized from choline via intracellular synthesis or from extracellular pools produced in the mitochondria (Pierce *et al.,* 1992; Yancey, 2005). Betaine is often accumulated as a response to stress, especially osmotic stress, and is the most important solute dictating osmolyte regulation in the California mussel, *Mytilus californianus,* where its concentration decreases in gill tissue under hypoosmotic conditions (Neufeld and Wright*,* 1996). Rapid increase in betaine is also seen in the ribbed mussel, *Geukensia demissa,* in hyperosmotic environments (Deaton, 2001). The Atlantic oyster, *Crassostrea virginica,* mobilizes glycine-betaine under osmotic stress, but this varies among populations (Pierce *et al.,* 1995). Sorbitol, often synthesized from glucose, has also been linked to osmotic regulation in invertebrates (Yancey, 2005). Betaine and sorbitol are both compatible solutes, meaning their concentrations can change dramatically without disrupting protein function. Betaine and sorbitol concentrations in bivalve tissue can be tested simultaneously using High Performance Liquid Chromatography (HPLC).

Not only is it important to understand the role of osmolytes at different salinity levels, but it is necessary to determine which tissues are responsible for osmotic regulation to further understand their basic biology. Most studies on bivalves have linked osmotic regulation to gill and mantle tissue. Bivalve gill tissue functions as a major site for gas exchange, having a high surface area-to-volume ratio, and has been linked to osmotic regulation. For example, osmotic regulation and changes in osmolyte concentration have been seen in the gills of *G. demissa*

(Deaton, 2001)*, Mytilus edulis* (Neufeld and Wright*,* 1996), *Mytilus trossulus, C. virginica* and

Mercenaria mercenaria (Van Winkle, 1972). The mantle also has high surface area-to-volume

contact with the external environment and the free amino acid pool changes with differing

salinity in the mantle of the bivalve *Corbula amurensis* (Paganini *et al.*, 2010).

The timing of osmolyte production, or how an organism responds under short-term stress

versus under long-term stress, is also important. Short-term changes are necessary for rapid

adjustment, as might happen in estuaries subject to quick tidal changes or runoff areas during a

rainstorm. These rapid internal solute changes happen via the break-down of existing proteins

into free amino acids or by disassembling other large molecules. In many bivalves, there is an

immediate short-term stress response to salinity change, sometimes referred to as initial shock

response. For example, within 12 hours *G. demissa* rapidly increases glycine and alanine when

transferred from hypoosmotic to sea water conditions (Deaton, 2001). Because *N. obscurata*

survives in estuaries and areas of freshwater seepages which have rapidly changing salinities it

likely has a short-term response. *N. obscurata* also survives for up to 3.75 days at 60 ppt and

over two weeks at 1 ppt salinity, which means that it has long-term methods of tolerating

osmotic stress (Wittes and Donovan, unpublished data). For this reason, I tested osmolyte

concentrations in both short-term (5-90 minute) and long-term (24 hour) exposure to different

salinities.

Filtration Rates

As bivalves alter their internal composition to compensate for changes in salinity they

may also undergo behavioral responses, such as change in filtration rate. Bivalves may reduce

5

their exposure to a salinity change by closing their shell or reducing filtration. This may help them save energy in an osmotic response (Shumway *et al*., 1977), but in return they must use energy in controlling shell closure and sacrifice optimal respiration and feeding during this time (Vernberg *et al..* 1969; Pierce, 1971; Riisgard *et al.,* 2003). Open siphons, active beating of gill cilia, and filtration serve in bivalve respiration and feeding. Therefore, shell-closing and a lower filtration rate may reduce a bivalve's respiration, metabolism, feeding rates, and reproduction (Navarro *et al.,* 2003; Gosling, 2003). Lower feeding and respiration rates may translate to lower growth and reproduction, and reduced expansion of the species (Newell and Bayne*,* 1980; Møhlenberg and Riisgård, 1981; Smaal and Widdows*,* 1994). Bivalves must balance their need to respond to osmotic stress by reducing filtration with the need to filter, respire, feed, grow, and reproduce.

Filtration rate varies among bivalve species, but is particularly high in some euryhaline species such as the Asian clam, *Corbicula fluminea*, which can filter up to 10 ml hr^{-1} g^{-1}, which in turn may have helped the populations expand so quickly (Elliott and zu Ermgassen, 2008). The invasive bivalve *Perna viridis* has nearly double the filtration rate of the native bivalve *C. virginica* under slightly hypoosmotic conditions (25 ppt to 35 ppt) (McFarland *et al.,* 2013). It is likely that *N. obscurata* filters at a high rate, which has helped the species grow and expand. *N. obscurata's* unique inhabitation of the high intertidal which limits the amount of time the species can filter feed.

Experimental Questions

My goals were to determine physiological and behavioral responses of *N. obscurata* and *L. staminea* to different salinities (1 ppt, 20 ppt, 30 ppt, 40 ppt, and 60 ppt). My research questions were:

1. How do levels of betaine and sorbitol differ in gill and mantle tissues of *N. obscurata* and *L. staminea* after clams are held for 24 hours at different salinities?

2. How do levels of betaine and sorbitol change in *N. obscurata* gill tissue after being held at different salinities over short-term exposure (5 min, 60 min, 90 min)?

3. How do filtration rates differ in *N. obscurata* and *L. staminea* under exposure to different salinities?

MATERIALS AND METHODS

Clams were collected either from Mud Bay in Bellingham, WA, (48.701861° N, -122.497041 ° W) during low tides in March through May 2013 or from Birch Bay, WA, (48.927° N 122.745° W; accessed north of Birch Bay State Park) during a low tide in February 2013. Both species were collected with hands and shovels from the first 0.5 meters of sediment. Clams were held in a 10-gallon holding tank of 30 ppt salt water for approximately 48 hours until the time of the experiment in Western Washington University's 10°C cold room. This is where all experiments took place to ensure the clams were tested at a constant temperature. Clams were not fed during this time. Clam mass was recorded before they underwent treatment.

Water of different salinity levels (1 ppt, 20 ppt, 30 ppt, 40 ppt, and 60 ppt) was prepared separately for every experiment. The salinity levels were chosen to represent one treatment near local ambient seawater conditions (30 ppt), two treatments in which *N. obscurata* osmoconforms (20 ppt and 40 ppt), and two treatments in extreme hypoosmotic and hyperosmotic environments in which *N. obscurata* osmoregulates (1 ppt and 60 ppt) (Wittes and Donovan, unpublished data)

Osmolyte Analysis

Osmolyte levels of whole animals under long-term conditions (24 hours)

After collection and the 48 hour holding time, clams (N = 50, 25 clams of each species) were haphazardly distributed individually into labeled 500 ml plastic containers filled with 250 ml of the treatment water (1 ppt, 20 ppt, 30 ppt, 40 ppt, or 60 ppt), resulting in five clams per salinity level per species. The plastic containers were arranged in a randomized block design and

stock solutions of water of each salinity were made by mixing Instant Ocean with DI water. Salinity was checked with a refractometer. Each container contained a clam and an air stone, set on low air flow. The clams were held in the treatment water for 24 hours.

Just prior to the experiment, a small hole was drilled with a Dremel drill into each clamshell on the posterior shell area. The hole ensured that at least some treatment water came in contact with gill and mantle tissue if the clam closed during treatment. Care was taken to avoid drilling into any of the clam's mantle or internal organs during this process, however mantle tissue was occasionally damaged in this process.

Gill and mantle tissues (25 gill and 25 mantle samples from each species) were dissected and to ensure standardization in dissection technique, a single lab assistant was selected to dissect all specimens for an entire experiment. Dissections were completed using surgical scissors, washed between dissections. After dissections, tissues were blotted dry, wet mass was recorded, and HPLC preparation and analysis was performed.

For HPLC preparation, dissected gill and mantle tissues were kept on ice until they were homogenized in 2 ml 10% trichloroacetic acid using a table-top homogenizer for 20 seconds per sample. The homogenizer was cleaned with DI water between samples. Samples were then centrifuged at 12,000 rpm for 15 minutes at 4°C. Samples were refrigerated for 2 hours at 1.6°C, allowing extra particulate matter to precipitate out of solution, and then centrifuged once again at 12,000 rpm for 15 minutes at 4°C. I then extracted 1.5 ml of the supernatant and neutralized it using 2.5M KOH to pH 6.2-7.8, checking accuracy with a micro pH meter, recalibrated every 20 samples. Data on the amount of KO (between 200 μL and 500 μL) and the exact pH of each sample were recorded for use in dilution calculations.

To remove remaining fats and isolate betaine and sorbitol, samples were passed through a Water's Sep-Pak C18 cartridge. This cartridge was first loaded with 2 ml HPLC grade methanol and filtered using a vacuum. After the methanol filtration, 2 mls of nano-pure water were also loaded and filtered through. During this process, the filter was never allowed to dry out and a meniscus of fluid was always left covering the filter. After the initial preparation of the filter, samples were loaded and filtered using a vacuum. These samples were then stored at -80ºC in HPLC vials until analysis. Storage time for the samples never exceeded 72 hours.

Each sample was passed through an HPLC column using an ion exclusion Rezex RCM Monosaccharide Ca^{+2} (8%, 300 x 7.8 mm) column manufactured by Phenomenex (PN 00H-0130-K0). I ran 50µl of each sample for 40 minutes. Each sample was run through the column at 85º C with a flow rate of 1 ml min^{-1} and a mobile phase of nano-pure water. The concentration outputs were read by a Reichert digital refractometer (Model #13106600). Before running any samples, the column was turned on and warmed for 4 to 5 hours and nano-pure water poured through the system until a baseline had stabilized. Column filters were replaced every 100 samples ensuring the column stayed clean of particulates.

For both osmolyte experiments, a negative and a positive control (internal standards) were included. Negative controls were samples that went through the entire preparation procedure but contained no clam tissue. Because these negative controls were free of osmolytes, they were used to indicate contamination between the samples. These negative controls were placed randomly throughout the samples, ensuring that there was one negative control for every 20 samples. Positive controls or internal standards contained 0.5 mM betaine and 0.5 mM sorbitol. There was one internal standard randomly placed for every 20 samples. These

standards showed consistent concentrations of osmolytes, thus ensuring no drift or change over time with the sample preparation, the HPLC column, or the refractometer.

Using Varian Inc.'s HPLC software, a chromatogram was generated for each tissue sample and the sample was then analyzed for concentrations of betaine and sorbitol. This information was gathered by first creating a series of chromatograms from standards, which contained known concentrations of betaine and sorbitol. A standard curve was created from this series of chromatographic standards. The samples were then compared to the standard curve to determine concentrations of betaine and sorbitol in each sample.

To account for the different mass of gill and mantle tissue in samples, wet tissue mass was converted into dry tissue mass. I derived a conversion factor for wet to dry tissue mass by dissecting gill and mantle tissues from three individuals of each clam species, weighing them while wet, then drying them at 100°C for 18 hours. Conversion rates were as follows: 0.17 for *N. obscurata* mantle tissue and 0.13 for gill tissue and 0.17 for *L. staminea* mantle tissue and 0.16 gill tissue with small amounts of variance between the dried samples. These data closely matched those of previously derived *L. staminea* wet to dry tissue conversion rates of 0.21 for all *L. staminea* tissue (Sidwell, 1981). The initial wet mass of each sample was then converted to dry mass and betaine and sorbitol were ultimately analyzed as μmole osmolytes per gram of dry tissue mass.

A three-way ANOVA was used to determine statistical differences between osmolyte levels (dependent variable) in the two tissues (gill and mantle) in the two species (*N. obscurata, L. staminea*) at the different salinities (1 ppt, 20 ppt, 30 ppt, 40 ppt, 60 ppt) (fixed independent variables). SPSS was used to analyze results of all experiments.

Osmolyte levels of gill tissue under short-term conditions in *N. obscurata* (5 to 90 minutes)

Due to time restrictions in processing the tissues for HPLC, only a single tissue type and a single species could be tested in this experiment. Previous research has shown that betaine can increase under hyperosmotic conditions and decrease under hypoosmotic conditions in bivalve gill tissue (Neufeld and Wright, 1996; Deaton, 2001) and I was most interested with the osmotic response of *N. obscurata.* Therefore I only tested betaine and sorbitol concentration in dissected gill tissue of *N. obscurata.*

Dissected gills from individual clams (N=75) were submerged in 25 ml treatment water (1 ppt, 20 ppt, 30 ppt, 40 ppt, or 60 ppt) in 50 ml plastic containers. The containers were arranged in a randomized block design as described above. Due to the small size of these containers and the short treatment time, no aerators were used. For each salinity level, the gills were held for 5, 30, or 90 minutes. Each treatment contained five clams per salinity per exposure time. Once the treatment time elapsed, the tissues were prepped for HPLC analysis, and osmolytes were extracted and analyzed as described previously. A two-way ANOVA was used to determine statistical differences between osmolyte levels (dependent variable) in *N. obscurata* at the different salinities and the different time periods (fixed independent variables). SPSS was used to analyze results of all experiments.

Filtration Rates

To determine behavioral responses in *N. obscurata* and *L. staminea* to a range of salinities, clams (N = 50, 25 of each species) were collected and placed individually into plastic

containers filled with 250 ml treatment water (1 ppt, 20 ppt, 30 ppt, 40 ppt, or 60 ppt), with five

clams per salinity treatment. Fluorometry was used to determine filtration rate. Fluorometry

reads the fluorescence of chlorophyll in algae. In my experiment, algae was added to an

experimental chamber and as the clam filtered this algae, the chlorophyll level decreased,

measuring a decrease in fluorescence. Preliminary studies showed that Tahitian *Isochrysis*

galbana, acquired from a local aquaculture facility, had the lowest death rate under extreme

salinities. Therefore, 14 mls of Tahitian *I. galbana* were added to each container containing a

clam and fluorometer readings were taken several times over the experiment (0 min, 60 min, 120

min, and 340 min). During the filtration experiment traits such as general appearance, siphon

exposure, tissue swelling, and shell-closure were noted.

Positive and negative controls were included in the experiment. For each salinity level,

there was a positive control that contained no clam, but did have algae added. For every salinity

treatment, there was also a negative control that contained a clam but no algae. The fluorometer

was checked with blanks containing DI water after every 20th sample to ensure there was no drift

in the fluorometer readings. Filtration rate was calculated using the equation:

$$Filtration\ rate = \frac{V(\log C_o - \log C_f)\,2.303}{t}$$

where C_o was the fluorometer reading at 60 minutes, C_f was the fluorometer reading at 340

minutes, *V* was volume, and *t* was time.

During all experiments, the positive controls generally showed a minor decrease in

fluorometry reading even though no filtration occurred. This reduced fluorometry reading was

likely due to algae death and settlement. Any experimental treatment that produced the same or

less change in filtration as the positive controls were considered treatments with no filtration and

were considered no filtration. These treatments were not included in the final analysis of

filtration rate. The negative controls showed almost no changes in the fluorometry readings.

Two-way ANOVAs were used to determine statistical differences between filtration rates

(dependent variable) in species (*N. obscurata* and *L. staminea*) at the different salinities (1 ppt,

20 ppt, 30 ppt, 40 ppt, 60 ppt) (fixed independent variables). SPSS was used to analyze results

of all experiments.

RESULTS

Osmolyte levels of whole animals under long-term conditions (24 hours)

Only betaine was consistently detected in gill and mantle tissue samples of *Nuttallia obscurata* and *Leukoma staminea*. Sorbitol was present in only 4.5% of all gill and mantle samples. No trends were seen in sorbitol concentration in species, salinity, or tissue type. Before analysis, betaine concentrations were transformed using the squared function to ensure equal variances. I used the Levene's test of equality of variances, which was verified. There was a significant interaction between species and salinity (Table 1). This means that that salinity and species' effects on betaine concentration were interdependent and that *N. obscurata's* betaine production responded differently to changes in salinity in comparison to *L. staminea*. The interaction is likely caused by the large amount of betaine in *N. obscurata* at 30 ppt salinity and the reduction in betaine under hypoosmotic and hyperosmotic conditions in *N. obscurata* (Fig.1). Betaine concentrations in *L. staminea* were low at near seawater conditions (30 ppt) and increased under hypoosmotic and hyperosmotic conditions. (Fig 1). There were no significant differences in betaine concentration between gill and mantle (Table 1).

Mean betaine concentrations were 191 μmole g^{-1} for *N. obscurata* and 154 μmole g^{-1} for *L. staminea* tissue over all salinities. Mean betaine concentrations in mantle tissue were 167 μmole g^{-1} for *N. obscurata* and 161 μmole g $^{-1}$ for *L. staminea*, while mean betaine concentrations in gill tissue were 215 μmole g^{-1} for *N. obscurata* and 148 μmole g^{-1} for *L. staminea* (Fig. 1).

Table 1: ANOVA results for the effects of species (*N. obscurata* and *L. staminea*), salinity treatment (1 ppt, 20 ppt, 30 ppt, 40 ppt, 60 ppt), and tissue type (gill and mantle) on squared betaine concentrations (μmole g^{-1}) in tissue held at different salinities over long-term exposure (24 hr).

Source	dF	F	p
Species	1	3.885	0.055
Salinity	4	0.859	0.497
Tissue Type	1	0.495	0.486
Species x Salinity	4	3.321	0.019
Species x Tissue Type	1	0.789	0.379
Salinity x Tissue Type	4	0.877	0.486
Species x Salinity x Tissue Type	4	1.126	0.357
Error	42		
Corrected Total	61		
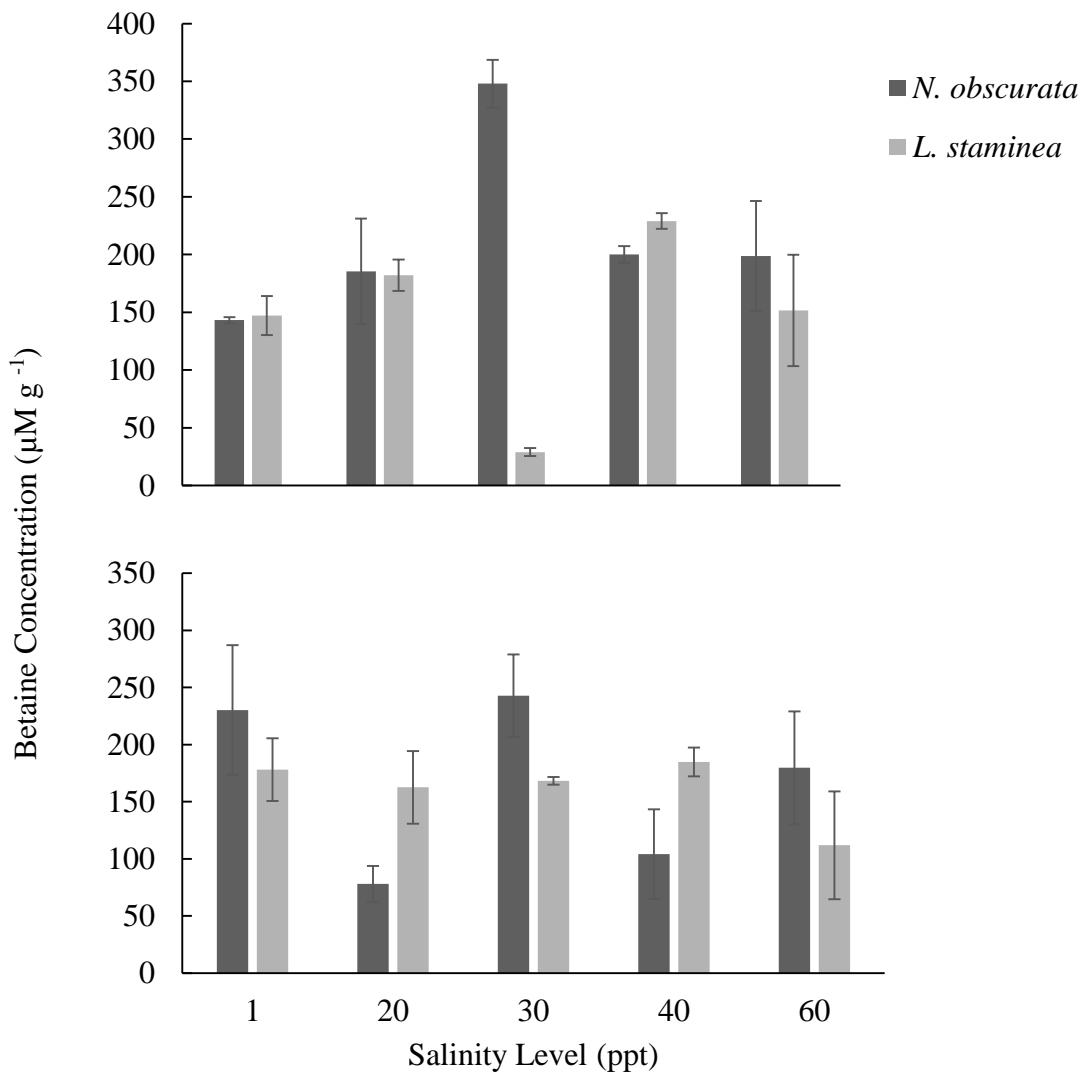
Figure 1. Average betaine concentrations (μmole g $^{-1}$) in gill (upper panel) and mantle (lower panel) tissue of *N. obscurata* and *L. staminea* held at different salinities (1 ppt, 20 ppt, 30 ppt, 40 ppt, 60 ppt) over long-term exposure (24 hr). Clams were collected from Mud Bay, WA. N =5 per treatment. Error bars indicate standard error.

Osmolyte levels of gill tissue under short-term conditions in *N. obscurata* (5 to 90 minutes)

Only betaine was consistently detected in excised gill tissue that had been exposed to different salinities for 5, 30, and 90 minutes. Sorbitol was present in only 2.5% of the samples. No trends were seen in sorbitol concentration in species, salinity, or tissue type. Before analyzing the results for betaine, I used an inverse transformation (1/y) to ensure equal variances under Levene's test which was verified.

Betaine concentration was significantly affected by time but not salinity under short-term exposure (Table 2). Post-hoc analysis using Bonferroni's method indicated that gill tissue exposed for five minutes had significantly less betaine than gill tissue exposed for 90 minutes (p > 0.001). Mean betaine concentrations were 33 μmole g^{-1}, 36 μmole g^{-1}, and 48 μmole g^{-1} after 5, 30, and 90 min, respectively (Fig. 2).

Under these short-term conditions, *N. obscurata* produced an average of 38 μmole g^{-1}, while over 24 hour exposure in the previous experiment *N. obscurata* produced 242 μmole g^{-1}, a difference of 536%.

Filtration Rates

Before the results were analyzed, I transformed the filtration rate values using the log$_{10}$ function to achieve equal variances according to Levene's test which was verified. Species and salinity significantly affected filtration rate (Table 3). *N. obscurata* actively filtered at 20 ppt, 30 ppt, and 40 ppt salinity, with mean values of 5.2 ml hr^{-1} g^{-1}, 7.9 ml hr^{-1} g^{-1}, and 5.8 ml hr^{-1} g^{-1} respectively. *L. staminea* only actively filtered at 30 ppt and 40 ppt salinity, with mean filtration

Table 2: ANOVA results for the effects of salinity (1 ppt, 20 ppt, 30 ppt, 40 ppt, 60 ppt) over short-term exposure periods (5 min, 30 min, and 90 min) on the inverse of betaine concentrations (μmole g $^{-1}$) in *N. obscurata* gill tissue.

Source	dF	F	P
Time	2	8.805	0.001
Salinity	4	0.199	0.937
Time x Salinity	8	0.753	0.645
Error	42		
Corrected Total	57		
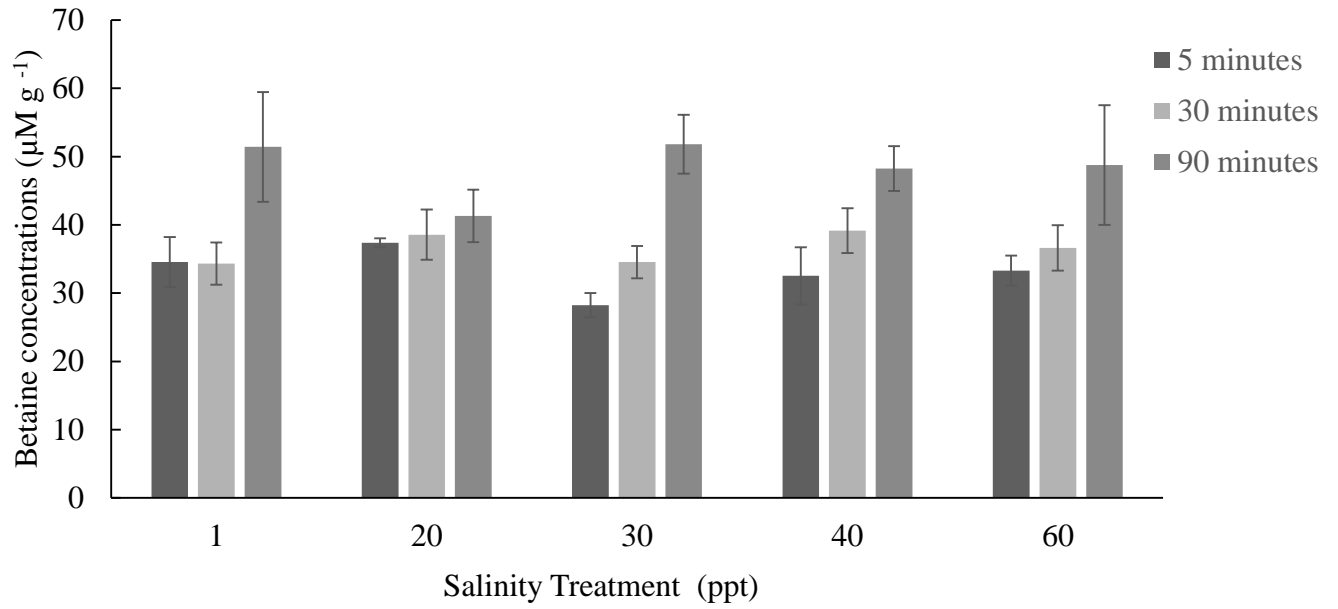
Figure 2. Average betaine concentrations (μmole g^{-1}) in *N. obscurata* gill tissue held at different salinities (1 ppt, 20 ppt, 30 ppt, 40 ppt, 60 ppt) over short-term exposure (5 min, 30 min, and 90 min). Clams were collected from Mud Bay, WA. N = 5 per treatment. Error bars indicate standard error.

Table 3: ANOVA results for the effects of salinity (1 ppt, 20 ppt, 30 ppt, 40 ppt, 60 ppt) and bivalve species (*N. obscurata*, *L. staminea*) on the square root of filtration rates over 340 minutes exposure time using Tahitian *Isochrysis galbana* algae.

Source	dF	F	P
Species	1	3.615	0.041
Salinity	4	4.002	>0.001
Salinity x Species	4	0.693	0.567
Error	38		
Corrected Total	47		

rates of 3.6 ml hr^{-1} g^{-1} and 6.7 ml hr^{-1} g^{-1}. Overall *N. obscurata* filtered at a higher rate than *L. staminea*. Species and salinity significantly affected filtration rate (Table 3). *N. obscurata* actively filtered at 20 ppt, 30 ppt, and 40 ppt salinity, with mean values of 5.2 ml hr^{-1} g^{-1}, 7.9 ml hr^{-1} g^{-1}, and 5.8 ml hr^{-1} g^{-1} respectively. Species and salinity significantly affected filtration rate (Table 3).

Species and salinity significantly affected filtration rate (Table 3). *N. obscurata* actively filtered at 20 ppt, 30 ppt, and 40 ppt salinity, with mean values of 5.2 ml hr^{-1} g^{-1}, 7.9 ml hr^{-1} g^{-1}, and 5.8 ml hr^{-1} g^{-1} respectively. *L. staminea* only actively filtered at 30 ppt and 40 ppt salinity, with mean filtration rates of 3.6 ml hr^{-1} g^{-1} and 6.7 ml hr^{-1} g^{-1}. Overall *N. obscurata* filtered at a higher rate than *L. staminea*. At 30 ppt and 40 ppt salinity, where both species filtered, *N. obscurata* filtered at an average rate of 8.5 ml hr^{-1} g^{-1} while *L. staminea* filtered at an average rate of 6.2 ml hr^{-1} g^{-1} (Fig 3). Filtration rates at 1 ppt and 60 ppt salinity were greatly reduced for both species. Post-hoc analysis using Bonferroni's method showed that filtration at 30 ppt was significantly higher than filtration at 1 ppt or 60 ppt salinity. There was great variability in the filtration rates of *N. obscurata* at 30 ppt and *L. staminea* at 40 ppt salinity because both groups contained a single outlier with very high filtration.

N. obscurata also exhibited less shell-closing and more exposed siphons in comparison to *L. staminea* at all salinity levels except at 1 ppt and 60 ppt salinity (Table 4). At 1 ppt and 60 ppt salinity both species exhibited shell-closing and unexposed siphons. Tissue swelling was noted in *L. staminea* at 1 ppt salinity.

Table 4: Quantitative results for the effects of salinity (1 ppt, 20 ppt, 30 ppt, 40 ppt, 60 ppt) and bivalve species (*N. obscurata, L. staminea*) on filtration rates over 340 minutes exposure time using Tahitian *Isochrysis galbana* algae. Plus plus (+ +) signifies that the action was observed to a great degree, plus (+) signifies that the action was observed to a lesser degree, and minus (-) signifies that the action was not observed.

Filtration Treatment	Active Filtration	Openness of Shell
N. obscurata 1 ppt	-	-
L. staminea 1 ppt	-	-
N. obscurata 10 ppt	+	+
L. staminea 20 ppt	-	-
N. obscurata 30 ppt	+ +	+ +
L. staminea 30 ppt	+	-
N. obscurata 40 ppt	+ +	+
L. staminea 40 ppt	+	-
N. obscurata 60 ppt	-	-
L. staminea 60 ppt	-	-
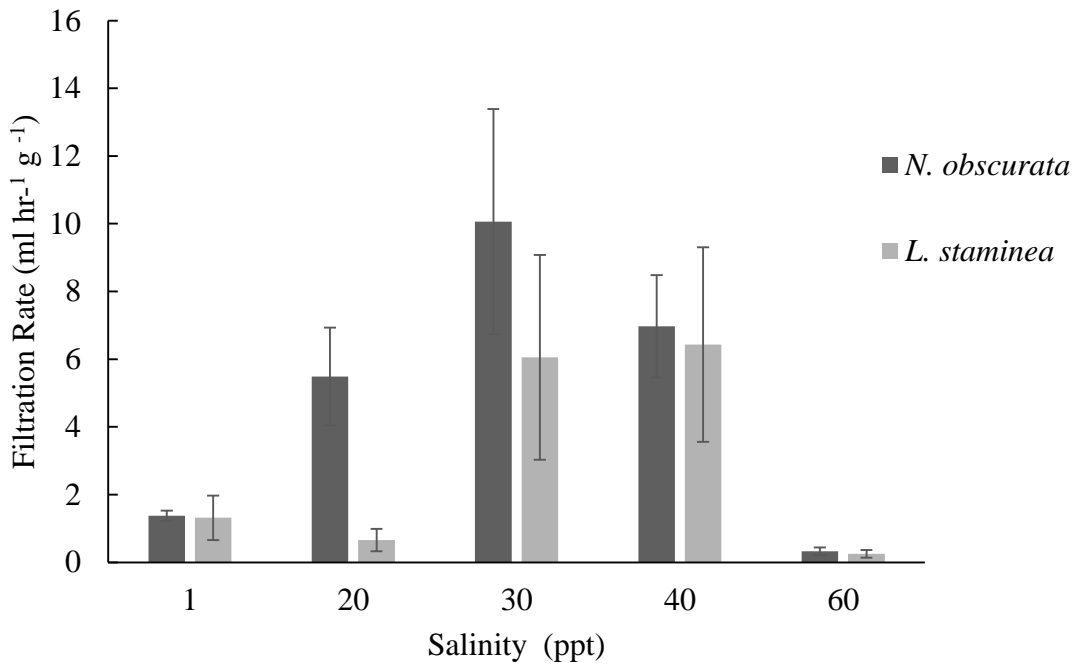
Figure 3. Average filtration rates (ml hr^{-1} g^{-1}) of *N. obscurata* and *L. staminea* held at different salinities (1 ppt, 20 ppt, 30 ppt, 40 ppt, 60 ppt) for 340 minutes. Tahitian *Isochrysis galbana* algae were used for filtration and clams were collected from Mud Bay in Bellingham, WA. N = 5 per treatment. Error bars indicate standard error.

DISCUSSION

Filtration Rate

Nuttallia obscurata filtered at a higher rate than *Leukoma staminea*. This significant difference is likely due to the slightly higher filtration rate of *N. obscurata* under ambient seawater conditions (30 ppt) and the dramatically higher filtration rate under brackish water conditions (20 ppt). *N. obscurata*'s high filtration rate may lead to higher growth and reproduction, contributing to this species' invasive settlement and fast population expansion (Newell and Bayne*,* 1980; Møhlenberg and Riisgård, 1981; Smaal and Widdows*,* 1994). Higher filtration rate also likely helped *N. obscurata* inhabit the open niche of the high intertidal, where feeding time is limited, enabling this species to survive even with reduced time to feed. Little is known about the effects of *N. obscurata's* invasion, however it does outcompete some local species, such as the manila clam, *Venerupis philippinarum,* in disturbed or heavily harvested areas (Dudas, 2005). *N. obscurata*'s high filtration rate is likely one characteristic which allowed the species to outcompete *V. philippinarum.*

Other invasive bivalves such as the Asian clam, *Corbicula fluminea*, also have high filtration rates of 10 ml hr^{-1} g^{-1} (Elliott and zu Ermgassen, 2008), comparable to the 8.6 ml hr^{-1} g^{-1} in *N. obscurata*. These behavioral characteristics allowed *C. fluminea* to cause great economic and environmental damage such as outcompeting local species, limiting resources and changing food web dynamics, changing water turbidity, and changing sediment characteristics (Elliott and zu Ermgassen, 2008). Morphological differences such as gill size and gill structure also play a role in filtration rate. *C. fluminea* has large gills in comparison to total body size and wide spacing between gill cirri resulting in high filtration rate (Neufeld and Yoder, 2011). The source of *N. obscurata's* high filtration is unknown, but it is possible that similar morphological

25

characteristics to *C. fluminea* such as total gill size or gill characteristics could help *N. obscurata* filter at a high rate. *C. fluminea* provides a cautionary tale with more than enough evidence to merit further research and understanding of how *N. obscurata* is able to filter at a high rate and the effects on growth rates, reproduction rates, high expansion rates, interspecies competition, and other economic and ecological impacts.

N. obscurata also filtered under a wider range of salinities than *L. staminea*. *N. obscurata*'s ability to filter under hypoosmotic conditions (20 ppt) may have allowed this invasive species to grow in the open niche areas of freshwater seepages and brackish waters. Inhabitation of these unique areas likely led to *N. obscurata's* fast population expansion along the eastern Pacific coast because these areas had limited competition and available space. *N. obscurata*'s ability to filter under 20 ppt, 30 ppt, and 40 ppt salinity is likely due to its euryhalinity adaptations and ability to cope with and adjust to osmotic stress. Such adaptations may have come from *N. obscurata's* inhabitation of hypoosmotic conditions in its native habitat where it commonly found from 8 ppt to 35 ppt salinity (Kolpakov and Kolpakov, 2004).

N. obscurata's ability to filter at a high rate under 20 ppt salinity and ability to filter under a wider range of salinities in comparison to *L. staminea* was expected. *L. staminea* typically lives in salinities ranging from 27 ppt to 35 ppt salinity (Shaw, 1986) and *N. obscurata* inhabits areas of 8 ppt to 35 ppt salinity (Kolpakov and Kolpakov, 2004). Previous research confirms that *N. obscurata* gill tissue survived for over two weeks at low salinity environments as low as 1 ppt salinity (Wittes and Donovan, unpublished data). This difference in filtration rate between *N. obscurata* and *L. staminea* at 20 ppt salinity is further supported by Siegrist's (2010) study on the effects of salinity on the health and survival of bivalve gill tissue. This study found that *N. obscurata* had no reduction in gill cilia activity at 20 ppt compared to activity at 30 ppt

salinity, while *L. staminea* gill cilia activity was significantly reduced under 20 ppt in comparison to 30 ppt salinity. In addition, Elsasser and Donovan (unpublished data) found that whole *N. obscurata* survived for 57.2 ± 2.8 days in 20 ppt salinity while *L. staminea* only survived for 35.0 ± 2.6 days. *N. obscurata*'s salinity tolerance merits further research to confirm differences between *N. obscurata* and local species and the impacts of these differences.

Both *N. obscurata* and *L. staminea* filtered at high rates compared to other bivalves previously studied. At 30 and 40 ppt salinity *L. staminea* filtered at 6 ml hr^{-1} g^{-1} while *N. obscurata* filtered at 10 ml hr^{-1} g^{-1}. Kryger and Riisgård (1988) examined six different bivalves and found that filtration rates varied between 1.2 and 1.9 ml hr^{-1} g^{-1} at 35 ppt salinity, much lower than *L. staminea* or *N. obscurata*. Even the incredibly invasive and fast growing euryhaline Zebra mussel, *Dreissena polymorpha,* filtered between 1.4 and 1.9 ml hr^{-1} g^{-1} (Kryger and Riisgård, 1988). *N. obscurata* and *L. staminea* filtration rates were comparable to invasive bivalves such as the Brown mussel, *Perna perna,* which filtered at a rate of 8.85 ml hr^{-1} g^{-1} at 35 ppt salinity (Berry and Schleyer, 1983) and the Asian clam, *C. fluminea*, which filtered at a rate of 10 ml hr^{-1} g^{-1} (Elliott and zu Ermgassen, 2008). The reason for high filtration rates in *N. obscurata* and *L. staminea* are unknown but may be due to favorable morphological differences (gill size or structure). Further research is needed to determine the cause of high filtration *N. obscurata* and *L. staminea*.

The high filtration of *N. obscurata* and *L. staminea* could be a result of experiment conditions and methodology. There are many ways of measuring filtration rate and even similar experimental procedures can result in different filtration rates (Møhlenberg and Riisgård, 1981; Riisgard *et al.,* 2003). Algae concentration can even effect filtration rate with very low or very high algae concentrations resulting in lower filtration rate as seen in *Mytilus edulis* (Riisgard *et*

al., 2011). Further experimentation is needed to confirm *N. obscurata* and *L. staminea* high filtration rates in comparison to other species without the bias of experimental methods.

Salinity significantly affected filtration rate and both species had greatly reduced filtration rate under 1 ppt and 60 ppt salinity. *N. obscurata* and *L. staminea* underwent additional behavioral and physical responses including shell-closing in both species and tissue swelling in *L. staminea*. Tissue swelling also occurs in *N. obscurata* under 1 ppt salinity (Wittes and Donovan, unpublished data). This implies that neither *N. obscurata* nor *L. staminea* have a sufficient osmotic response to survive in freshwater. These species were unable to combat the uptake of water at this very low salinity via regulatory volume decrease, which is common in salinity stressed invertebrates (Pierce and Greenberg, 1973). Swelling is detrimental to a cell's vital functions, often resulting in cell death (Yancey, 2001). This means that neither *N. obscurata* nor *L. staminea* have adapted to survive in freshwater environments.

The geographical range of *N. obscurata* and *L. staminea* populations are likely related in part to their filtration rates and salinity tolerance. *N. obscurata* inhabits areas of freshwater seepage, estuaries, river mouths and seawater. This species is unlikely to spread to freshwater lakes and rivers because it cannot filter or survive long-term exposure to freshwater. Since its discovery in 1991, *N. obscurata* has moved from Northern Vancouver Island (discovered in 1993) to Crescent City, California (Dudas, 2005; Cureton, 2015). The geographical range of *N. obscurata* is likely also dictated by temperature and competitive species. It has been hypothesized that *N. obscurata* has a northern limit of British Columbia, as it can survive in brackish frozen Russian waters that even freeze over (Kolpakov and Kolpakov, 2004; Dudas, 2005). *N. obscurata* has moved as far south as Northern California and it has been hypothesized that *N. obscurata* will not move into southern California because of a similar native species

Nuttallia Nuttallia, that may compete with *N. obscurata* (Dudas, 2005; Cureton, 2015*). L. staminea* only filtered in salinities close to ambient seawater (30 and 40 ppt). This closely matches the previously reported optimal range of *L. staminea* of 27 ppt to 30 ppt salinity (Shaw, 1986). This explains why *L. staminea* has not moved into areas of the high intertidal, brackish water or freshwater. Current population studies and field collections are needed to confirm the current geographical range and preferred microclimates of *N. obscurata,* which would lead to a more complete understanding of geographical range of this growing invasive species.

The high filtration rate of *N. obscurata* may have great environmental impacts. Other bivalves with high filtration rates, like *C. fluminea,* altered eutrophication, increased water clarity, limited food availability, increased levels of nitrogen and phosphorus through excretion, acted as vectors for parasites, and accumulated contaminants (Nichols *et al.,* 1990; Elliott and zu Ermgassen 2008; Sousa *et al.,* 2008). The invasive mussel, *Perna perna*, has populations that filter an estimated 144,000,000 to 157,000,000 $l\,m^{-2}$ of water annually on South Africa's coast, filtering over 454,000 $g\,m^{-2}$ of organic material. This rate of filtration significantly changed composition of the water column and the local ecosystem on the Natal Coast (Berry and Schleyer, 1983). Bivalves with high filtration rates also filter larval invertebrates. This has been demonstrated in the oyster, *Crassostrea virginica,* where high filtration rates contributed to the outcompeting of other bivalve species by filtering larvae, therefore decreasing recruitment of competitor species (Gosling, 2003). There is also evidence that the increase in *N. obscurata* increases ammonium. This may increase eutrophication, which is already increasing in the areas of freshwater seepages due to increased pollution in areas of freshwater runoff (Chan and Bendell, 2013). *N. obscurata* has been labeled an invasive species and already shows signs of outcompeting the economically important Manila clam, *V. philippinarum,* in disturbed areas

(Dudas, 2005). *V. philippinarum* accounts for 50% of commercial clam sales in Washington State and is highly valued by recreational and commercial harvesters (WDFW, 2015). It is possible that *N. obscurata's* high filtration rate is in part responsible for this out-competition.

High filtration rates and growth rates for *N. obscurata* and *L. staminea* may also be of economic interest. Increased filtration may lead to higher growth rate and shorter incubation to sale time. There is little economic research, market research, or consumer data available on *N. obscurata,* however the clams are sold in fish markets and grocery stores from Canada to California. *N. obscurata* has been marketed as the "savory clam" and is harvested from naturally seeded populations from British Columbia to Oregon. *N. obscurata's* salinity tolerance could increase the area of harvesting for *N. obscurata*.

N. obscurata's ability to survive in disturbed areas is of special concern as an increased number of beaches and tidal zones undergo human disturbance. *N. obscurata* may become a more prominent species in these expanding disturbed areas. Such areas may also be affected by pollutants and urban runoff and *N. obscurata*'s high filtration rate may lead to a high level of toxin acquisition for recreation and commercial harvesters. *N. obscurata* also thrives in areas susceptible to harmful algal blooms and there is some evidence that *N. obscurata* accumulates and stores toxins longer than other local clam species (WDFW, 2015). This acquisition of toxins would be of great interest to the commercial and recreational harvesters. Future work should explore the market viability of *N. obscurata,* while keeping in mind the possibility of higher toxin acquisition and invasive nature of the species.

Both species underwent shell-closing to reduce exposure to the external environment at 1 ppt and 60 ppt salinity, creating a temporary microclimate in the mantle cavity which minimized hypoosmotic stress on tissues. Shell-closing likely helped *N. obscurata* and *L. staminea* survive

30

such conditions by preventing osmotic shock and reducing the need for a strong osmotic response. These results were expected as neither *N. obscurata* or *L. staminea* are found in freshwater environments or in extreme hyperosmotic conditions (60 ppt), an environment that does not exist on the Pacific coast. Shell-closing is expected as Hoyaux *et al.* (1976) found that three species of bivalves (*M. edulis*, *Scrobicularia plana*, and *Glycymeris glycymeris*) closed their shells when transferred to hypoosmotic conditions. Lack of filtration and shell-closing alter cellular responses and internal biochemistry and are likely only used as short-term responses to low salinity exposure. For example, reduced filtration and shell-closing led to reduced respiration in *Mytilus sp.* (Potts and Perry, 1964) and increased anaerobic activity leading to changes in chemical composition and free amino acid pool in up to eight different bivalves (Shumway *et al.*, 1977). In Shumway *et al.'s* (1977) study, eight species of bivalves (*M. edulis, Mercenaria mercenaria, Crassostrea gigas*, *Modiolus modiolus, Scrobicularia plana* and *Mya arenaria*) closed their shells under low salinity conditions. These factors may be detrimental to normal cell function and the long-term reduction filtration or shell-closing will likely result in poor cell health or cell death. Therefore, the behavioral responses of *N. obscurata* and *L. staminea* in my experiments were likely short-term responses.

Filtration rate is also of great importance because it, in part, determines the amount of exposure clams have with the external environment and the extent of osmotic response needed by the clam. Therefore, examining the effects of salinity on filtration rate should be studied in tandem with the effects of salinity on osmotic response (osmolyte concentration) in *N. obscurata* and *L. staminea*. Although my filtration rate experiment was run separately from my osmolyte concentration experiment, it is imperative to understand that these biological systems are

interdependent and related. Behavioral responses such as filtration rate affect physiological responses such as osmolyte concentrations.

Osmolyte Concentrations

Betaine was present in all gill and mantle tissues tested, ranging in concentration from 6 to 377 μmole g^{-1}. Therefore, betaine was an intracellular component in both *N. obscurata* and *L. staminea.* This was expected as betaine is a common osmolyte in many organisms (Cayley *et al.,* 1992; Pierce *et al.,* 1992; Craig, 2004; Asraf and Foolad, 2007). Betaine is also a compatible solute, having the ability to increase or decrease dramatically in concentration without disrupting proteins (Yancey, 2005). Betaine can also increase the water retention of cells, replace inorganic salts, and protect intracellular enzymes against osmotic or thermal stress (Craig, 2004). Betaine has also been shown to reduce drought and thermal stress in plant cells (Asraf and Foolad, 2007), stabilize protein structure (Cayley *et al.,* 1992), and help water balance in bacteria cells (Cayley *et al.,* 1992). *N. obscurata* had a very high amount of betaine at 30 ppt salinity in gill and mantle tissue after 24 hour exposure. These large stores of betaine in *N. obscurata* gill tissue are present because *N. obscurata* is an extremely tolerant species and may need to utilize betaine as an osmoprotectant under many stressful conditions on a regular basis. Betaine also likely allows *N. obscurata* to undergo normal cell function while being very tolerant to changes in salinity, temperature, and oxygen. This high amount of betaine may imply that the choline to betaine synthesis pathway is working a high rate under normal seawater conditions (Pierce *et al.,* 1992; Yancey, 2005). Sorbitol was not detected in a high number of samples. Although sorbitol is known as a common osmoprotectant, its absence is not surprising as some studies suggest that a

decrease in sorbitol synthesis is compensated by increase betaine synthesis in mammalian cells, which may also be true for bivalves (Yancey and Burg, 1990).

There was a reduction in betaine under hypoosmotic conditions in gill tissue in *N. obscurata* in comparison to ambient seawater conditions under 24 hours of exposure. This reduction in betaine was likely caused by betaine breakdown and/or exportation or the reduction in the choline to betaine production pathway. This is expected as Neufeld and Wright (1996) found that the mussel, *Mytulis californianus,* had a 37% decrease in betaine concentrations after gill tissue was exposed to 20 ppt seawater for one hour. Further research is needed to determine the cellular mechanism used to decrease betaine in gill tissue.

There was also a reduction in betaine under hyperosmotic conditions in *N. obscurata* gill tissue over 24 hours. This reduction was unexpected as *N. obscurata,* an osmoconformer at 40 ppt salinity, should increase osmolyte concentrations under hyperosmotic conditions. I suspect that these cells produced other osmolytes or free amino acids to help compensate for the hyperosmotic surroundings. It is also possible that *N. obscurata* was already undergoing cell death at 60 ppt salinity as previous studies show that cell death occurs after 3.75 days (Wittes and Donovan, unpublished data). Betaine concentration was low in *L. staminea* gill tissues at seawater conditions. This is expected as *L. staminea* does not undergo environmental stresses regularly and would not need a storage of betaine used for osmotic or other stresses. *L. staminea* did produce betaine under all hypoosmotic and hyperosmotic conditions. The increase in betaine under hyperosmotic conditions likely accumulated as an osmotic response, increasing solute concentration in gill cells to closely match that of the external environments, allowing *L. staminea* to try and osmoconform at these levels. Betaine concentration also decreased in hypoosmotic conditions likely accumulating to help stabilize proteins under these low salinity

stressful conditions. The betaine concentrations of both gill and mantle tissue in both species were relatively constant and no trends were seen in this data. This may mean that gill tissue dictates osmotic or stress response more than mantle tissue. These differences in species and tissue reaction to salinity likely let to the interaction seen in this experiment and is a result of the different trends in *N. obscurata* and constant betaine concentrations in *L. staminea*.

Betaine levels in *N. obscurata* gill tissue increased over time by 46%, from 33 μmole g^{-1} at 5 minutes to 48 μmole g^{-1} at 90 minutes. This shows that betaine can increase in gills quickly. It is unclear whether the increase in betaine was part of normal betaine synthesis or betaine production due to stress. Betaine is produced as a general stress response as it is involved with drought and thermal stress in plant cells (Asraf and Foolad, 2007), stabilization of protein structure in invertebrates (Cayley *et al.,* 1992), and water balance in bacteria cells (Cayley *et al.,* 1992). In my experiment, clams were transferred to different environments and tissues were removed. These environmental changes and dissection of the gills undoubtedly caused stress to the clams. There is also evidence in my study that betaine accumulates over a longer time period. Mean betaine levels in gill tissue were 242 μmole g^{-1} after 24 hours of exposure in comparison to an average of 48 μmole g^{-1} in 90 minutes. Although these experiments were run independently and cannot be directly compared, this may be an indication that betaine concentration increases over 24 hours. Again, this betaine accumulation could be a result of normal betaine synthesis or a result of increased production due to the stress caused by the experimental treatment process. Further research is needed to determine why betaine was produced and to test the difference between short-term and long-term betaine production.

Betaine did not accumulate under hyperosmotic conditions after 24 hours in *N. obscurata*, but it is clear that the species survives at 40 ppt salinity and survives for at least 3.75

days under 60 ppt salinity. It is likely that other osmolytes such as taurine, alanine, glycine or proline help *N. obscurata* survive in these conditions. Taurine is one of the most abundant intracellular osmolytes present in the bivalves *M. californianus* (Silva and Wright, 1994) and *Saxidomus giganteus* (Yancey, 2005). Other osmolytes and amino acids such as proline, glycine and alanine also contribute to coping with osmotic stress in bivalves. Deaton (2001) determined that the ribbed mussel, *G. demissa*, produced alanine, glycine, and proline in gill tissue exposed to hyperosmotic salinities. I had originally planned to measure taurine levels in tandem with sorbitol and betaine. The HPLC chromatogram outputs showed that at least 20% of samples contained a compound that was likely taurine. However, the taurine in the samples reacted with the HPLC preparation compounds or the HPLC column. These interactions created broad and tailing peaks in the chromatograph, making it difficult to compare my sample to my taurine standard. Therefore, I was unable to determine the amount of taurine in my samples. Further experiments should determine the relationship between osmotic stress and the concentration of taurine, alanine, glycine and proline in *N. obscurata* and *L. staminea* and develop HPLC preparation and analysis that allows researchers to look at these compounds in tandem.

Conclusion

N. obscurata's unique physiological and behavioral characteristics represent an important example of how euryhalinity can aide invasive clam species in settlement and expansion. *N. obscurata*'s large stores of betaine under normal conditions and ability to reduce these stores under hypoosmotic conditions likely helped *N. obscurata* to be a tolerant species to abiotic stressors and have relatively high filtration rates under hypoosmotic conditions. *N. obscurata*'s tolerance and ability to filter at a high rate and over a wide range of salinity allowed it to feed

and grow in open niche areas and expand quickly. Further research on *N. obscurata* should

continue to help us understand this uniquely tolerant species and how its tolerance and high

filtration rate lead to high growth and production and well as understand the species'

environmental and economic impacts.

LITERATURE CITED

Asraf, M., M.R. Foolad. 2007. Roles of glycine betaine and proline in improving plan abiotic stress resistance. *Environmental and Experimental Biology*. 59(2), 206-216.

Bax, N., A. Williamson, M. Aguero, E. Gonzalez, W. Geeves. 2003. Marine invasive alien species: a threat to global biodiversity. *Marine Policy*. 27(4), 313-323.

Berry, P.F., M.F. Schleyer. 1983. The brown mussel *Perna perna* on the Natal coast, South Africa: utilization of available food and energy budget. *Marine Ecology*. 13, 201-210.

Byers, J.E. 2002. Physical habitat attribute mediates biotic resistance to non-indigenous species invasion. *Oecologia*, 130(1). 146-156.

Byers, J.E. 2005. Marine reserves enhance abundance but not competitive impacts of a harvested nonindigenous species. *Ecology*. 86(2), 487-500.

Carlton, J.T., J.B. Geller. 1993. Ecological roulette: the global transport of nonindigenous marine organisms. *Science*. 261(5117). 78-82.

Cayley S., B.A. Lewis, J.M.T. Record. 1992. Origins of the osmoprotective properties of betaine and proline in *Escherichia coli* K-12. *Journal of Bacteria*. 174, 1586 -1595.

Chan L., Bendell L.I. 2013. Potential effects of an invasive bivalve, *Nuttallia obscurata*, on select sediment attributes within the intertidal region of coastal British Columbia. *Journal of Experimental Biology and Ecology*. 444 66–72.

Craig, S. 2004. Betaine in human nutrition. *American Journal of Clinical Nutrition.* 80, 539–549.

Cureton, E. 2015. Alien Invaders. *The Del Norte Triplicate*. < www.triplicate.com/News/Local-News/Alien-Invaders >. October 2015.

Deaton, L.E. 2001. Hyperosmotic volume regulation in the gills of the ribbed mussel, *Geukensia demissa*: rapid accumulation of betaine and alanine. *Journal of Experimental Marine Biology and Ecology*. 260(2), 185-197.

Elsasser P.A., Donovan, D. Wittes, J.W. 2010. Broad salinity tolerances of the invasive clam *Nuttallia obscurata.* Western Washington University and Swarthmore College. Unpublished.

Dudas, S.E. 2005. Invasion dynamics of a non-indigenous bivalve, *Nuttallia obscurata* in the Northeast Pacific. PhD Dissertation. University of Victoria, Victoria, British Columbia, Canada.

Dudas, S.E., J.F. Dower, B. Anholt. 2007. Invasion dynamics of the varnish clam (*Nuttallia*

obscurata): A matrix demographic modeling approach. *Ecology*. 88(8), 2084-2093.

Elliott P., P.S.E. zu Ermgassen. 2008. The Asian clam (*Corbicula fluminea*) in the River Thames, London, England. *Aquatic Invasions.* 3(1), 54-60.

Fisheries and Oceans Canada. 2000. Shellfish. <http://www.pac.dfo-mpo.gc.ca/index-eng.html>. June 1, 2015.

Gillespie, G.E., M. Parker and B. Merilees. 1999. Distribution, abundance, biology and fisheries potential of the exotic varnish clam (*Nuttallia obscurata*) in British Columbia. *Canadian Stock Assessment Secretariat Research*. 99, 193-239.

Gillespie, G.E., B. Rusch, S.J. Gormican, R. Marshall, D. Munroe. 2001. Further investigations of the fisheries potential of the exotic clam (*Nuttallia obscurata*) in British Columbia. *Canadian Stock Assessment Secretariat Research.* 143.

Gosling, E. 2003. Bivalve Molluscs; Biology, Ecology and Culture. 2nd ed. Blackwell publishing. Oxford, United Kingdom.

Grosholz, E.D. 2005. Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. *Proceedings of the National Academy of Sciences of the United States of America*. 102(4), 1088-1091.

Hill, R.W., G.A. Wyse, M. Anderson. 2008. Animal Physiology. 2nd ed. Sinauer Associates, Inc. Sunderland, Massachusetts.

Hoyaux, J., R. Gilles, C, Jeuniaux. 1976. Osmoregulation in molluscs of the intertidal zone. *Comparative Biochemistry.* 53(4), 361-365.

Kolpakov, E.V., N.V. Kolpakov. 2005. Population size, age structure, and growth of the subtropical bivalve *Nuttallia obscurata* in Primorye waters at the north boundary of its geographic range. *Russian Journal of Marine Biology*. 31(3), 164-67.

Kryger, J., H. Riisgård. 1988. Filtration rate capacities in six species of European freshwater bivalves. *Oecologia* 77, 34–38.

Lodge, D.M. 1993. Biological invasions: lessons for ecology. *Trends in Ecology & Evolution.* 8, 133-137.

Mack, R.N., D. Simberloff, W.M. Lonsdale, H. Evans, M. Clout, and F.A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications.* 10, 689-710.

McFarland, K., L.Donaghy, A. Voltey. 2013. Effect of short-term salinity changes on hemolymph osmolality and clearance rate of the non-native mussel, *Perna viridis*, and the native oyster, *Crassostrea virginica*, in Southwest Florida. *Aquatic Invasions*. 8(3). 299 -

310.

Mills, C.E. 2002. *Nuttallia obscurata,* the purple varnish clam or the purple mahogany clam <Http://faculty.washington.edu/cemills/Nuttallia.html> . March 2012.

Mooney, H.A., R.N. Mack, J.A. McNeely, L.E. Neville, P.J. Schei, J.L. Waage. 2005. Invasive alien species: a new synthesis. Island Press, Washington. DC.

Møhlenberg F., H. Riisgård. 1979. Filtration rate, using a new indirect technique, in thirteen species of suspension feeding bivalves. *Marine Biology*. 54, 143–148.

Navarro J.M., U. Labarta, M. Fernandex-Reinz, A. Velasco. 2003. Feeding behavior and differential absorption of biochemical components by the infaunal bivalve *Mulinia edulis* and the epibenthic *Mytilus chilensis* in response to changes in food regimes. *Journal of Experimental Marine Biology and Ecology.* 287(1), 13-35.

Neufeld, D.S., S.H. Wright. 1996. Response of cell volume in *Mytilus* gill to acute salinity change. *Journal of Experimental Biology*. 199(2), 473-484.

Neufeld D.S., J. Yoder. 2011. The role of feeding adaptations in resource competition between invasive and native Clams. *Proceedings of the Association for Biology Laboratory Education.* 32.78-87.

Newell R.I.E., Bayne B.L. 1980. Seasonal changes in physiology, reproductive condition and carbohydrate content of the cockle *Cardium (=Cerastoderma) edule* (Bivalvia: Cardiidae). *Marine Biology*. 56, 11-19.

Nichols, F.H., J. Thompson, L. Schemel. 1990. Remarkable invasion of the San Francisco Bay (Claifornia, USA) by the Asian clam *Potamocorbula amurensis*. I. Introduction and dispersal. *Marine Ecology Progress Series*. 66(1-2), 81-94.

Office of Technology U.S. Congress. 1993. Harmful non-indigenous species in the United States. *OTA Publication.* F-565, U.S. Government Printing Office, Washington, D.C.

Paganini, A., W.J. Kimmerer, J.H. Stillman. 2010. Metabolic responses to environmental salinity in the invasive clam *Corbula amurensis*. *Aquatic Biology.* 11, 139–147.

Pierce, S.K. 1971. Volume regulation and valve movements in marine mussels. *Comparative Biochemistry and Physiolog*y. 39, 103-117.

Pierce, S.K., M.J. Greenberg. 1973. The initiation and control of free amino acid regulation of cell volume in salinity-stressed bivalves. *Journal of Experimental Biology*. 59, 435-440.

Pierce, S.K., L.M. Rowland-Faux, S.M. O'Brien. 1992. Different salinity tolerant mechanisms in Atlantic and Chesapeake Bay conspecific oysters: glycine betaine and amino acid pool variations. *Marine Biology*. 113(1), 107-115.

Pierce, S.K., L.M. Rowland-Faux, B.N. Crombie, 1995. The mechanism of glycine betaine regulation in response to hyperosmotic stress in oyster mitochondria: A comparative study of Atlantic and Chesapeake Bay oysters. *Journal of Experimental Zoology*. 271(3), 161-170.

Pimentel, D., L. Lach. 2000. Environmental and economic costs of nonindigenous species in the United States. *Bioscience*. 50(1), 53-65.

Pimentel, D., R. Zuniga. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*. 52(3), 273-288.

Potts, W.T., G. Perry. 1964. Osmotic and Ionic Regulation in Animals: International Series of Monographs on Pure and Applied Biology. Pergamon Press. London.

Riisgard, H.U., C. Kittner, S. Seerup. 2003. Regulation of opening state and filtration rate in filter-feeding bivalves (*Cardium edule, Mytilus edulis, Mya arenaria*) in response to low algal concentration. *Journal of Experimental Marine Biology and Ecology*. 284 (1-2), 105-127.

Riisgard, H.U., P. Egede, I. Saavedra. 2011. Feeding behaviour of the mussel, *Mytilus edulis*: new observations, with a mini review of current knowledge. *Journal of Marine Biology*. Article ID 312459.

Ruiz, G.M., J.T. Carlton, E.D. Grosholz, A.H. Hines. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: Mechanisms, extent, and consequences. *American Zoologist*. 37(6), 621-63.

Ruiz, G.M., P.W. Fofonoff, J.T. Carlton, M.J. Wonham, A.H. Hines. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annual Review of Ecology and Systematics*. 31, 481-531.

Shaw, W.N. 1986. Species profiles: 1ife histories and environmental requirements of coastal fishes and invertebrates (Pacific Southwest)—common littleneck clam. U.S. Fish Wildlife Service Biological Report 82(11.46). U.S. Army Corps of Engineers.

Shumway, S.E., P.A. Gabbott, Youngson, A.. 1977. The effect of fluctuating salinity on the concentrations of free amino acids and ninhydrin positive substances in the adductor muscles of eight species of bivalve molluscs. *Journal of Experimental Marine Biology and Ecology*. 29, 131-150.

Shumway, S. 1977. The effect of fluctuating salinity on the concentrations of free amino acids

and ninhydrin-positive substances in the adductor muscle of eight species of molluscs. *Journal of Experimental Marine Biology and Ecology*. 29(2) 131-150.

Sidwell, V.D. 1981. Chemical and nutritional composition of finfishes, whales, crustaceans mollusks and their products. *NOAA Technical Memorandum.* NMFS Sec 11.

Simmons B., Sterling J. Watson J. 2014. Species and size-selective predation by raccoons (*Procyon lotor)* preying on introduced intertidal clams. *Canadian Journal of Zoology.* 91(12), 11059-1065.

Siegrist, Z.C. 2010. Broad physiological tolerances of the invasive clam, *Nuttallia obscurata*. Thesis. Western Washington University. Bellingham, Washington.

Silva, A.L., S.H. Wright. 1994. Short-term cell volume regulation in *Mytilus californianus* gill. *Journal of Experimental Biology*. 194, 47-68.

Smaal, A.C., J. Widdows. 1994. The scope for growth of bivalves as an integrated response parameter in biological monitoring. *Biomonitoring of coastal waters and estuaries*. CRC Press Inc. Boca Raton, Florida. 247–267.

Sousa, R., C. Antunes, and L.Guihermino. 2008. Ecology of the invasive Asian clam *Corbicula fluminea* in aquatic ecosystems: an overview. *Annales De Limnologie-International Journal of Limnology*. 44(2), 85-94.

Van Winkle, W. 1972. Ciliary activity and oxygen consumption of excised bivalve gill tissue. *Comparative Biochemistry and Physiology.* 42, 473-485.

Verling, E., G.M. Ruiz, D.L. Smith, B. Galil, A.W. Miller, and K.R. Murphy. 2005. Supply-side invasion ecology: characterizing propagule pressure in coastal ecosystems. Proceedings of the Royal Society. *Biological Sciences.* 272, 1249-1257.

Vernberg, F.J., C. Schlieper, D.E. Schneider. 1969. The influence of temperature and salinity on ciliary activity of excised gill tissue of molluscs from North Carolina. *Comparative Biochemistry and Physiology*. 8.3, 271-285.

Washington Department of Fish and Wildlife. 2000. Shellfish. <http://wdfw.wa.gov/fish/ shelfish/beachreg/1clam>. January 13, 2015.

Wittes, J., D. Donovan. 2009. Broad physiological tolerances of the invasive clam, *Nuttallia obscurata.* Unpublished. Western Washington University. Bellingham, Washington.

Yancey, P.H. 2001. Water stress, osmolytes and proteins. *American Zoologist*. 41(4), 699-709.

Yancey, P.H. 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *The Journal of Experimental*

Biology. 208, 2819-2830.

Yancey, P.H., M.B. Burg. 1990. Counteracting effects of urea and betaine on colony-forming efficiency of mammalian cells in culture. *American Journal of Physiology*. 258, 198-204.

Zenetos, A., M.E. Cinar, M.A. Pancucci-Papadopoulou, J.G. Harmelin, G. Furnari, F. Andaloro, N. Bellou, N. Streftaris, H. Zibrowius. 2005. Annotated list of marine alien species in the Mediterranean with records of the worst invasive species. *Mediterranean Marine Science*. 6(2), 63-118.