Physiological adaptations and feeding mechanisms of the invasive purple varnish clam, Nuttallia obscurata

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PHYSIOLOGICAL ADAPTATIONS AND FEEDING MECHANISMS OF THE INVASIVE PURPLE VARNISH CLAM, *NUTTALLIA OBSCURATA*

by

Leesa E. Sorber

Accepted in Partial Completion

of the Requirements for the Degree

Master of Science

Kathleen L. Kitto, Dean of the Graduate School

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Leesa E. Sorber
29 April 2013
PHYSIOLOGICAL ADAPTATIONS AND FEEDING MECHANISMS
OF THE PURPLE VARNISH CLAM, *NUTTALLIA OBSCURATA*

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Leesa E. Sorber

March 2013
ABSTRACT

The Purple Varnish Clam, *Nuttallia obscurata*, is an example of a recent successful invader to the Pacific Northwest. *Nuttallia obscurata* reside in the high intertidal zone where it must adapt to daily and seasonal fluctuations in salinity and food availability. I investigated their physiological and morphological adaptations to these conditions.

The acute physiological response of the invasive, *N. obscurata* and the native, *L. staminea*’s excised gill tissue’s respiratory metabolism (measured as oxygen consumption) in 5, 30, and 55 ppt salinities were measured in a closed system, Gilson Differential Respirometer and compared. The excised gill tissue of both species displayed the highest rate of oxygen consumption in the hyposaline (5 ppt) treatment and the lowest rate in the hypersaline (55 ppt) treatment. In addition, the excised gill tissue of *N. obscurata* maintained a lower overall rate of oxygen consumption in all salinity treatments than *L. staminea*. GLM modeling supported these results, indicating that both salinity and species were factors describing the rate of oxygen consumption.

*Nuttallia obscurata* also must have adaptations in feeding structures or strategies to survive in the high intertidal zone where there is limited food availability during exposure at low tide. I analyzed sediment characteristics and the relative dry tissue mass of *N. obscurata*’s gills and palps, and therefore, the gill-to-palp mass ratio, from high and mid tide zones from two field sites. Due to *N. obscurata*’s alternate modes of feeding, I also analyzed the relative dry tissue mass of the foot and siphon tissue.

*Nuttallia obscurata* from Birch Bay had 30% larger gill tissue and 14.8% larger siphon tissue at high tide than at mid tide potentially due to increased filter feeding during limited submersion in the expansive high intertidal zone. There was no difference in palp of
foot mass from Birch Bay *N. obscurata*. The log gill-to-palp mass ratio placed Birch Bay *N. obscurata*, on the continuum of bivalve feeding strategies, closer to other known filter feeders. *Nuttallia obscurata* from Clayton Beach displayed no difference in the gill, palp, or siphon mass at different tide levels perhaps due to the small exposed tidal flat. There was a 9.5% increase in foot mass at high tide but there was no evidence to support that it indicates increased pedal feeding. The gill-to-palp mass ratio was 79% and 41% smaller than *N. obscurata* from high tide and mid, respectively, from Birch Bay. This difference is likely due to the coarse sediment grain size (0.380-2.0 mm) found at Clayton Beach, which required larger palps for increased sorting needs. The log gill-to-palp mass ratio placed Clayton Beach *N. obscurata* on the continuum of bivalve feeding strategies, closer to other known deposit feeders.

During the course of this research, I noticed a large number (57 out of 100) of *N. obscurata* collected from Clayton Beach were infected with the mantle pea crab, *Pinnixa faba*, while a small number (1 out of 100) of the *N. obscurata* from Birch Bay were infected. Difference in infection rate between sites may be due to increased mud flat exposure at Birch Bay and course sediment found at Clayton Beach. A one-way ANOVA showed there was no effect of infection by *P. faba* on the gill-to-palp mass ratio of *N. obscurata*.

The change in respiratory metabolism in different salinities, the varied feeding structures with tide height and sediment grain size as well as the symbiotic relationships with native organisms may well contribute information regarding *N. obscurata*’s success as an invasive species in the high intertidal zone of the Pacific Northwest.
ACKNOWLEDGMENTS

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CHAPTER 1
LITERATURE REVIEW

Biological invasions have reshaped the structure, dynamics, and function of biological communities throughout the world (Carlton, 1989; Lodge, 1993; Mack et al., 2000; Verling, et al., 2005). In 1999, the U.S. Federal Executive Order 13112 defined an invasive species as “an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health.” The most harmful of these invasions can displace native species, change community structure and food webs, and alter nutrient cycling and sedimentation. In the marine environment, they can damage economies by diminishing fisheries, fouling ship’s hulls and clogging intake pipes (Molnar et al., 2008). A 1993 report by the U.S. Congressional Office of Technology Assessment estimated that there were approximately 4,500 non-indigenous species (NIS) (species that are introduced to a new environment but do not cause harm) established in the United States. Approximately 15% of these NIS are considered nuisance species that have significant ecological and/or economic impact (OTA, 1993; Ruiz et al., 1997).

Some extreme examples of invasive species are the Green Crab, Carcinus maenas, along the Pacific and Atlantic coasts (Grosholz, 1996), Atlantic Cordgrass, Spartina alterniflora, in Puget Sound (Stiller and Denton, 1995), and Zebra Mussels, Dreissena polymorpha, in the Great Lakes (Charlton, 1994). The Green Crab is an aggressive omnivore with rapidly spreading populations that occupy large coastal areas (Grosholz, 1996), drive out native species (Moulton and Gustafson, 1956), compete for available food sources, invade fisheries beds (Jamieson et al., 1998), and alter the productivity of habitats (Grosholz and Ruiz, 1995). Atlantic Cordgrass invaded Puget Sound in the early 1920’s in association
with the Atlantic oyster, and over the past 40 years, has had a population explosion. The increase in population has altered the topography of mud flats by increasing the rate of sedimentation and changed nutrient availability for native species (Stiller and Denton, 1995). Economic impacts can occur when fouling species such as the Zebra Mussel clog drainage pipes, severely foul boats and docks (MacIsaac, 1996) and decrease water quality (Charlton, 1994). Great Lakes officials estimate that as much as $5 billion will be spent in 10 years on zebra mussel control (O’Neil et al., 1991).

The introduction of NIS to coastal regions has been occurring on a global scale for centuries (Carlton, 1979, 1989); however, the impacts of NIS in marine environments have received much less attention in the past than terrestrial and fresh water habitats (Carlton, 1989; OTA, 1993; Ruiz et al., 1997). This has changed in recent years due to research that indicates NIS are common in marine ecosystems and have the potential to cause ecological changes (Carlton, 1979, 1989; Hutchings, 1992; Cohen and Carlton, 1996; Ruiz et al., 1997). Introductions can occur intentionally, to create new markets for commercially valuable organisms (e.g., fish or shellfish), or unintentionally, as a by-product of ship transport, traffic in seafood and ocean products, pet trade, or scientific research (Carlton, 1985).

In coastal marine ecosystems, the discharging of ballast water in commercial shipping is considered the largest single transfer mechanism for NIS, resulting in hundreds to thousands of invasions worldwide. Ballast water is water taken on in one port, used for stability during voyages, and discharged at other ports of call. In 1991, the U.S. alone received >70 million metric tons of ballast water from foreign ports (Ruiz et al., 2001). The use of ballast water by ships results unintentionally in the dispersal of species around the globe (Ruiz et al., 2001). The increase in ballast water invasions is caused by increased
shipping between ports with larger vessels that hold more ballast water and are able to arrive at various ports in less time due to increased speed (Carlton et al., 1995). Many species that are present in ballast tanks are viable upon arrival to a new port and are capable of invasion when released (Carlton and Gellar, 1993; Smith et al., 1996).

Successful invaders tend to have a short generation time, high fecundity, a long planktonic stage, broad diet, and broad environmental tolerances (Ehrlich, 1986; Dudas, 2005). The recipient regions tend to have low native species diversity (Stachowicz et al., 1999), recent disturbance (Hobbs and Huenneke, 1992), and anthropomorphic changes (Ruiz et al., 1997). The recipient region must have a favorable climate and appropriate habitat for the invading species (Swincer, 1986). For marine invertebrates with a planktonic larval stage, the recipient’s regional oceanography is important concerning circulation patterns, temperature, and salinity factors (Dudas, 2005).

A recent study by Molnar et al. (2008) investigated the threat of marine NIS through ecoregions and invasion pathways. The Temperate Northern Pacific ecoregion (including British Columbia, Washington, and Oregon) had 123 known marine invasions, 63% of which were harmful to the native ecosystem. Of those harmful invaders, 68% were transported via shipping/ballast water (Molner et al., 2008). The majority of invaders are marine invertebrates, of which a large number are molluscs (Ruiz et al., 2000; Wonham and Carlton, 2005; Molner et al., 2008).

The Purple Varnish Clam, Nuttallia obscurata, is an example of a recent successful invader to the Pacific Northwest. Nuttallia obscurata is an infaunal species originally native to Japan, Korea and China (Dudas and Dower, 2006) that was first reported in the Pacific
Northwest in 1991 near Vancouver, British Columbia (Forsythe, 1993) and was likely introduced via ballast water disposal in Vancouver Harbor (Gillespie et al., 1999). Since its introduction, *N. obscurata* has spread rapidly throughout the Strait of Georgia, northward along the west coast of Vancouver Island, and southward along the Washington and Oregon coasts (including interior estuaries) attaining adult densities of up to 800 m\(^{-2}\) (Dudas, 2005). As of 2006, the geographical limits of the populations were reported as Smith Sound (51° 17.07’ N) in British Columbia to the north (Dudas and Dower, 2006; Gillespie and Bourne, 2004), and Coos Bay (43° 20.27’ N), Oregon, to the south (Dudas and Dower, 2006) (Figure 1).

*Nuttallia obscurata* is found on beaches with mixed sand, gravel and mud substratum (DFO, 1999; Gillespie et al., 1999, 2001). They typically are found higher in the intertidal zone than other clam species, such as the native littleneck, *Leukoma staminea*, and the introduced Manila clam, *Venerupis philippinarum*. *Nuttallia obscurata* is also often associated with freshwater runoff or seepage (Gillespie et al., 2001). When *N. obscurata* and *V. philippinarum* populations do overlap, *N. obscurata* is found deeper (up to 20 cm) in the substratum (Gillespie et al., 1999; 2001; Miyawaki and Sekiguchi, 1999).

Studies have shown that *N. obscurata* displays the characteristics generally associated with successful invaders (i.e., short generation time, high fecundity, long planktonic stage, broad diet, and broad environmental tolerances). According to Dudas and Dower (2006), the varnish clam has a short generation time, reaching maturity at sizes ranging from 1.6 to 2.3 cm within one year. This is younger than co-occurring native bivalves that typically do not reach maturity for 2 to 3 years.
Figure 1. The population range of *Nuttallia obscurata* in the Pacific Northwest. Black bars represent current distribution limits. Black arrows represent the direction of distribution. Source: Gillespie *et al.* (2001) and Gillespie and Bourne (2004).
Nuttallia obscurata also exhibits a high fecundity with $4 \times 10^{4}$ to $6 \times 10^{6}$ eggs per individual female each spawning season, which typically lasts from late spring to early fall (Dudas and Dower, 2006). Once the eggs are fertilized, the planktonic duration of *N. obscurata* is at least 3 weeks but can be up to 8 weeks. This long planktonic duration has implications for its dispersal southward into Washington and Oregon based on the oceanographic circulation patterns in the recipient region (Dudas and Dower, 2006).

*Nuttallia obscurata* has a broad diet in that they not only filter feed suspended particles from the water column, but also utilize organic detritus in the substratum by pedal-sweep feeding (DFO, 1999, 2001; Gillespie *et al.*, 1999, 2001). Additionally, reports indicate they are siphonal deposit feeders, sweeping the inhalant siphon over broad areas of substratum to collect deposited materials (Tsuchiya and Kurihara, 1980). These additional modes of feeding may be an advantage to *N. obscurata* during extended low tides, which bring decreased food availability and may facilitate their successful invasion.

*Nuttallia obscurata* has been shown to have extremely broad tolerances to numerous environmental factors. Siegrist (2010) demonstrated *N. obscurata*’s hardiness in conditions of high temperature and low salinities. He found that *N. obscurata* gill tissue could survive for 60 minutes at 41.1°C and extrapolated that to whole organism survival. This was 0.5°C higher that *V. philippinarum* and 5.0°C higher than *L. staminea*. In addition, excised gill tissue of *N. obscurata* tolerated much lowers salinities than the other two clams, surviving with no decline of ciliary activity for at least two weeks in salinity treatments from 1- 30 ppt. This finding helped to explain the population distribution studies that show high densities of *N. obscurata* in association with streams, freshwater runoff, or groundwater seepages (DFO, 1999, 2001; Gillespie *et al.*, 1999, 2001). Unpublished data from our lab in 2009
demonstrated that gill tissue tolerance of *N. obscurata* is not limited to low salinities, as gill tissue survived for 3.75 days at 60 ppt.

The 2009 study found that intact *N. obscurata* has tolerances for both hypo- and hyperosmotic conditions. The study conducted on the internal hemolymph of whole organisms revealed that *N. obscurata* has the ability to osmoregulate at salinities of 5 ppt and 55 ppt and to osmoconform at 30 ppt. The ability to osmoregulate at the extremes of their salinity range may help to explain their successful invasion of the high intertidal zone in the Pacific Northwest where wide salinity fluctuations occur both daily and seasonally.

The invasibility of the recipient region also influences the progression of an invasion. There are several bivalves species that inhabit the intertidal zone of the Pacific Northwest but they are found primarily in the mid to low to subtidal zones. This distribution of native and introduced bivalve species creates low biodiversity in the high intertidal zone; *N. obscurata* has filled this niche quite rapidly. The low biodiversity decreases *Nuttallia’s* need to compete for space or food resources. The chance of predation also decreases with tidal height, as many bivalve predators such as Cancrid crabs prefer to feed when submerged by the tides (Byers, 2002).

The oceanographic circulation patterns of the recipient region are another factor in the dispersal and survival of invasive species. The North Equatorial Current becomes the Alaska Current to the north and the California Current to the south just west of Vancouver Island, British Columbia. This water mass movement around Vancouver Island and to the south during the spawning period likely facilitated *N. obscurata’s* invasion by dispersing the larvae throughout the regions where suitable habitat is abundant (Dudas, 2005). These circulation
patterns also played a role in past exotic species introductions. *Venerupis philippinarum*, also a native to Japan, followed a similar invasion route after its introduction to Vancouver Island in the 1930’s (Bourne, 1982). When invertebrate species with extended planktonic larval stages (*N. obscurata* larval stage is 3-8 weeks) are introduced into this area, they are likely to be rapidly dispersed throughout not only British Columbia, but also southwards towards Washington and Oregon (Dudas, 2005).

The two main steps in invasions are initial introduction and successful establishment of the species, followed by the expansion of populations in the new region (Vermeji, 1996; Williamson and Fitter, 1996; Dudas, 2005). *Nuttallia obscurata*’s small sizes at maturity, high fecundity, lengthy planktonic phase, broad environmental tolerances, as well as regional ocean currents, have all played a role in the successful invasion and dispersal. The dispersal of the *N. obscurata* has remained primarily in the high intertidal zone throughout its entire range. Even in areas devoid of clam species typically found in the mid to low intertidal zones, *N. obscurata* is found in the greatest densities in the high intertidal zone (Byers, 2002).

The primary focus of this research was to determine if specific physiological and morphological characteristics found in *N. obscurata* have contributed to its success as an invasive species in the high intertidal zone of the Pacific Northwest. In Chapter 2, I examine the rate at which excised gill tissue from both the invasive, *N. obscurata* and the native, *L. staminea*, consume oxygen in different salinity treatments. The outcome of this study was to determine (1) if the rate of oxygen consumption of excised gill tissue differed with varying salinity concentrations, and (2) if there was a relationship between the rate of consumption and energy metabolism needed for osmoregulation as oxygen uptake increases with increased
metabolism (Vernberg, 1963). In Chapter 3, I investigate the role of tidal zonation on the feeding structures of *N. obscurata*. I examined the relative mass of the *N. obscurata*’s gill, palp, foot and siphon tissue as well as the overall gill-to-palp mass ratio at different tidal heights and different beach types. The outcome of this study was to determine (1) if the relative mass of gill, palp, foot, or siphon tissue varied with tidal height, (2) if the overall gill to palp mass ratio varied with tidal height or sediment type, and (3) if changes in feeding structures correlated with the different feeding strategies utilized by *N. obscurata* in the high intertidal zone. In Chapter 4, I investigated the effects of infection by the mantle pea crab, *Pinnixa faba*, on the gill-to-palp mass ratio of *N. obscurata*. This chapter came about following the discovery of a high number of infected *N. obscurata* during the dissection phase of Chapter 3. In Chapter 5, I discuss the overall conclusions ascertained from this study.
CHAPTER 2

THE EFFECT OF DIFFERENT SALINITY LEVELS ON THE RATE OF OXYGEN CONSUMPTION IN EXCISED GILL TISSUE OF NUTTALLIA OBSCURATA AND LEUKOMA STAMINEA

2.1 INTRODUCTION

Salinity is one of the most important environmental factors in aquatic habitats. It is relatively constant in the open seas, but it varies considerably in intertidal zones and estuaries. Many intertidal aquatic environments experience salinity variations due to the opposing effects of tidal inundation and fresh water run-off from land (Wheatly, 1988). Intertidal organisms are subjected to diurnal, seasonal and spatial salinity fluctuations, which may be gradual or abrupt (Wells, 1983). The magnitude of these fluctuations will depend on the nature of the freshwater source, the morphology of the basin and the effect of the tides and current (Wheatly, 1988). The ability to exist at varying salinities depends on different adaptations. One adaptation is osmoregulation based on active ion transport mechanisms (Berger and Kharazova, 1997). However, many osmoconformers, which lack the ability to regulate the osmotic pressure of the internal medium, still demonstrate considerable adaptations to fluctuations in salinity (Berger and Kharazova, 1997).

Salinity may influence the organism in two ways, by osmotic concentration and by chemical components dissolved in water (Bielawski, 1961). For estuarine organisms, the blood or hemolymph osmolality typically varies directly with that of the external solution, and cells will shrink or swell during a salinity change unless cellular mechanisms are invoked to counteract the change in cell size (Neufeld and Wright, 1996). Tissues of estuarine organisms do encounter large and frequent fluctuations in salinity. In the face of such osmotic challenges, one might expect the cells of tissues in estuarine organisms to have a
well-developed ability to regulate their volume by either losing or gaining osmotically active solutes (Neufeld and Wright, 1996). The preservation of an independent internal medium requires energy, which is supported by the processes of respiratory metabolism (Bielawski, 1961).

Because respiratory metabolism, measured as oxygen consumption, can be expected to change with the demand for energy for biological activities, it can also be expected that a change in salinity of the surrounding medium will affect the rate of oxygen consumption of an organism (Kutty et al., 1971). Oxygen consumption reflects energy metabolism and can be a sensitive indicator of physiological health of an organism (Modlin and Froelich, 1997). There is evidence to indicate that the energy spent on osmotic regulation can be extrapolated from the oxygen consumption of an organism (Rao, 1968). Changes in the external or internal environment of an organism, even slightly beyond its range of acclimation, can dramatically shift the rate of oxygen consumption (Gilchrist, 1956; Vernberg and Vernberg, 1972; Simmons and Knight, 1975; Modlin and Jayne, 1981; Modlin, 1983; Burggren and Roberts, 1993). In many invertebrates, a change of salinity causes a change in the intensity of respiration (Bielawski, 1961). Kinne (1966) found that in crustaceans salinity affected the intensity of respiration (oxygen consumption) in one of four ways: (1) increased in subnormal salinities or reduced in supranormal salinities, (2) increased in both sub- and supranormal salinities, (3) reduced both in sub- and supranormal salinities, or (4) unaffected.

Although many studies emphasize the response of the intact organism to various combinations of temperature and salinity, observations of isolated tissues have described adaptations to these factors at the cellular level (Schlieper et al., 1960; Vernberg, 1960). Gill tissue is important in feeding, respiration, and excretion in all bivalves, and due to its
importance, it has been the object of several ecologically oriented physiological studies (Bielawski, 1961; Vernberg et al., 1963; Dehnel and McCaughran, 1964; King, 1965; van Winkle, 1968; Engel and Eggert, 1974; Engel et al., 1975). Because gills are directly involved in osmotic and ionic regulation, they should give some indication of the metabolic effects of salinity (Koch et al., 1954; Engel and Eggert, 1974).

The majority of studies dealing with the effects of salinity on oxygen consumption come from crustacean research. Whole organisms, gills, and isolated mitochondria from gills in crustaceans have demonstrated a relationship between the respiration rate and the salinity of the incubation medium (Dehnel and McCaughran, 1964; King, 1965; Thabrew et al., 1973; Engel and Eggert, 1974). The relationship between salinity and gill tissue respiration is inverse for most crustaceans (i.e., the lower the salinity, the higher the respiration rate of the tissue) (Engel et al., 1975). However, Schwabe (1933) demonstrated that with decreased salinity, there was a decreased rate of respiration in spider crabs (Labinia sp.).

Bivalves seem to show similar results to those found in crustaceans. Van Winkle (1968) observed increased rates of respiration in the gill tissue from both Mercenaria mercenaria and Modiolus demissus at 5 ppt as compared to 30 ppt. In Crassostrea virginica, whole organism oxygen consumption increased with decreasing salinity (28 ppt to 7 ppt) at both 10°C and 20°C, similar to reports for isolated gill tissue (Persey et al., 1971; Bass, 1977; Shumway and Koehn, 1982). Lange (1968) demonstrated a similar response in the blue mussel, Mytilus edulis. Bielawski (1960) observed that in the zebra mussel, Dreissena polymorpha, a lower salinity (tap water) caused an increase of oxygen consumption and a higher salinity (30 ppt) caused a decrease in oxygen consumption.
In aquatic ecosystems, salinity also is considered a major environmental property that limits the distribution of invertebrates (Gunter, 1957; Kinne, 1971). Because adult bivalves are relatively immobile, their distribution along a salinity gradient and their survival in habitats of varying salinity must reflect their adaptive capabilities at both the whole animal and tissue level (Van Winkle, 1972). These adaptive physiological responses to environmental factors will influence the biological fitness of individuals of a population and will ultimately affect the distributional limits of the species (Nelson et al., 1977).

*Nuttallia obscurata* is a relatively recent invader to the Pacific Northwest that has established populations primarily in the high intertidal zones where there is limited competition with other introduced or native clam species such as *Venerupis philippinarum* and *Leukoma staminea*. The tradeoff for less interspecific competition is living in an environmentally harsh habitat. The high intertidal zone is subjected to daily air exposure during low tides. Fluctuations in salinity come with this exposure. The exposure to air can change the salinity of the substratum by increasing it during the warm summer months due to evaporation or decreasing it due to freshwater influxes caused by precipitation. If a species is able to tolerate salinity (osmotic) stress better than others species, it may have a competitive advantage as those conditions increase in duration and intensity as tide levels increase.

A 2009 study from our lab on hemolymph osmolality and tissue hydration of both gill tissue and intact *N. obscurata* in a salinity range of 1 ppt to 60 ppt revealed that under osmotic stress, the water content of its tissues remained relatively constant (roughly 80-90%) during day 4. There is literature demonstrating that cells of most estuarine bivalve tissues can invoke a regulatory volume decrease or increase in response to osmotic stress (Chamberlin and Strange, 1989) that would mitigate the functional consequences of the cell
volume change experienced during frequent fluctuations in salinity that they experience. Deaton (2001) found that *Geukensia demissa* exposed to high (32 ppt) salinities experienced a rapid loss of gill tissue volume under hyperosmotic stress, but restored tissue volume through a quick release of the osmolytes betaine and alanine. The ability of *N. obscurata* to maintain a relatively constant tissue volume in an extreme range of salinities may be evidence of adaptive mechanisms to counteract fluctuations of salinity found in the high intertidal zone.

The 2009 study from our lab also demonstrated that at salinities of 1-10 ppt, the internal osmolality (tested from hemolymph in the adductor muscle) of intact *N. obscurata* was higher than that of the surrounding medium for the duration of the experiment (14 days). This was an indication that the organisms were osmoregulating at these low salinities. *Nuttallia obscurata* had decreased internal osmolality at 50 and 60 ppt for day one of the experiment, indicating possible osmoregulation at the higher salinities. The organisms at the higher salinities began to deteriorate after prolonged exposure to these salinities, resulting in mortality before day 14. Several authors have suggested that increased respiratory metabolism at salinities differing from the iso-osmotic point as indicative of increased energy cost due to osmoregulation (Beadle, 1931; Dehnel and McCaughran, 1964; Kutty *et al.*, 1971; Lofts, 1956; Nelson *et al.*, 1977).

To determine if the osmoregulation results demonstrated by our lab’s 2009 study were related to increased respiratory metabolism, I examined the rate of mass-specific oxygen consumption of excised gill tissue from *N. obscurata* acutely exposed to salinities ranging from 5 to 55 ppt. I compared the metabolism of *N. obscurata* to that of the native, *L. staminea*. 
Leukoma staminea typically is found in the low intertidal zones of the Pacific Northwest where fluctuations in salinity, both daily and seasonally, are less than those in the high intertidal zone. The low intertidal zone is less affected by freshwater influences and remains exposed to air for less time during low tides. Molluscs residing in this location may not need physiological adaptations, such as osmoregulation capabilities, to counteract osmotic stress caused by salinity fluctuations. Bivalves can also avoid unfavorable environmental conditions by tightly clamping their valves shut (Vernberg, 1963). This adaptation may be enough for low intertidal organisms to counteract the consequences of changes in salinity.

Historically, studies of salinity tolerances and the effects of those tolerances have focused on low and fluctuating salinity, within the normal range of ambient seawater, on various marine and estuarine organisms. Previously published studies investigating the effects of hypersaline solutions on the physiology of molluscs could not be found. In this experiment, I investigated the following experimental questions:

**Question 1:** Does the rate of oxygen consumption in excised gill tissue of *N. obscurata* differ with different salinities?

**Hypothesis 1.1:** Gill tissues exposed to high and low salinities will have an increase in the rate of oxygen consumption compared to gill tissue exposed to ambient seawater salinity.

**Question 2:** Does the rate of oxygen consumption in excised gill tissue of the invasive *N. obscurata* differ from that of the native *L. staminea* in different salinities?

**Hypothesis 2.1:** Gill tissue of *N. obscurata* will differ in the overall rate of oxygen consumption than gill tissue from *L. staminea*. 
2.2 MATERIALS AND METHODS

Study sites and collection techniques

Two Northwest Washington sites were selected for this study: Birch Bay (48.927° N 122.745° W; accessed north of Birch Bay State Park) and Mud Bay (48.701° N 122.499° W; northern part of Chuckanut Bay). Each site was visited three times at low tide between July and August 2011.

*Nuttallia obscurata* was collected from the high intertidal zone and *L. staminea* was collected from the mid to low intertidal zone. Clams were collected using shovels and hands and typically were found in the top 15 cm of substratum. Sediment was collected from each site for the substratum base (2-3 inches) in three 10-gallon holding aquaria. Typically, 30-60 clams of both species were haphazardly placed in the three 10-gallon laboratory aquaria and were maintained in Biology Department’s 10ºC cold room at Western Washington University in 30 ppt seawater from Shannon Point Marine Center, Anacortes, WA. Vernberg *et al.* (1963) showed with other salt-water molluscs, significant acclimation occurs within 3-5 days, therefore, the clams were acclimated to the above conditions for one week prior to beginning the experiments. After the seven day acclimation period, two tablespoons of PhycoPure Reef Blend© were added to each 10-gallon aquaria to feed the clams every other day until the completion of the experiments.

Gill preparation and experimental methods

Excised gill tissue from both *N. obscurata* and *L. staminea* was used to measure the amount of oxygen consumed in different salinities. After the seven-day acclimation period, six clams from each species were haphazardly removed from the acclimation tanks. Whole
clams were dried with paper towels and weighed to the nearest 0.01g using a Mettler Toledo B502 electric scale. In addition, the shell length (in centimeters) of each clam was recorded using calipers, where length was considered the longest distance of the shell from the anterior to the posterior. The clams were opened using a scalpel to sever the anterior or posterior adductor muscles. Each of the four gill lamellae was carefully removed and placed in labeled, species-specific, 6-well tissue culture dishes that were filled with 5ml of seawater from the acclimation tank. The culture dishes were placed in a 10ºC chiller box until the dissections were completed to maintain all gill tissue at the acclimation temperature.

Experimental salinities of 5, 30, and 55 ppt were prepared by adding de-ionized water or artificial sea salt to the 30 ppt unfiltered seawater from SPMC and stored in aerated 2.5-gallon laboratory aquaria in the 10º C cold room for the duration of the experiments. Salinities were tested daily using a refractometer to maintain salinities to a precision of ~ 0.1 ppt.

Oxygen consumption of excised gill tissues was measured using a Gilson Differential Respirometer (GDR), which is a closed system in which the reaction vessels are separated from the compensation chamber by a manometer (Umbreit et al., 1957). Six reaction vessels were filled with 5 ml of one experimental salinity treatments (18 vessels). All reaction vessels received 0.2 ml of 20% potassium hydroxide in the sidearm to absorb carbon dioxide released by the gill tissue during respiration. This ensured that any change in manomeric pressure was due to the consumption of oxygen. Using forceps, the dissected gill tissue from a *N. obscurata* or a *L. staminea* was placed in a reaction vessel. There were enough reaction vessels on the GDR that there were two individuals per species in each of the three salinity treatments in each run. The remaining six reaction vessels served as controls for each
salinity treatment. I randomly assigned each of the 18 reaction vessels onto the GDR for each of the runs.

The GDR was cooled to 10ºC and a shake rate of 60-65 strokes minute\(^{-1}\) was employed to maintain oxygen in equilibrium between the air and the aqueous medium. After the reaction vessels were attached to the GDR and lowered into the respirometer bath, there was a 20 minute acclimation period to allow the gill tissue to recover from handling before closing the respirometer valves. Readings were taken every thirty minutes for two hours. At the completion of each two hour run, the gills were rinsed with deionized water and placed onto paper towels to remove excess salts and water. The gill tissues were placed on pre-weighed (to the nearest 0.0001 g measured using a Sartorius AC1215 scientific scale) aluminum squares and dried at 60º C until the mass no longer changed. I corrected all respiratory readings for any fluctuations in the control flasks, and then further corrected to standard temperature and barometric pressure. I converted the measurements to microliters of oxygen consumed per gram of dry tissue weight per hour (µl g\(^{-1}\)h\(^{-1}\)).

**Independent gill tissue study**

In order to ensure that tissue death was not affecting the rate of oxygen consumption within each salinity treatment during the two hour testing period, an initial experiment was conducted prior to measuring oxygen consumption of gill tissue. Gills were dissected and gill pieces from *N. obscurata* and *L. staminea* were placed in the GDR as explained above. Every thirty minutes for two hours I removed gill strips from each treatment and examined under a compound light microscope at magnifications of 40, 100, and 400 X to determine if the gill tissue was still alive. Terminal cilia activity was observed and rated on a quantitative
scale, with 3 being most active and 0 being dead, as described by Vernberg et al. (1963).

The results of this study showed that all terminal cilia from both *N. obscurata* and *L.
staminea* were healthy and active after two hours in 5 ppt, 30 ppt, and 55 ppt salinity
treatments. This ensured that tissue death would not be a factor in the results of the oxygen
consumption study.

**Statistical Analysis**

To determine the effects of the response (experimental) variables, generalized linear
mixed effect models (GLMM) were fit to the data using the package “lme4” for the statistical
program R. GLMMs account for the nested design of this experiment by allowing both fixed
and random effects in models. The response variable for this experiment was oxygen
consumption (µl g⁻¹ hr⁻¹) of *N. obscurata* and *L. staminea*. The fixed factors were clam
species and salinity and random factors were beach and experimental block. I compared the
full model with the reduced and null models. I used Akaike Information Criterion (AIC) to
determine which model best fit the data. The best-fit model is judged as the one with the
lowest AIC value. The presence of a factor or interaction in the best-fit model is analogous
to being associated with a significant p-value in ANOVA.

**2.3 RESULTS**

Changes in oxygen consumption of isolated gill tissue differed by clam species and
salinity. These results are supported by the best fit GLMM, which showed that both “Clam
species” and “Salinity” were factors describing changes in oxygen consumption (Table 1). Interactions between the fixed factors were absent from the best fit model.

Oxygen consumption of both N. obscurata and L. staminea decreased with increasing salinity. The highest overall oxygen consumption occurred in the 5 ppt treatment and lowest at 55 ppt (Figure 2). Nuttallia obscurata had a 9% increase in oxygen consumption from 30 ppt to 5 ppt and a 21% decrease from 30 ppt to 55 ppt. Leukoma staminea had a 13% increase in oxygen consumption from 30 ppt to 5 ppt and a 22% decrease from 30 ppt to 55 ppt (Figure 2).

In all salinities treatments, L. staminea had a higher rate of oxygen consumption than N. obscurata. In the 5 ppt treatment, there was a 39% higher rate of oxygen consumption and a 35% and 33% higher rate in 30 ppt and 55 ppt respectively (Figure 2).
Table 1. The best-fit generalized linear mixed effects models describing changes in oxygen consumption of *N. obscurata* and *L. staminea*. Clam species = C, salinity = S. The best-fit model is denoted by the lowest Akaike Information Criterion (AIC) value and is listed first. Models include the main factors and interactions between factors that describe changes in oxygen consumption. The presence of a factor or interaction in the best-fit model is analogous to being associated with a significant p-value in ANOVA. “Block” and “Beach” were included as random factors in all models, but not listed in the table.

<table>
<thead>
<tr>
<th>Response variable</th>
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</tr>
<tr>
<td></td>
<td>S * C</td>
<td>6226.3</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>6280.5</td>
</tr>
<tr>
<td></td>
<td>S</td>
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Figure 2. Mean oxygen consumption (µl g⁻¹ h⁻¹) of excised gill tissue of *Nuttallia obscurata* and *Leukoma staminea* in 5, 30, and 55 ppt seawater after two hours in a Gilson Differential Respirometer. N=77 for *N. obscurata* and *L. staminea* in all treatments. Error bars indicate +/- one standard error.
2.4 DISCUSSION

In this study, I found that the rate of oxygen consumption in the excised gill tissue for both *N. obscurata* and *L. staminea* increased with decreasing salinity, with the highest level of oxygen consumption at 5 ppt and the lowest at 55 ppt (Figure 2). Although the tissue reacted similarly within the two species, *L. staminea* had a higher overall rate of consumption than *N. obscurata* in all salinity treatments. These results did not support my hypothesis that oxygen consumption would be elevated at high and low salinity concentrations where increased metabolic demands might be expected.

Several studies conducted on the rate of oxygen consumption in crustaceans (Dehnel and McCaughran, 1964; King, 1965; Thabrew *et al*., 1973; Engel and Eggert, 1974, 1975) also found this relationship (i.e., the lower the salinity the higher the respiration rate of the tissue). Van Winkle (1968) observed increased rates of respiration at low salinities in the gill tissue of molluscs as well, specifically *M. mercinaria*, and *M. demissus*. However, in all of these studies, high salinity was considered ambient seawater and low salinity was typically 5 ppt, but went as low as distilled or tap water in several of the experiments. There is no literature that discusses the effects of oxygen consumption of crustacean or mollusc gill tissue at salinities higher than 40 ppt.

In the case of both crustaceans and molluscs, osmotic swelling is indicated as a cause of accelerated respiration (Kinne, 1971). In the current study, both *N. obscurata* and *L. staminea* increased their rate of oxygen consumption at the lower (5 ppt) salinity treatment, which is in accordance with previous findings. In the 2009 study from our lab, *N. obscurata* osmoregulated at low salinities, such as 5 ppt, so an increase in respiration rate was expected for this species. *Nuttallia obscurata* also are often found in high densities in areas of
freshwater influx, so they were expected to have mechanisms in place to contend with low and fluctuating salinities that they encounter in their invaded environment.

*Leukoma staminea* are osmoconformers in ambient seawater and I hypothesized that they would not show an elevation in oxygen consumption levels at lower salinities. Osmoconformers allow their internal hemolymph to change with the external environment making their internal fluids isotonic and do not need extra metabolic energy for this process. Although osmoconforming requires less energy, there are disadvantages. Changes in salinity can alter the amount of solutes in internal tissues that can effect pH and ion concentration (Willmer *et al.*, 2005). This change in pH and ion concentration can alter biochemical pathways by denaturing protein’s structure making them less efficient (Wilmer *et al.*, 2005).

Perhaps *L. staminea* are not osmoregulators under normal environmental conditions but they have mechanisms in place to regulate cell volume in dire situations, such as very low salinities levels and this is why an increase in oxygen consumption was seen in the experimental salinity treatment of 5 ppt. At such an extreme salinity, their options are to osmoregulate and regulate their cell volume by producing osmolytes and free amino acids, which requires increased metabolic energy, or to expire. Under normal conditions, *L. staminea* would most likely close its valves during times of such osmotic stress.

It appears that both species tried to prevent osmosis from occurring at the low experimental salinity. One mechanism to maintain cell volume is to regulate the concentrations of free amino acids, which are the building blocks of proteins. When the salinities of the external medium decline, cells actively transport free amino acids (glutamate, alanine, threonine, and serine) out of the cell, but ions remain in the cell at normal concentrations (Hanson and Dietz, 1976). The result is the total concentration of
dissolved molecules (ions plus free amino acids) declines along with the external salinity, preventing the gain of water by osmosis and swelling of the cells. This process requires energy metabolism in the form of oxygen consumption, which could explain why both *N. obscurata* and *L. staminea* displayed increased oxygen consumption at 5 ppt.

The 2009 study in our lab indicated that intact *N. obscurata* had decreased internal osmolality at 50 and 60 ppt for day one of the experiment, indicating possible osmoregulation at the higher salinities. The organisms at the higher salinities began to deteriorate after prolonged exposure to these salinities, resulting in mortality before day 14. Based on these results, I hypothesized that *N. obscurata* would have a short-term increase in the rate of oxygen consumption at the experimental salinity treatment of 55 ppt due to extra metabolic energy demands required for osmoregulation. Interestingly, the current study did not support this hypothesis. *Nuttallia obscurata* demonstrated the lowest level of oxygen consumption in the 55 ppt salinity treatment at both sites (Figure 2). *Leukoma staminea* also displayed the lowest level of oxygen consumption in the 55 ppt treatment.

Published research concerning the effect of hypersaline environments on the physiology of molluscs could not be found. There is limited research dealing with whole organism oxygen consumption when organisms are placed in salinities greater than saltwater. In hypersaline environments, a reduction in oxygen consumption has been seen in Tilapia (salinity range of 35 to 95 ppt) (Sardella *et al.*, 2004). Salinity based reduction in oxygen consumption has also been observed in the Dead Sea killifish, *Aphinius dispar*, (Plaut, 2000), sheepshead minnow, *Cyprindion variegatus*, (Hanely and Nordlie, 1997) and the milkfish, *Chanos chanos*, (Swanson, 1998). However, it was hypothesized that these reductions in oxygen consumption were caused by reduced activity of individual fish and reaching their
upper salinity tolerances (Sardella and Brauner, 2008). This differs from the current study in that the depression of oxygen consumption found in the hypersaline (55 ppt) treatment of excised gill tissue of both *N. obscurata* and *L. staminea* are not due to activity of the whole organism. The lowered oxygen consumption was caused by physiological changes on the tissue or cellular level.

It initially appeared that one possibility for the decreased rate of oxygen consumption of both species in the current study was due to decreased oxygen tension in the 55 ppt salinity treatment. Bielawski (1960) found that the oxygen concentration of the environment might be a factor influencing the intensity of respiration. Oxygen solubility at 10°C and salinities of 5, 30, and 55 ppt is 69.9, 59.7, and 50.9 in µg O₂ liter⁻¹ mmHg⁻¹ respectively (Green and Carritt, 1967). Generally, it is thought that bivalves with opened valves rely on aerobic metabolism, fueled mostly by glycogen (Ortmann and Grieshaber, 2003). Once bivalves close their valves, the enclosed oxygen is consumed within a few minutes (Widdows, 1987), causing the partial pressure of oxygen to decrease rapidly in the mantle cavity, as measured in *Arctica islandica* (Taylor, 1976) and *Mytilus edulis* (Davenport and Woolmington, 1982). It has been suggested that anaerobic metabolism begins as soon as the partial pressure of oxygen falls below between 20 and 50 mmHg (Ortmann and Grieshaber, 2003; Pörtner et al., 1985). The experimental chambers in the Gilson Differential Respirometer are a closed system used to measure oxygen consumption, which can be compared to the closed shells of bivalves, in that the amount of water and oxygen contained in the chambers is all that is available until the system is opened. Perhaps, as the gill tissue in the 55 ppt treatment respired, they decreased the amount of dissolved oxygen contained in the system to a level known to initiate anaerobic metabolism.
Anaerobic metabolism may be a plausible explanation for the decrease in the rate of oxygen consumption in the 55 ppt treatment. *Nuttallia obscurata* and *L. staminea*, like all bivalves, have the ability to utilize anaerobic metabolism in unfavorable conditions. If the oxygen concentration of the water becomes too low (hypoxic or anoxic), the concentration difference between the water and the gills will not be large enough and the transfer of oxygen will cease. In their natural environment clams will close their shell (Vernberg, 1963), and begin to use anaerobic metabolism, converting fuel reserves to energy without using oxygen. However, anaerobic metabolism produces about 6% of the energy that aerobic metabolism does. Bivalves reduce their metabolic rate by about 95% to make their reserves last longer during anaerobic metabolism (Weber *et al.*, 2011). While their shells are closed and metabolic rates are low, the bivalves are not feeding, growing, or putting energy into reproduction. Persistent low oxygen concentrations can lead to a reduction of biomass and, eventually, death (Weber *et al.*, 2011). My study was conducted on excised gill tissue and therefore the clams did not have the option of valve closure to avoid unfavorable conditions. Even though research from our lab indicated that intact *N. obscurata* did in fact osmoregulate at higher salinities of 50-60 ppt, there was no increased oxygen consumption recorded from *N. obscurata* or *L. staminea* in the current study. Whether they are metabolic conformers or regulators, it initially appeared that the amount of available oxygen may have decreased passed a critical level, which may have caused both *N. obscurata* and *L. staminea* to enter a hypometabolic state.

Calculations on the amount of available oxygen in the experimental flasks as well as the amount of oxygen consumed by *N. obscurata* and *L. staminea’s* gill tissue in the 55 ppt salinity treatment do not support this hypothesis. Water at 10°C and 55 ppt contains 5.5 ml
of O₂ per liter of H₂O, which would be 0.0055 ml O₂ per ml H₂O. There were 5 ml of water in the experimental flask, making the amount of oxygen in the water sample 0.0275 ml. Oxygen consumption of the gill tissue was measured in microliters (µl) of O₂ making the total available oxygen in the 55 ppt water 27.5 µl (Table 2).

The gill tissue of both species consumed oxygen that was measured in µl of O₂ per gram of dry gill tissue per hour. Using these units I was able to determine the amount of oxygen used by the gill tissue of each species after each of the 2 hour trials (Table 2).

*Leukoma staminea* consumed 490 µl O₂ g⁻¹ hr⁻¹:

\[
490 \text{ µl O}_2 \text{ g}^{-1} \text{ hr}^{-1} \times 2 \text{ hours} \times 0.077 \text{ grams} = 75.4 \text{ µl O}_2 \text{ consumed}
\]

*Nuttallia obscurata* consumed 369 µl O₂ g⁻¹ hr⁻¹:

\[
369 \text{ µl O}_2 \text{ g}^{-1} \text{ hr}^{-1} \times 2 \text{ hours} \times 0.038 \text{ grams} = 28.0 \text{ µl O}_2 \text{ consumed}
\]

According to these calculations, *L. staminea* consumed almost three times the amount of oxygen available to them in the water and *N. obscurata* consumed over 100% of the available oxygen. This would lead to the conclusion that oxygen tension fell below a critical level and anaerobic respiration had occurred in the 55 ppt salinity treatment, explaining the recorded decrease in oxygen consumption. These calculations did not take into account the amount of oxygen available in the air of the experimental flasks. The Gilson Differential Respirometer maintained a shake rate of 60-65 strokes minute⁻¹ was to maintain oxygen in equilibrium between the air and the aqueous medium. This allowed the oxygen in the air to diffuse into the water during the two hour trials. 15 ml experimental flasks were used in the experiment; 5 ml were filled by the 55 ppt water sample, leaving just under 10 ml of air in the closed chamber. Air is 20.9% oxygen gas by volume, meaning there was approximately 2100 µl O₂ available in the air above the water in the experimental flask (Table 2).
Table 2. Comparison of the amount of oxygen (µl) available in 5 ml of each experimental salinity treatment (5, 30, 55 ppt) and 10 ml of air as well as the amount of oxygen consumed (total µl O₂ consumed in two hours) by the gill tissue of *N. obscurata* and *L. staminea* in all three treatments.

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 ppt</td>
</tr>
<tr>
<td>µl of O₂/5 ml H₂O</td>
<td>39.5</td>
</tr>
<tr>
<td>µl of O₂/10 ml air</td>
<td>2100</td>
</tr>
<tr>
<td>Total available O₂ (µl)</td>
<td>2139.5</td>
</tr>
<tr>
<td>O₂ consumed (µl)</td>
<td></td>
</tr>
<tr>
<td><em>Leukoma staminea</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Nuttallia obscurata</em></td>
<td>33</td>
</tr>
</tbody>
</table>
There was enough oxygen available during the two-hour trials for the gill tissue to utilize during aerobic respiration (Table 2). Therefore, lowered oxygen tension and anaerobic respiration are not viable explanations for the decrease in the rate of oxygen consumption of both *N. obscurata* and *L. staminea* gill tissue in the 55 ppt water treatment.

The independent gill tissue study showed that all terminal cilia from both *N. obscurata* and *L. staminea* were healthy and active after two hours in 5 ppt, 30 ppt, and 55 ppt salinity treatments. This ensured that tissue death was not be a factor in the results of the oxygen consumption study. Perhaps, the terminal cilia on the gills were still functioning but the hypersaline conditions in the 55 ppt treatment caused individual cell death. This cell death could have disrupted protein synthesis or breakdown, changed the pH of the cells or detrimentally affected the overall functioning of the gill tissue leading to decreased oxygen consumption.

The differences in the rate of oxygen consumption between *N. obscurata* and *L. staminea* in each treatment did support the hypothesis that *N. obscurata* would have an overall different rate of oxygen consumption than *L. staminea*. *Leukoma staminea* had a higher rate of oxygen consumption at all experimental salinities. It is interesting to note that not only did *L. staminea* have a higher overall rate of oxygen consumption then *N. obscurata* in all salinity treatments, but also it had a larger decrease from one treatment to the other (Table 2).

One explanation for the overall lower metabolic rate seen in *N. obscurata* could be due to their location in the high intertidal zone. In intertidal environments, the amount of food available to filter feeding organisms depends on the amount of water coverage during high tides. Water coverage decreases with increased height in the intertidal zone. Even
though *N. obscurata* has been shown to utilize pedal or siphonal feeding during times of exposure, they inhabit the highest intertidal zone in the Pacific Northwest and have a more limited supply of food available to them due to the length of time they are exposed during low tides. This may cause their overall energy budget to be lower than *L. staminea* to compensate for the potential food deficiency. An organism’s energy budget is used to describe how ingested energy is partitioned into different components, such as metabolic processes, growth or reproduction or waste products. The general energy budget formula is

\[ E_{\text{food}} = E_{\text{feces}} + E_{\text{urine}} + E_m + E_p \]

where \( E_m \) is energy lost to maintain bodily function, for locomotion, metabolism, and for digestion, absorption, and processing of food and \( E_p \) is for somatic growth and reproductive growth (production of gametes) (Wilmer et al., 2005). The lowered amount of available food in the high intertidal decreases the overall energy budget, which decreases the amount that can be partitioned into each component. *Nuttallia obscurata* has a low overall metabolic rate, which was seen in the lower oxygen consumption in all experimental salinities. This low metabolic rate may allow more energy to be partitioned into \( E_p \). *Nuttallia obscurata* are known to have short generation time, reaching maturity at sizes ranging from 1.6 to 2.3 cm within one year and a high fecundity (4 x 10^4 to 6 x 10^6 eggs per individual female each spawning season) (Dudas, 2005) which are important characteristics of a successful invasive species and require a large amount of the partitioned energy.

**Conclusions/Further studies**

This study was conducted to determine the effect of salinity differences on oxygen consumption. The effect of any single environmental variable does not realistically reflect
the natural environment. The combined action of several factors produces a greater effect than that caused by a single factor (Galtsoff, 1964). In future studies, the combined effects of salinity and temperature on the rate of oxygen consumption may be a better indicator of natural changes. It would also be beneficial to study the effects of salinity and temperature on the oxygen consumption of both excised gill tissue and intact *N. obscurata* and *L. staminea* to compare the previous findings of osmoregulation of whole organisms and potential anaerobic metabolism of gill tissue. King (1965) found that in crustacean gill tissues did not always correspond with that of whole organisms experiments with both may help elucidate differences that may be occurring in the metabolism or mechanism used in oxygen consumption.

A current study in our lab is examining the cellular mechanisms that allow *N. obscurata* to cope with and survive in both hypo- and hypersaline environments. Particularly, determining how the production of betaine and taurine in different salinities and tissues aid *N. obscurata* in coping with the osmotic stress and cell volume regulation.

This study did find that the ability to survive long periods of exposure, food shortages, salinity fluctuations, and potential anoxic/hypoxic conditions would hold a competitive advantage over similar species found in the same area. As an invasive species, *N. obscurata*, has adapted to life at the very high intertidal. To survive here, they must have excellent tolerance mechanisms that most likely would include osmoregulation capabilities, the utilization of different anaerobic pathways, and compartmentalized energy budgets.
CHAPTER 3

THE EFFECT OF TIDAL ZONATION AND SEDIMENT SIZE ON THE FEEDING STRUCTURES IN *NUTTALLIA OBSCURATA*

3.1 INTRODUCTION

The ctenidia (gills) of bivalves are important for many reasons. Although involved in gas exchange, gills provide only a small part of the total oxygen demand of bivalves (Jørgensen *et al*., 1986; Eble and Scro, 1996; Honkoop *et al*., 2003). The main task of the gills is to filter suspended particulate matter and to transport it towards the alimentary canals by the action of frontal cilia (Morton, 1983; Ward *et al*., 1991; Ward and Shumway, 2004; Navarro *et al*., 2010). The frontal cilia create a current that enables the gills to replace the water within the shell with water from outside the shell. The gills capture suspended particulate matter from this water and select edible particles (Ward *et al*., 1994; Eble and Scro, 1996; Honkoop, 2003). The edible particles are transported further to the labial palps where a secondary sorting process takes place. Rejected particles are voided as pseudofaeces, agglomerations of mucus and filtered particles, which are rejected instead of ingested (Bayne *et al*., 1995); accepted particles are carried further down the alimentary canal to the mouth (Shumway *et al*., 1985; Ward *et al*., 1998; Drent *et al*., 2004). The area of bivalve gills is proportional to the rate of filtration (i.e., the volume of water cleared of particles per unit time) (Meyhöfer, 1985; Riisgård, 1988; Jones *et al*., 1992; Honkoop, 2003). Larger gills collect more particles per unit time than smaller gills. The pumping and collecting capacity of the gill is proportional to its size as is the sorting capacity of the labial palps.
Functional traits are those that define species in terms of their ecological roles - how they interact with the environment and with other species (Diaz and Cabido, 2001). An example of a clear functional trait is the feeding morphology of bivalves that is represented by the dimensionless ratio of the size of the pumping (gills) to sorting (labial palps) apparatus. This gill-to-palp mass ratio reflects a continuum between the two main feeding (suspension and deposit) strategies in bivalves. The size of both the gills and labial palps has different functional roles in suspension-feeding and deposit-feeding bivalves (Navarro et al., 2010).

In suspension feeding bivalves, intraspecific differences in gill and palp size have been described (Theisen, 1982; Mettan, 1993; Payne and Miller; 1995; Ward et al., 1995; Barille et al., 2000; Drent et al., 2004). The differences in the size of the gills and palps are related to the relative suspended particulate matter in the water. In areas with high concentrations of suspended particulate matter the gills tend to be smaller and the palps larger. The pumping and collecting capacity of the gill, which is proportional to its size (Møhlenberg and Riisgård, 1979; Meyhöfer, 1985; Jones et al., 1992; Drent et al., 2004), will be smaller, and the sorting capacity of the labial palps, which is also proportional to its size (Theisen, 1977; Kiøboe and Møhlenberg, 1981), will be larger. In areas with a low concentration of suspended particulate matter the gills will tend to be larger and the labial palps smaller because the pumping and sorting needs have shifted (Figure 3).

In deposit feeding bivalves, it is the sediment characteristics of the area and not the concentration of the suspended particulate matter in the water that determines the relationship in the gill-to-palp mass ratio (Yonge, 1949). Meyhöfer (1985) and Specht and Lee (1989) found that the deposit feeding *Macoma balthica* uses its long inhalant siphons to inhale
deposited material into the mantle cavity. The small homorhabdic eulamellibranch gill (Atkins, 1937) does not sort material, instead only captures the food particles in mucus and transfers them to the palps for sorting (Levinton et al., 1996), therefore, the palps are larger than gills in such deposit-feeding bivalves. Bivalves with large palps and small gills will have a smaller gill-to-palp mass ratio than bivalves with large gills and small palps (Figure 3).

The gill-to-palp mass ratio is phenotypically plastic (i.e., adjustable within individuals) (Essink et al., 1989; Honkoop et al., 2003; Piersma and Drent, 2003). We can therefore assume that both gills and labial palps respond to changes in suspended particulate matter and sediment characteristics. Differences in the gill-to-palp mass ratio based on the quantity and quality of available food have been observed. Foster-Smith (1978) and Barillé et al. (2000) found in Mytilus edulis and Crassostrea gigas, the size of the palp increased in
areas of high turbidity and the size of the gills increased in areas of low turbidity. Yonge (1949) investigated modes of feeding and demonstrated that deposit feeders have larger labial palps than do suspension feeders. The ratio of gill-to-palp surface area was significantly smaller in bivalve species in areas with a high concentration of suspended particulate matter compared to those living in areas with low concentrations (Theisen, 1977; Essink et al., 1989; Payne et al., 1995).

Another factor in determining the size of gills is feeding time. Franz (1993) demonstrated that the ribbed mussel, Geukensia demissa, had relatively larger gills at higher than lower intertidal levels. He attributed this difference to decreased submersion at the high intertidal zones and therefore reduced feeding time for the mussels.

Because bivalves are relatively sessile organisms, their ability to obtain food is dependent on specific feeding structures and submersion time. Nuttallia obscurata is a semi-sessile filter-feeding bivalve that inhabits the high intertidal zone and therefore is alternately covered by water and exposed to air during the tidal cycle. During periods of submersion, N. obscurata actively collects suspended particulate matter from the water column via siphons through filter feeding. The gills transport the particulate matter to the labial palps for sorting, ingestion, and excretion.

During times of air exposure, many filter-feeding bivalves utilize the adaptive strategy of shell closure to avoid desiccation and potential terrestrial predation. Nuttallia obscurata have large unfused siphons that allow them to burrow up to 20 cm into the substratum to avoid predation and desiccation. During these times of exposure, when filter feeding from the water column is not an option, N. obscurata can also utilize organic detritus in the substratum by pedal-sweep feeding (DFO, 1999, 2001; Gillespie et al., 1999, 2001).
Reports indicate they are also siphonal deposit feeders, sweeping the inhalant siphon over broad areas of substratum to collect deposited materials (Tsuchiya and Kurihara, 1980). These alternate modes of feeding may be an advantage to *N. obscurata* surviving in the high intertidal zone during extended low tides, which bring decreased food availability. These modes of feeding may also be a cause of the plasticity seen in the gill-to-palp mass ratio as pedal and siphonal feeding bring large quantities of sediment into the mantle cavity. This excess sediment needs to be sorted into ingestible organic food or pseudofaeces by the labial palps. Therefore, the size of the labial palps may increase with increased pedal and siphonal feeding. This increase in palp size would decrease the overall gill-to-palp mass ratio.

The purpose of this study was to determine if the relative mass of the dry gill, palp, foot, or siphon tissue of *N. obscurata* varied with different tidal heights or sediment characteristics and to determine if that led to a difference in the gill-to-palp mass ratio. The relative mass was calculated as dry tissue mass of gill, palp, foot, or siphon as a proportion of total dry tissue mass of the individual specimen. The gill-to-palp mass ratio would perhaps be higher (larger gills compared to palps) in instances of increased suspension feeding and smaller (larger palps compared to gills) in instances of increased deposit feeding. Current literature only details the role of labial palps and gills in the feeding behavior of bivalves. The relative foot and siphon mass of *N. obscurata* at different tidal heights were analyzed in this study to determine if there was an increase in tissue mass with tidal height due to increased pedal and siphonal feeding. The sediment size and distribution at different beaches were analyzed to determine if they played a role in the plasticity of the gill-to-palp mass ratio. *Nuttallia obscurata*’s ability to sustain dense populations at the high intertidal zones, led to the following experimental questions.
**Question 1:** Is there a change in the gill-to-palp mass ratio of *N. obscurata* in relation to tidal height?

**Hypothesis:** Individuals found in the high intertidal zone will have a smaller gill-to-palp mass ratio than those individuals found in low and mid intertidal zones due to the increase in pedal and siphonal feeding.

**Question 2:** Is there a change in relative mass of foot or siphon tissue of *N. obscurata* in relation to tidal height?

**Hypothesis:** Individuals found in the high intertidal zone will have a larger relative foot and siphon mass than those individuals found in low and mid intertidal zones due to the increase of particle gathering.

**Question 3:** Is there a relationship between the gill-to-palp mass ratio and sediment grain size at different sites?

**Hypothesis:** There will be a smaller gill-to-palp mass ratio at sites with course sediments than at sites with fine sediments.

### 3.2 MATERIALS AND METHODS

**Study sites and collection techniques**

Two Northwest Washington sites were selected to use in this study: Birch Bay (48.927° N 122.745° W; accessed north of Birch Bay State Park), and Clayton Beach (48.382° N 122.29° W; part of Larrabee State Park). *Nuttallia obscurata* were collected between October 2011 and April 2012. At both sites, differences in feeding structure morphology based on tidal height were investigated and therefore a tide specific quadrat system of collection was designed. At both beaches, *N. obscurata* was collected from five
0.25 m² quadrats at three tidal zones (high, mid and low). Distance to shore was used to determine the high tide zone (shore was defined as the apparent high tide line indicated by tidal wrack or rock obstructions). Distance between zones differed at each site based on available mud flat exposed at low tide. Once the quadrats were established, an Electronic Self-Leveling Rotary Laser (CST/berger LM1000X) was used to determine the elevation difference between the three tidal zones (high, mid and low). Each quadrat was excavated to 20 cm or until no more clams were found. The type and number of clams from each quadrat were recorded and 10-20 similar-sized *N. obscurata* were placed in 1-quart quadrat and tide specific zip lock bags for transport back to WWU. Sediment samples from each quadrat were collected in 1-quart quadrat and tide level specific zip lock bags, transported to WWU, and placed in a laboratory freezer to be analyzed for sediment grain size later. Clams were placed in 15-quadrat and tide level specific, randomly distributed, 2.5-gallon aquaria in WWU’s 10°C cold room in 30 ppt unfiltered seawater from SPMC. There was no acclimation period for the clams and experiments began the day after collection. There were no *N. obscurata* found in the low tide zone at Birch Bay or Clayton Beach.

**Gill preparation and experimental methods**

Specimens were maintained in 2.5-gallon aquaria resting on 2-3 inches of quadrat specific sediment. Ten *N. obscurata* were haphazardly selected from quadrat and zone specific aquaria twice a day for five days until all dissections were completed. The specimens were dried with paper towels to remove excess water, weighed (to the nearest 0.01g), and measured to the same specifications as the previous study. I opened the specimens by severing the anterior and posterior adductor muscles. Using forceps, scalpels
and surgical scissors, the four gill lamellae, two labial pals, two siphons, foot, and visceral mass from each specimen were placed in labeled holding containers containing de-ionized water to remove excess salt from the tissue (an independent study was conducted to ensure the de-ionized water did not cause tissue damage prior to drying). All tissue samples were placed on pre-weighed (to the nearest 0.0001g) and labeled aluminum squares after being placed on paper towels to remove any excess water. Tissue samples were dried in a 60º C drying oven until the mass was constant and were then reweighed.

**Substratum Study**

To determine differences between tidal height and collection sites the sediment samples from each quadrat from both study sites were place in individual aluminum weigh boats and dried at 60º C in a drying oven for no less than 24 hours. Once the sediment samples were dried, they were transported to the Geology Building on WWU campus to be separated using the RoTap Testing Sieve Shaker (W.S. Tyler Company #20242). A 150 gram sediment sample from each individual quadrat was placed in sorting sieves with screen sizes of 2.0 mm, 1.19 mm, 0.5 mm, 0.3 mm, 0.125 mm, and 0.063 mm. The sieves were placed in RoTap shaker and set for 15 minutes. The sorted sediment was transferred to individual pre-weighed weigh boats and weighed to the nearest 0.001g. Sediments were analyzed using the program GRADISTAT (Blott and Pye, 2001).

**Statistical Analysis**

Univariate Analysis of Variance (ANOVA) on untransformed data was used for all statistical analyses dealing with relative dry gill, palp, foot, and siphon tissue mass as well as
the gill-to-palp mass ratios. The program GRADISTAT (Blott and Pye, 2001) was used to analyze the sediment grain size.

3.3 RESULTS

Gill-to-palp mass ratio

There was a significant tide effect in the gill-to-palp mass ratio of *N. obscurata* from Birch Bay, WA (F_{1,98} = 28.0, p<0.01, ANOVA) (Figure 4). *Nuttalia obscurata* from the high tide zone had a 36% larger gill-to-palp mass ratio compared to *N. obscurata* from the mid tide zone (Figure 4). The average relative mass of *N. obscurata*’s dry gill tissue varied significantly between tide levels (F_{1,98} = 34.69, p<0.01, ANOVA), with the high tide gill tissue mass 30% larger than that from the mid intertidal zone (Figure 5). There was no significant difference in the relative dry palp tissue mass of *N. obscurata* at high tide or mid tide zones (Figure 5). There was no significant tide effect in the gill-to-palp mass ratio of *N. obscurata* collected from Clayton Beach, WA (Figure 4). There was no significant tide effect on the relative dry gill or palp tissue mass (Figure 5).

The gill-to-palp mass ratio differed significantly between *N. obscurata* collected from Birch Bay and Clayton Beach, WA (Figure 4). There was a significant interaction between beach*tide (F_{1,19} = 9.4, p =0.003, ANOVA) therefore two separate ANOVAs were conducted on the different tide levels from both sites. There was a significant difference between high tides (F_{1,99} = 71.1, p < 0.01, ANOVA) at Birch Bay and Clayton Beach as well as at mid tides (F_{1,99} = 40.1, p < 0.01, ANOVA) for both sites. The gill-to-palp mass ratio from Birch Bay *N. obscurata* was 79% larger at high tide and 41% larger at mid tide zones than that of Clayton Beach *N. obscurata*. 

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Figure 4. Average gill-to-palp mass ratio between *Nuttallia obscurata* collected from high and mid tidal zones from Birch Bay and Clayton Beach, WA. N= 50 for each tide zone (N = 100 per beach). Error bars indicate +/- one standard error.
Figure 5. Comparison of the relative dry tissue mass of gill, palp, foot, and siphon of *Nuttallia obscurata* collected from high and mid tide transects from Birch Bay and Clayton Beach, WA. Relative mass was calculated as the average dry mass of individual tissue/average total dry tissue mass of the organism. N = 50 for all samples. Error bars indicate +/- one standard error.
Foot and Siphon mass

Tide had a significant effect on the relative dry foot mass ($F_{1,98} = 6.27$, $p = 0.014$, ANOVA) of *N. obscurata* at Clayton Beach (Figure 5). The relative dry foot tissue mass collected from *N. obscurata* at high tide was 9.5% greater than tissue collected from mid tide. Tide did not have a significant effect on the relative dry foot mass of *N. obscurata* collected from Birch Bay (Figure 5, Table 2).

Tide had a significant effect on the relative dry siphon mass ($F_{1,99} = 6.49$, $p = 0.012$, ANOVA) of *N. obscurata* at Birch Bay, WA (Figure 5). The relative dry siphon tissue mass collected from *N. obscurata* at high tide was 14.8% greater than tissue collected from mid tide. Tide did not have a significant effect on the relative dry siphon tissue mass of *N. obscurata* collected from Clayton Beach, WA (Figure 5, Table 2).

Substratum analysis

Sediment characteristics between Birch Bay and Clayton Beach were substantially different (Figure 6), reflecting the difference in the sedimentary environment between the two intertidal zones. Birch Bay consisted of 84-90% fine sand (0.063-0.125 mm) and Clayton Beach consisted of 26-37% very fine gravel, 30% coarse sand, and 18-26% medium sand (0.380-2.0 mm). Birch Bay was uniform in its sediment size distribution whereas Clayton Beach had a larger grain size and more diversity in grain size (Figure 6).
Sediment characteristics at high and mid tidal zones at Birch Bay and Clayton Beach. Sediment that would not pass through 0.0625 mm sieve is very fine sand; through 0.125 mm is fine sand; through 0.380 mm is medium sand; through 0.5 mm is coarse sand; through 1.18 mm is very coarse sand and through 2.0 mm is very fine gravel. All percentages were calculated by weight using the program GRADISTAT (Blott and Pye 2001).
3.4 DISCUSSION

*Nuttallia obscurata* demonstrated differences in their feeding structures related to tidal height, site, and sediment type. The gill-to-palp mass ratio of *N. obscurata* collected from the high intertidal zone was 30% larger compared to that from the mid intertidal zone from Birch Bay. I hypothesized that there would be a decrease in the gill-to-palp mass ratio of *N. obscurata* collected from the high intertidal zone caused by the dual feeding modes that may be employed to counteract the lack of suspended particulate matter during exposure at low tides. Pedal or siphonal feeding brings a large quantity of sediment into the mantle cavity, which needs to be sorted into ingestible organic food or pseudofaeces by the labial palps. The hypothesized decrease in the gill-to-palp mass ratio in the high tide zone would be caused by an increase in the overall palp mass needed to sort the increased volume of sediment brought into the mantle cavity during pedal and siphonal feeding. Kiørboe and Møhlenberg (1981) showed that the degree to which food and nonfood particles are sorted is positively correlated with palp size. An increase in palp size in the high intertidal zone was not seen in the current study. In fact, it was the 30% increase in the relative dry gill tissue mass at high tide (Figure 5) that caused an increase in the gill-to-palp mass ratio. There was a difference in the feeding structures based on tidal height with the increase in relative dry gill tissue mass at high tide and no significant change in the relative dry palp tissue mass (Table 2). Ward *et al.* (1998) showed that *Crassostrea gigas* and *Crassostrea glomerata* can process a large amount of particles without affecting the efficiency of the palps and did not see a change in the size of palps regardless of the amount of suspended particulate matter.

One possibility for the increased size of the gill tissue may be caused by the feeding time available to *N. obscurata*. Franz (1993) demonstrated that *Geukensia demissa* had
larger gills at higher than lower intertidal levels. He attributed this difference to decreased
submersion at the high intertidal zones and therefore reduced feeding time for the mussels.
Perhaps *N. obscurata* residing in the high intertidal zone of Birch Bay utilize larger gills for
increased filtering of suspended particulate matter during shorter periods of water coverage
each day. On the day of collection at Birch Bay, the total distance of exposure at low tide
was 165 M with a 1-foot elevation decrease between high and mid tide. However, Birch Bay
exhibits large exposed tidal flats during all low tides with in a tidal cycle. Therefore, *N.
obscurata* residing at this site would contend with decreased food availability during the
course of a tide cycle.

This may also help to explain the lack of significant difference in the gill-to-palp
mass ratio of *N. obscurata* with relation to tidal height found at Clayton beach (Figure 4,
Table 2). The total distance of exposure on the day of collection was 50 M with a 1-foot
elevation decrease between tide levels. Clayton Beach exhibits a much smaller exposed tidal
flat regardless of the tidal cycle than Birch Bay. *Nuttallia obscurata* found at the high and
mid tide zones at Clayton Beach (as measured from the exposed portion of substratum)
would be unlikely to experience different conditions during low tide exposure. This may due
to the small exposed mud flat and therefore little variation in exposure time between tide
levels leading to the lack of variation in their feeding structures (i.e., size of gill and palp
tissue) to coincide with different feeding techniques (i.e., filter or deposit feeding) potentially
caused by exposure.

It has been suggested (Pohlo, 1967; Theisen, 1977; Essink et al., 1989; Payne et al.,
1995) that the amount of suspended particulate matter determines the size of the gills and
palps in bivalves. Kiørboe and Møhlenberg (1981) found that species have large palps and
small gills (small gill-to-palp mass ratio) in environments with a high resuspension events. These resuspension events occurred on beaches with fine sand with a high resuspension rate. Species had larger gills and smaller palps (higher gill-to-palp mass ratio) in environments with gravel or coarse sand that is more difficult to resuspend (Navarro et al. 2010). The data from this study contradicts these findings. *Nuttallia obscurata* that were collected from Birch Bay where the sediment was uniform and much finer in sediment grain size (Figure 6) had a larger gill-to-palp mass ratio (larger gills and smaller palps) than *N. obscurata* collected from Clayton Beach where the sediment was much coarser and less uniform (Figure 6).

There was a difference in the gill-to-palp mass ratio between sites, with *N. obscurata* collected from high tide at Birch Bay having an 79% larger gill-to-palp mass ratio than those collected from high tide at Clayton Beach and a 41% larger gill-to-palp mass ratio from those collected at mid tide. There were differences not only in the gill-to-palp mass ratios found in *N. obscurata* collected from both sites but also in the breakdown of the components of the ratio (i.e. the relative size of the dry gill and palp tissue mass) (Table 2). I hypothesized that there could be a smaller gill-to-palp mass ratio at sites with course sediments than sites with fine sediments. This smaller gill-to-palp ratio would be caused if *N. obscurata* were indeed pedal or siphonal feeding, the palps would need to be larger at sites with coarse sediment to handle the increased sorting needs. Clayton Beach had more coarse sediment (Figure 6) and the *N. obscurata* found there had a smaller gill-to-palp mass ratio than from Birch Bay (Figure 4, Table 2). Larger palps and smaller gills did not drive the lower gill-to-palp mass ratio found at Clayton Beach. The gills in *N. obscurata* collected from Clayton Beach were smaller than those collected from Birch Bay and that was causing the smaller gill-to-palp
Table 3. Comparison of relative dry gill, palp, foot, and siphon tissue mass (as a percent of total dry tissue mass) and gill-to-palp mass ratio of *N. obscurata* collected from high tide and mid tide levels at Birch Bay and Clayton Beach.

<table>
<thead>
<tr>
<th></th>
<th>Birch Bay</th>
<th></th>
<th>Clayton Beach</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>High Tide</td>
<td>Mid Tide</td>
<td>High Tide</td>
<td>Mid Tide</td>
</tr>
<tr>
<td>Relative gill tissue mass</td>
<td>4.6%</td>
<td>3.5%</td>
<td>2.8%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Relative palp tissue mass</td>
<td>1.8%</td>
<td>1.8%</td>
<td>2.0%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Relative foot tissue mass</td>
<td>8.1%</td>
<td>8.3%</td>
<td>9.5%</td>
<td>8.7%</td>
</tr>
<tr>
<td>Relative siphon tissue mass</td>
<td>10.6%</td>
<td>9.3%</td>
<td>7.2%</td>
<td>6.9%</td>
</tr>
<tr>
<td>Gill-to-palp mass ratio</td>
<td>2.6</td>
<td>2.0</td>
<td>1.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>
mass ratio. However, when comparing the individual components of the ratio (i.e., size of gill and palp tissue) it is clear that the relative dry palp tissue mass makes up a larger percent of total tissue mass when compared to the relative dry gill tissue mass in *N. obscurata* collected from Clayton Beach. On average (between tide levels), the relative dry gill tissue makes up 2.65% of the total body tissue and the relative dry palp tissue makes up 2%. This is considerably closer to a 1:1 ratio than is seen in *N. obscurata* collected from Birch Bay were the relative dry gill tissue makes up (on average) 4.1% of the total body tissue and the relative dry palp tissue only 1.8% of the total. In comparison to Clayton Beach, the relative dry gill tissue at Birch Bay is more than twice as large as the relative dry palp tissue. Perhaps this difference in percent tissue mass was caused by *N. obscurata* at Clayton Beach utilizing pedal or siphonal feeding more than Birch Bay or perhaps it is due solely to the increased grain size found at Clayton Beach that must be sorted regardless of feeding method (i.e., filter or deposit feeding).

It also was hypothesized that if *N. obscurata* were utilizing pedal or siphonal feeding in order to better survive conditions in the high intertidal zone that they would have increased foot and siphon tissue mass to support these feeding methods. The data from this study does not support this hypothesis. There was a significant tide effect on relative dry foot tissue mass in *N. obscurata* collected from Clayton Beach, WA (Figure 5, Table 2). There was a 9.5% increase in relative foot mass found in *N. obscurata* collected from high tide as compared to mid tide from Clayton Beach. The relative foot mass of Clayton Beach high tide *N. obscurata* was 17.3% higher than from high tide at Birch Bay and 4.8% higher at mid tide. The increase relative foot mass from Clayton Beach may be due to the increase in grain size found at that site. *Nuttallia obscurata* has the ability to burrow up to at 20 cm into the
substrate, much further than any native clam. Perhaps the different grain sizes found at each site places different burrowing pressures on *N. obscurata* and the foot mass varies to contend with those pressures.

There was no significant tide effect on the relative foot mass of *N. obscurata* found at Birch Bay (Figure 5, Table 2). This is perhaps indication that *N. obscurata* are not participating in pedal feeding in the high intertidal zone during times of exposure. Alternatively, an increase in relative foot mass may not be necessary for pedal feeding to occur.

There was a significant tide effect in the relative dry siphon tissue mass in *N. obscurata* collected from Birch Bay, WA (Figure 5, Table 2). *Nuttallia obscurata* collected from the high tide zone had 14.8% larger relative dry siphon tissue mass than *N. obscurata* collected from the mid tide at Birch Bay. This increase in siphon tissue may be related to the increase in relative dry gill tissue mass seen in high tide *N. obscurata* from Birch Bay, WA. If *N. obscurata* in the high tide zones have larger gill tissue to compensate for decreased submersion time like Franz (1993) found for *G. demissa*, then it follows that there would be an increase in siphon tissue as well. To insure an increased rate of filter-feeding needed during times of submersion, larger siphons may help facilitate that.

The gill-to-palp mass ratio and relative size of gill, palp, foot, and siphon tissue varied in *N. obscurata* collected from Birch Bay and Clayton Beach but the specific mechanisms driving this difference were not uncovered in this study. Perhaps it is due to the increase exposure time at Birch Bay caused by the large exposed mud flat that decreases feeding time, which leads to an increase in the size of the gill tissue as found by Franz (1983) or it may be due to the difference in sediment grain size found between the two sites. Further
studies need to be conducted on the plasticity of feeding structures in *N. obscurata* collected from different sites with varied exposure time and sediment characteristics to elucidate their significance.

Even though studies have shown the gill-to-palp mass ratio in bivalves to be a phenotypically plastic trait (Essink *et al.*, 1989; Honkoop *et al.*, 2003; Piersma and Drent, 2003), it is also possible that the differences in the feeding structure found at Birch Bay and Clayton Beach were instead due to differential mortality (species sorting). Perhaps the environmental conditions found at Birch Bay, fine sediment and large tidal exposure, were suited to *N. obscurata* with larger gills and smaller palps (a large gill-to-palp mass ratio). Conversely, the environmental conditions found at Clayton Beach, coarse sediment and small tidal exposure, were suited to *N. obscurata* with smaller gills and relatively large palps (a small gill-to-palp mass ratio). If this were the case, the *N. obscurata* not suited to the environmental conditions at each site would die, causing distributions similar to the results of this study. Further research needs to be conducted to determine if plasticity or differential mortality is the driving force behind the difference in the gill-to-palp ratio found in *N. obscurata*.

**Conclusions/Further Studies**

Compton *et al.* (2007) found that when the gill-to-palp mass ratio of several species of bivalves inhabiting two intertidal zones in the Netherlands and Australia was log transformed they fell along a continuous gradient from typically deposit feeders to suspension (filter) feeders (Figure 7). *Nuttallia obscurata* is considered a filter-feeding bivalve that is also capable of pedal or siphonal feeding (mechanisms of deposit feeders).
Figure 7. Ranked log gill-to-palp mass ratios of bivalves in Roebuck Bay and the Wadden Sea display a gradient between mostly sorting (deposit feeding) and mostly pumping (suspension feeding), within the total range of morphospace occupied at each location, as indicated by the vertical dashed lines. Number of individuals (n) collected for each species is shown. From Compton et al., 2007. Boxes represent the log gill-to-palp mass ratio of *N. obscurata* from Birch Bay and Clayton Beach.
The log gill-to-palp mass ratio for *N. obscurata* at Birch Bay is 0.4 and 0.3 for high tide and mid tide respectively and 0.15 and 0.14 for high tide and mid tide at Clayton Beach (Figure 7). According to the log gill-to-palp mass ratio continuum from Compton *et al.* (2007), *N. obscurata* from Birch Bay are closer to filter feeding bivalves with larger gills compared to palps. Clayton Beach *N. obscurata* are placed near deposit feeding individuals from Family Tellinidae on the continuum. This is interesting taxonomically because *N. obscurata* is in the same Superfamily as Tellinidae, which contains the deposit feeding *Macoma sp.* (the only species known to co-occur in the high intertidal zone with *N. obscurata*). This close taxonomic classification as well as the ability to thrive in the high intertidal zone along with known deposit feeders may indicate that *N. obscurata* is more often a deposit feeder.

There is very little published research regarding the percent relative mass of gill or palps with regard to total dry tissue mass. Honkoop *et al.* (2003) reported that for *Crassostrea gigas* the relative percent gill tissue was between 12-21% and palp tissue between 4-7%. For *Saccostrea glomerata*, the percent gill tissue was between 12-18% and palp tissue between 4-6%. Both of the mentioned species are known filter feeders. They have a larger percentage (relative to total body mass) of gill tissue than palp tissue. Tsuchiya (1981) found the percent gill tissue in filter feeding *Patinopectin yessoensis* to be 13% and Pouvreau *et al.* (1999) found that 30% of the total body weight was from the gill tissue of *Pinctada margaritifera*.

Using this information, the comparison between the percent gill and palp tissue per total body weight of *N. obscurata* is extremely interesting. At Clayton Beach, the percent gill tissue ranges from 1.6-2.0% and the palps 1.2-1.4%. At Birch Bay, the percent gill tissue
is between 3.9-7.7% and the palps between 1.9-2.8%. These values show a much lower overall percentage of gill tissue when compared to known filter feeders. This could indicate that *N. obscurata* is more of a deposit feeding bivalve than a filter feeding one.

It would be helpful to collect data on the gill-to-palp mass ratios of the deposit feeding, *Macoma nasuta*, and filter feeding, *L. staminea*, to obtain comparisons for the data collected and analyzed for *N. obscurata*. 
CHAPTER 4
THE EFFECT OF THE MANTLE PEA CRAB, PINNIXIA FABA
ON THE GILL-TO-PALP MASS RATIO OF NUTTALLIA OBSCURATA

4.1 INTRODUCTION

Pinnotherids, or pea crabs, are a group of decapod crustaceans adapted for life within other marine animals (Bierbaum and Ferson, 1986) and commonly found in the mantle cavity of marine molluscs (Bierbaum and Shumway, 1988). Pea crabs are considered commensals by some (Rathbun, 1918; Dales, 1957; Barnes, 1980) and parasites by others (Orton 1920; Stauber, 1945; Pearce, 1966; Chenge, 1967). The pea crab positions itself on the gills of host bivalves and uses its chelae to pick up mucous food strands that pass by on their way to the palps to be ingested (Bierbaum and Ferson, 1986). This behavior has detrimental effects on the bivalve host. McDermott (1961) and Flower and McDermott (1952) found gill lesions in infested Mytilus edulis and Crassostrea virginica, respectively. Krucyznski (1972) noted a reduction in the dry tissue mass of infested scallops as compared to uninfected scallops of the same size. Pregenzer (1979) demonstrated that particles were cleared at a slower rate in mussels that were infected with pea crabs than those that were uninfected.

Miller et al. (2008) demonstrated the ability of Pinnothetes sp., which typically infest native bivalves, to infest the introduced bivalve, Musculista sehousia, in New Zealand. Although the preference in selection still fell to the native bivalves, in areas with increased numbers of the introduced bivalve there were increased cases of infection by the pea crab (Miller et al, 2008).
Nuttallia obscurata is a recent invasive species whose population has increased rapidly in the estuaries as well as along the coastlines of British Columbia, Washington, and Oregon. Nuttallia obscurata have established populations in some areas since the early 1990s and several organisms have learned to prey on this invasive species. In the northeastern Pacific Ocean N. obscurata are preyed upon by moonsnails, gulls, crows, black oystercatchers, several crab species (e.g., Cancer sp., Hemigrapsus sp.) and scoters (Gillespie et al., 2001; Lewis et al., 2007). Nuttallia obscurata has been a host to the native, mantle pea crab, Pinnixa faba, since N. obscurata’s invasion (Gillespie et al. 1999; DFO, 1999; DFO, 2001; Gillespie et al., 2001; Dudas and Dower, 2006) but there are no published studies investigating the effects of pea crabs on N. obscurata’s physiology or morphology.

The discovery of a high infection percentage of pea crabs in the mantle cavity of N. obscurata collected from Clayton Beach, WA, during the dissection procedures for Chapter 3 of this thesis, led to the following experimental questions:

**Question 1:** Does infection by the P. faba affect the overall gill-to-palp mass ratio?

**Hypothesis:** Infection by P. faba will cause an increase in the overall gill-to-palp mass ratio

### 4.2 MATERIALS AND METHODS

After the specimens were opened by severing the anterior and posterior adductor muscles in the experiments in Chapter 3, the mantle cavity was examined to determine if there was P. faba present. If I found P. faba in the mantle cavity, they were removed using forceps and measured to the nearest 0.01 cm using calipers. The number and length of each
*P. faba* was recorded along with the corresponding *N. obscurata* data used in the other experiments.

**Statistical Analysis**

One way Analysis of Variance (ANOVA) was used to determine if infection by *P. faba* in the mantle cavity of *N. obscurata* had an effect on the overall gill-to-palp ratio and the relative mass of gill and palp tissue. For all statistical tests, $\alpha$ was set at 0.05. All ANOVA analyses were conducted using IBM SPSS Statistics 20.

**4.3 RESULTS**

There was a large discrepancy in infection rates between sites. Of the one hundred *N. obscurata* collected from Birch Bay, WA, only one was infected with the pea crab, *P. faba*, as compared to the 57% (57 out of 100) infected from Clayton Beach, WA. Of the infected clams at Clayton Beach, 49% contained one pea crab and 8% contained two pea crabs. Therefore, all *P. faba* analyses were conducted with data collected from Clayton Beach. There was no significant difference in the size of the relative dry gill or palp tissue mass of *N. obscurata* infected with *P. faba* (Figure 8) and therefore no significant difference in the overall gill-to-palp mass ratio (Figure 9).
Figure 8. Comparison of the relative gill and palp dry tissue mass of *Nuttallia obscurata* collected from Clayton Beach, WA with (n= 57) or without (n= 43) infection of *Pinnixa faba* in their mantle cavity. Error bars indicate +/- one standard error.
Figure 9. Comparison of the relative gill-to-palp mass ratio of *Nuttallia obscurata* collected from Clayton Beach, WA with (n=57) or without (n=43) infection of *Pinnixa faba* in their mantle cavity. Error bars indicate +/- one standard error.
4.4 DISCUSSION

*Nuttallia obscurata* collected from Clayton Beach had a high infection rate of *P. faba*; out of one hundred, 49% contained one pea crab, and 8% contained two. *Nuttallia obscurata* collected from Birch Bay, WA did not exhibit this same type of infection rate, only one out of one hundred clams was infected from that site. This disparity in infection between sites may be due to the larger size of individual clams, small exposed intertidal zone, and larger sediment grain size found at Clayton Beach.

Miller *et al.* (2008) demonstrated that of two mussel species, the smaller *Xenostrobus pulex* added growth constraints on any pea crabs inhabiting it due to decreased open mantle space and therefore *X. pulex* had a lower infection rate. The primary host for the female *P. faba* or mating pair in the Pacific Northwest tends to be *Tresus capax*, which are much larger than *N. obscurata*. They can be up to 20 cm and weigh between 3-4 lbs. (Gillespie *et al.*, 2001). *Nuttallia obscurata* collected from both sites were much smaller than *T. capax*, the preferred host of *P. faba*. Birch Bay clams were 54.2 % smaller than Clayton Beach clams. Perhaps the smaller size of *N. obscurata* found at Birch Bay was a contributing factor to the low infection rate due to potential growth constraints on *P. faba*. McLay (1988) noted that because pea crabs feed on food particles collected from the host, a larger host could potentially increase food availability to the pea crab.

The much larger exposed tidal flat found at Birch Bay may shed light on the different infections rates at both sites. It is doubtful whether *P. faba* venture high enough on the shore or encounter *N. obscurata* in the mid to high intertidal zones at Birch Bay for long enough to infect them, as pea crabs tend to occur low in the intertidal (McLay 1988). In addition, the short feeding time of *N. obscurata* in the high intertidal zone reduces food available for pea
crabs (Miller _et al._, 2008), which may help to explain why minimal _P. faba_ infection was evident in Birch Bay _N. obscurata_. Clayton Beach had a much shorter distance from high tide to low tide and had a shorter duration of exposure at high tide than does Birch Bay. _Pinnixa faba_ may have more access to all _N. obscurata_ located at Clayton Beach, which may have caused the increased infection rate at that site. Miller _et al._ (2008) found that pea crab infection only occurred on the lower half of the shore (mid to low intertidal zones), where hosts were submerged for most of the tidal cycle; therefore pea crab prevalence increases with decreasing tidal height.

Sediment characteristics between Birch Bay and Clayton Beach were substantially different (Figure 6). Birch Bay consisted of 84-90% fine sand and Clayton Beach consisted of 26-37% very fine gravel, 30% coarse sand, and 18-26% medium sand. Birch Bay was uniform in its sediment size distribution whereas Clayton Beach had larger sand grain and less uniform size distribution (Figure 6). This larger grain size at Clayton Beach creates more interstitial space, which may afford easier movement of _P. faba_ through the substratum. The larger grain size may also slow the closing time of the valves in _N. obscurata_. _Nuttallia obscurata_ may need to utilize its foot to remove large particles from the shell prior to complete closure, which may allow the crabs more access into and out of the mantle.

_Further studies_

In this study, only the number and size of _P. faba_ were recorded. In future studies, the sex of the _P. faba_ should be noted as they exhibit sexual dimorphism (Orton, 1920; Atkins, 1926) and may have markedly different detrimental effects on _N. obscurata_. McDermott (1961) cited gill and palp erosion on _Mytilus edulis_ caused by pea crab infection.
This erosion is presumably caused by the hard-shelled morphs of males and juvenile female
P. faba. As the crabs mature, the male’s carapace remains hard shelled whereas the female
undergoes a metamorphosis after mating that causes the shell to decalcify and become
translucent (Becker et al., 2011). After mating, the females also appear to adapt to a parasitic
lifestyle and no longer leaves the host whereas adult males are found within the host and free
living (Christensen 1959) throughout their life. Hart (1982) found that even though pea crabs
infect other intertidal bivalves, mature females are generally found only in Tresus capax. If
males and immature females are the only morphs present in N. obscurata it may indicate that
the invasive species is only an intermediate host to the native parasite.

It has been noted that infection with parasites can have marked effects on hosts and
their communities (Mouritsen and Poulin 2002). Understanding how a native generalist
symbiont affects an invasive species may help to elucidate the symbiont’s influence on the
population structure of the species and the possibility of reducing impacts caused by the
invasive species to the novel community (Mouritsen and Poulin 2002; Miller et al., 2008). It
would also be of great importance to determine if the interaction between N. obscurata and
P. faba is one of commensalism or parasitism. In order to determine this, long-term studies
of the effects of P. faba on the size and health of N. obscurata’s gill and palp tissue will need
to be studied as well as growth rate, oxygen consumption and filtration rate of the clam.
CHAPTER 5

GENERAL CONCLUSIONS

The Purple Varnish Clam, *Nuttallia obscurata*, is an example of a recent successful invader to the Pacific Northwest. Studies have shown that *N. obscurata* display the characteristics generally associated with successful invaders. *Nuttallia obscurata*’s small size at maturity, high fecundity, lengthy planktonic phase, broad environmental tolerances, as well as regional ocean currents, have all played a role in its successful invasion and dispersal since its introduction via ballast water in the late 1980’s. *Nuttallia obscurata* has attained adult populations of 800-1200 clams per m$^2$ in the high intertidal zones of the Pacific Northwest where they must contend with limited food availability and daily salinity fluctuations. Does the location, sediment grain size, or exposed tidal flat of individual sites play a role in the rate of oxygen consumption, the gill-to-palp mass ratio, feeding strategies, or infection rates of *P. faba* in *N. obscurata*?

The research contained in this thesis along with previous studies has begun to shed light on the adaptive capabilities of *N. obscurata*. Whole organisms have the ability to osmoconform within the normal range of ambient seawater as well as osmoregulate at both high and low salinity concentrations. The ability to osmoregulate is important for an organism that has established populations in areas of fresh water influxes and generally in the high intertidal zone in an area with high yearly precipitation, which causes salinity fluctuations. This study indicated that gill tissue of *N. obscurata* increased its rate of oxygen consumption, and therefore, metabolic activity, in a hyposaline environment. This supports
whole organism osmoregulation data, which showed increased hemolymph osmolality in lower salinities.

Interestingly, the metabolic rate observed for the invasive *N. obscurata* was lower than that of the native *L. staminea* in all experimental salinities. This may be advantageous to *N. obscurata* residing in the high intertidal zone. There is limited food available to them thereby lowering their overall metabolic rate. This lower metabolic rate may leave more energy to be allocated to somatic and reproductive growth, which is essential in order to remain a successful invader.

There is possible plasticity in *N. obscurata*’s feeding structures, which may indicate their ability to utilize both filter feeding and suspension feeding techniques. This may facilitate *N. obscurata*’s success in the high intertidal zone that is often devoid of food in the form of suspended particulate matter during low tides. This study showed that the potential plasticity in feeding structures may be due to the area of tidal flat exposed at low tide. *Nuttallia obscurata* residing in the upper limits of the expansive tidal flat located at Birch Bay, WA had larger gill structure, presumably to capture the highest quantity of suspended particulate matter during submersion at high tide. The potential plasticity may be due also to the size and distribution of the sediment. *Nuttallia obscurata* residing at Clayton Beach had a smaller gill-to-palp mass ratio and overall larger palps in comparison to gills than ones residing at Birch Bay did. There was a considerable difference in the sediment composition of both sites, with Clayton Beach composed of coarse sand to fine gravel. Perhaps fine sediment promotes filter feeding and coarse sediment promotes deposit feeding. The difference in the gill-to-palp mass ratio at the two sites seems to support this hypothesis.
*Nuttallia obscurata* residing at Clayton Beach had a high infection rate of *P. faba* in their mantle cavities. *Pinnixa faba*, are a symbiotic organism typically found in the low to mid intertidal zone in the mantle cavities of the horse clam, *T. capax*. There are conflicting studies dealing with the effects of *P. faba* on its host. Some studies indicate a commensal relationship while others indicate a parasitic relationship with adverse effects on the growth and filtration rate of the host as well as deterioration of the gill structures. The current study did not find evidence to support either relationship. It appears that infection in *N. obscurata* may be due to the size of tidal exposure and sediment grain size as well. Birch Bay has fine sediment and a large tidal flat, *P. faba* are known to only inhabit the low to mid intertidal zones and therefore did not infect *N. obscurata* residing in the highest intertidal zone. Clayton beach had a smaller exposed tidal flat and coarse sediment that may have allowed *P. faba* more accessibility to all clams.

It appears that *N. obscurata* is here to stay in the Pacific Northwest, and will likely continue to spread, due to its high abiotic and physiological tolerances. This study has shown that *N. obscurata* have different rates of oxygen consumption in different salinities, the ability to alter the size of their feeding structures and perhaps feeding strategies. These abilities aid *N. obscurata* in successfully inhabiting the high intertidal zone not populated by other bivalve species, which enables them to grow in population size. Further research still needs to be conducted to explore the specific mechanisms that allow *N. obscurata* to be a successful invader.
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