



2010

Broad physiological tolerances of the invasive clam *Nuttallia obscurata*

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**BROAD PHYSIOLOGICAL TOLERANCES OF THE INVASIVE CLAM
*NUTTALLIA OBSCURATA***

by

Zachary C. Siegrist

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

Moheb A. Ghali, Dean of the Graduate School

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MASTER'S THESIS

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Zachary C. Siegrist

February 22, 2010

BROAD PHYSIOLOGICAL TOLERANCES OF THE INVASIVE 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
Zachary C. Siegrist
January 2010

ABSTRACT

Nuttallia obscurata, or the purple varnish clam, is a non-native species that has spread recently and rapidly in the coastal Northeastern Pacific. Attempts at commercial marketing have largely failed. This is in contrast to *Venerupis philippinarum*, the Manila clam, which was accidentally introduced to the region in the 1930s but is now very important to commercial shellfish industry. Finally, *Leukoma staminea* is the local littleneck clam, another popular edible bivalve. These three ecologically important species were studied and their physiological tolerances were compared to help determine why *N. obscurata* is succeeding as an invasive species. To study physiological tolerances, specimens collected from multiple field sites were used in controlled experiments with altered seawater temperature and salinity levels. Gill tissue tolerance was used as a correlate for whole organism tolerance; thus, time to tissue death was evaluated for each species under different conditions. Regressions were used to interpolate specific temperatures at which each species survived for 60 minutes: 41.1 °C for *N. obscurata*, 40.6 °C for *V. philippinarum* and 36.1 °C for *L. staminea*, with *N. obscurata* having the highest overall tolerance to high temperatures. *Nuttallia obscurata* also tolerated significantly lower salinities than the other two clams, with the native *L. staminea* having the least tolerance to decreased salinities. In addition, excised gill tissue of *N. obscurata* survived in a wide range of salinities far longer than did *V. philippinarum* and *L. staminea*. Tissue of *V. philippinarum* and *L. staminea* did not survive for more than 48 hours, while all *N. obscurata* tissue survived for 2+ weeks, even at extremely low salinities. The higher abiotic tolerances of *N. obscurata* may well contribute to its success as an invasive species.

ACKNOWLEDGEMENTS

I would like to thank Western Washington University's Research and Sponsored Programs for providing funding for this project, and my committee members for support over the past two years. In particular, I'd like to thank my advisor, Deb Donovan, for providing me this opportunity and giving me all the help I needed in every stage of this project.

Cold midnight clam digs and countless hours spent excising gill cilia in the lab were significantly shortened by several undergraduate researchers, including Elizabeth Anderson, Tristan Biando, Hailie Colson, Christina Myer, Alecia Pitt and Cristina Wolf. I was also aided on several collection expeditions in the field by other Western Washington University graduate students including Phillip Dugger, Virginia Selz, Austen Thomas, Colin Wahl and Amanda Winans. Rachel Allee was also particularly helpful throughout this project as we continually helped each other with research ideas, fieldwork and statistical analysis with our very similar theses. Jeannie Gilbert and Peter Thut provided essential support from the Biology stockroom, and Stefan Freelan from WWU's Huxley College provided me with several great custom-made regional maps.

Finally, I'd like to thank my family for steadfast support, keeping me sane and being there whenever needed.

TABLE OF CONTENTS

ABSTRACT.....	iv
ACKNOWLEDGEMENTS.....	v
LIST OF FIGURES	viii
LIST OF TABLES.....	ix
INTRODUCTION	1
Overview.....	1
Study species.....	3
Experimental approach	6
METHODS	8
Collection of animals and study sites.....	8
Gill tissue assay.....	11
Temperature tolerance	13
Salinity tolerance	16
Long-term survivorship	17
RESULTS	19
Site parameters.....	19
Temperature tolerance	21
Salinity tolerance	26
Survivorship of gill tissue over time.....	29
DISCUSSION.....	31
Broad physiological tolerances	31

Temperature	31
Salinity	35
Survivorship.....	41
<i>Nuttallia obscurata</i> as an invasive species	42
Further investigations and recommendations	45
LITERATURE CITED	48

LIST OF FIGURES

- Figure 1. Map of Northwest Washington state displaying all three study sites. From north to south, sites are Birch Bay, Mud Bay and Padilla Bay. Source: Stefan Freelan, Huxley College, WWU..... 10
- Figure 2. Diagram of gill tissue excision procedure. Clockwise from upper left: 1) Clam removed from acclimation aquaria. 2) Clam cut open with scalpel; tweezers and scissors used to isolate and remove each of four gill lobes per individual. Two lobes are indicated in upper right photo. 3) Gill lobes are spread out in petri dish. 4) Gills cut into strips using razor blade and scalpel. Technique averages 20-30 gill pieces per clam..... 12
- Figure 3. A picture of the temperature-controlled water baths used in the experiment: 1) Water bath; 2) Erlenmeyer flask suspended in water bath; 3) Air stone to circulate water and ensure even temperatures; 4) Water heater with looped heating coil resting on bottom of water bath, linked to 5) Thermoregulator; 6) External thermometer to allow temperature adjustments. 15
- Figure 4. Natural log of survival time in minutes of each species at temperatures ranging from 35.5 °C to 42 °C. Each data point represents the natural log of an average time to death for all gill tissue in a water bath, not per individual clam. The results from all sites were combined. Regression lines (Table 2) are surrounded by 95% confidence intervals. The ln(60 minutes) line provides a visual representation of the interpolations in Table 2. 22
- Figure 5. Correlations between time to death and clam length. Each data point represents one individual. Correlation coefficients listed at right of corresponding lines..... 25
- Figure 6. Gill ciliary activity levels after 24 h of exposure to environments of varying salinity. Activity level is from 0-3, where 3 is fully active and 0 is total death. Error bars represent \pm one standard deviation (n = 9 per bar; 3 per salinity level per site per species). Data from all sites have been combined in this figure..... 27
- Figure 7. Gill ciliary activity levels after 24 h exposure to environments of varying salinity, with distinct results from each site shown (n = 3 per bar). Error bars represent \pm one standard deviation. 28
- Figure 8. Commercially-sold *N. obscurata* mixed with *V. philippinarum* in a market on Granville Island, Vancouver, British Columbia. 47

LIST OF TABLES

Table 1. Descriptive measures taken at each study site on multiple collection days. Approximate intertidal locations are self-defined. Temperatures were not taken on every collection trip. Collection depth and salinity measurements were taken on every collection trip. Error represents \pm one standard error.	20
Table 2. Regressions calculated for each study species, where s represents survival time in minutes and t represents temperature in degrees Celsius. The column at farthest right shows the interpolated temperature at which gill tissue of each species can survive for 60 minutes.	22

INTRODUCTION

Overview

Every year, marine and estuarine species are introduced into United States waters; many have little or no ecological effect, but some have the potential to affect ecosystems, fisheries, human infrastructure and human health on a large scale (Padilla and Williams, 2004; Ray, 2005). More than 500 species of marine invaders have been established in coastal waters of the U.S., and impacts of invasive species are predicted to continue to grow as human globalization increases and more non-indigenous species are established in new areas (Grosholz and Tilman, 2005; Sakai *et al.*, 2001). The costs of marine invasive species in the United States have been estimated at well over \$1 billion; however, because much less is currently known about the extent and effects of marine invasives than about their terrestrial counterparts, the true economic costs may be far greater (Bax *et al.*, 2001; Pimentel *et al.*, 2000).

In the Northeastern Pacific, as in other cool-temperate coastal environments around the world, the number of invasive marine and estuarine species is increasing at an exponential rate (Ruiz *et al.*, 2000; Wonham and Carlton, 2005). According to Molnar *et al.* (2008), the temperate Northern Pacific Ocean has one of the highest degrees of invasion in the world. More specifically, the Northeastern Pacific has the fourth highest documented number of harmful marine invasive species of any region on Earth, with 41 different species classified as those which disrupt multiple other species or wider ecosystems (Molnar *et al.*, 2008). Estuarine zones along the Pacific Coast have significantly higher numbers of invasive species than does the open coast, due to their geologically-young age and high anthropogenic

influence (Wasson *et al.*, 2005). These estuarine areas have a number of different invasive species transport vectors. The most common invasion pathways include shipping, aquaculture and shellfish and finfish imports; the majority of invaders are marine invertebrates, of which a large number are molluscs (Molnar *et al.*, 2008; Ruiz *et al.*, 2000; Wonham and Carlton, 2005). Invasive species survive due to a variety of different potential factors, including broad physiological tolerances (Lee and Bell, 1999; Mann and Harding, 2003; Marchetti *et al.*, 2004; Ray, 2005). Here, tolerance is defined as the ability to withstand different environmental conditions; an organism with greater tolerances can more easily survive environmental fluctuations while avoiding stress or death.

Estuarine and intertidal zones typically have greatly varying abiotic characteristics, including large fluctuations in temperature and salinity (Helmuth and Hofmann, 2001; Kneib, 1984; Tomanek and Helmuth, 2002). These large fluctuations are found in both hard-substrate (e.g., rocky intertidal) and soft-substrate (e.g., tidal mudflat) environments (Kneib, 1984). Although the rocky intertidal often poses the most striking examples of abiotic fluctuation, soft-substrate intertidal ecosystems are also exposed to extreme and sometimes unpredictable abiotic variations (Kneib, 1984). Species distribution in such soft-substrate intertidal environments is determined in large part by temperature and salinity, as well as other factors such as predation, which is also influenced by various abiotic factors (Gunter, 1961; Seitz *et al.*, 2001, Vernberg *et al.*, 1963). Organisms that can tolerate fluctuations in temperature and salinity will likely survive better than more limited-tolerance species in estuarine and intertidal zones; temperature and salinity gradients within an environment may

also determine species survivability (Boesch *et al.*, 1976; Jassby *et al.*, 1995; Peck *et al.*, 2004).

Study species

In Northeastern Pacific estuarine and intertidal areas, there are a variety of commercially important bivalve species, among them the native local littleneck clam, *Leukoma staminea* (formerly *Protothaca staminea*), and the nonnative Manila clam, *Venerupis philippinarum*. The latter was accidentally introduced to the region in the 1930s and now accounts for half of hard-shell clam commercial harvest in the state of Washington, while the former is a favorite of amateur harvesters but is still important to commercial industry (Washington Department of Fish and Wildlife, 2000).

Within the last two decades, the introduced Japanese purple varnish clam, *Nuttallia obscurata* (syn. *Nuttallia olivacea*), has become one of the dominant bivalves in the intertidal sands of coastal Oregon to British Columbia (Dudas and Dower, 2006; Ray, 2005; Roth, 1978). This species is native to northern Asia (Korea, China, Japan and Russia), where it lives in a wide range of environments, including oceanic areas, low-salinity estuaries and even brackish lakes (Kolpakov and Kolpakov, 2005). Tomiyama and Ito (2006) report the varnish clam living in areas experiencing fluctuations from 5 to 27 °C, and from 1 to 30 PSU (approximately 1 to 30 ppt); in two other studies, *N. obscurata* was collected from Japan's Gamo Lagoon, an estuary with salinities ranging from near 0 to over 30 PSU (Kanaya *et al.*, 2005; Kanaya *et al.*, 2007). The varnish clam was first reported in the northeastern Pacific in 1991 and was likely introduced into British Columbia via ballast water (Dudas, 2005; Mills,

2002). Since its introduction near Vancouver, B.C., it has spread rapidly, expanding over 500 km and reaching densities of over 800 per m² (Dudas, 2005).

Specific impacts of *N. obscurata* on Northeastern Pacific ecosystems are still largely unknown, but previous research has shown that a variety of native predators prey on the varnish clam, potentially affecting local population dynamics (Department of Fisheries and Oceans, 2001). In addition, the high densities attained by *N. obscurata* may significantly affect sediment composition and nutrient fluxes (Dudas *et al.*, 2007). The varnish clam frequently occurs on Manila clam farms and has raised industry concerns that it might compete with or displace the commercially-important Manila clam; however, there has so far been little evidence to support this (Dudas, 2005).

Limited commercial trials were conducted with *N. obscurata*, marketed as the “Savoury Clam,” and recreational harvesting is allowed in Washington State; however, it is unlikely that a commercial fishery will be created in Washington in the near future, due to a variety of reasons (Gillespie *et al.*, 2001; Meacham, 2008; Ray, 2005). Larger *N. obscurata* are prone to infestation by the pea crab *Pinnixia faba*, which may be unappetizing to the consumer as well as dangerous to those with crustacean allergies (Gillespie, 1999). Size limits may help curtail crab infestation, but harvesting smaller *N. obscurata* may lead to incidental harvest of undersize *V. philippinarum*. It is also unknown if smaller *N. obscurata* are as marketable as larger individuals. Vendors are hesitant to develop a market without assurance of a consistent supply (Meacham, 2008).

All three bivalves – *N. obscurata*, *L. staminea* and *V. philippinarum* – prefer partly sandy, low wave-energy environments (Byers, 2005). There are competition effects between

N. obscurata and *V. philippinarum*, with the varnish clam having a competitive advantage for space in the high intertidal zone and Manila clams having the advantage in the mid-intertidal zone (Department of Fisheries and Oceans, 2001; Yates, 1999). It is currently unknown why *N. obscurata* has an advantage over *V. philippinarum* in the high intertidal, but may be due to superior burrowing ability, reproductive capability or abiotic tolerances (Dudas, 2005; Ray, 2005). It appears that *N. obscurata* will not displace *V. philippinarum* in an undisturbed environment, but may increase in abundance after a *V. philippinarum* harvest, consequently reducing subsequent *V. philippinarum* populations (Gillespie, 1999).

Because *N. obscurata* is a fairly recent arrival to the Northeastern Pacific, there has been little local research done on it. Larval ecology, adult population dynamics and ecological interactions with native species have been investigated (Dudas, 2005; Dudas and Dower, 2006; Dudas *et al.*, 2007). Byers (2002) studied how physical habitat preferences mediate biotic resistance to *N. obscurata* invasion; Marshall *et al.* (2003) examined parasitic and symbiotic fauna present in *N. obscurata*. However, no studies have examined temperature and salinity tolerances of adult *N. obscurata*. By determining *N. obscurata* temperature and salinity tolerances, by studying interactions between temperature and salinity and by comparing the tolerances of *N. obscurata* relative to those of *L. staminea* and *V. philippinarum*, important information may be gained as to how temperature and salinity variation might influence habitat and competition of these three prevalent Northeastern Pacific bivalves. In turn, this information may aid future attempts to manage and control *N. obscurata* populations and their effects on local, commercially-important species.

Experimental approach

The goal of this project was to compare temperature and salinity tolerances of *N. obscurata*, *L. staminea* and *V. philippinarum* collected from several locations in Northwest Washington. Survivorship of *N. obscurata* gill tissue in varying salinities was also studied. Pilot studies were run to determine appropriate temperature and salinity ranges for each species. All experiments used procedures pioneered by Vernberg *et al.* (1963), in which bivalve gill tissue is used as a proxy for the entire organism. In these procedures, the organism's gill tissue is excised and cut into strips, which are then exposed to seawater of controlled temperature or salinity. Gill ciliary activity may be compared across species, sites and other experimental factors. Using this approach, time until cell death can be accurately measured, and is indicated by total cessation of gill ciliary movement. Gill ciliary activity can be considered a correlate for whole organism tolerances and provides an accurate way of measuring tolerance adaptation at the cellular level (Vernberg *et al.*, 1963).

Likewise, an organism's lethal temperatures can directly correlate to conditions it experiences in its normal environment (Hodgson, 1999). Numerous examples of this have been found in marine ecosystems. Examples include multiple species of porcelain crab that show significant correlations between upper lethal temperature and maximal habitat temperature (Somero, 2002) and several Chesapeake bivalves whose lethal temperatures reflect habitat location and upper temperature tolerances (Kennedy and Mihursky, 1971). Although the lethal temperatures of our study were higher than those typically found in the local environment, they can therefore still be directly correlated to environmental temperatures and habitat preference of the three study species.

There were three hypotheses considered:

- *Nuttallia obscurata* gill tissue will have significantly higher temperature tolerance than gill tissue of *V. philippinarum*; likewise, *V. philippinarum* gill tissue will have a higher temperature tolerance than gill tissue of *L. staminea*.
- *Nuttallia obscurata* gill tissue will have a higher tolerance for low salinities than that of the other study species.
- *Nuttallia obscurata* gill tissue will survive for a longer period of time in different salinities relative to the other study species.

These hypotheses were based both on previous studies and on research suggesting that effective invasive species often have broad abiotic tolerances (Lee and Bell, 1999; Mann and Harding, 2003; Marchetti *et al.*, 2004). Other factors such as site were not expected to yield any significant differences in physiological tolerance, as a common laboratory acclimation period used for all three species should standardize tolerance responses.

METHODS

Collection of animals and study sites

Based on preliminary research and availability of all three species, three Northwest Washington sites were selected for use in this study: Padilla Bay (48.489° N 122.483° W; accessed at Bay View State Park), Mud Bay (48.701° N 122.499° W; part of Chuckanut Bay) and Birch Bay (48.904° N 122.771° W; accessed at Birch Bay State Park) (Figure 1). Specimens of *Leukoma staminea*, *Venerupis philippinarum* and *Nuttallia obscurata* were collected at each site in different quantities, depending on experimental need.

Each site was visited multiple times at low tide between July 2008 and January 2009. Within this time frame, three distinct experiments were run consecutively, so that clams used in a single experiment were collected only during a two to three month window, rather than spread throughout the entire seven-month period. It was not possible to collect clams from all three sites in a single day, but efforts were made to collect from the three sites during a tidal cycle. Clams were collected using shovels and hands, and were typically found in the top 15 cm of substratum. Upon removal, the clams were placed in a bucket containing enough seawater to provide a covering layer. The specimens were then brought back to Western Washington University and were rinsed under cold tap water to remove any standing mud and debris, after which they were placed into aerated 10-gallon laboratory aquaria maintained at 15 °C at approximately 30 ppt salinity. Aquaria were filled with unfiltered seawater collected at Shannon Point Marine Center in Anacortes, WA.

During each collection, several environmental parameters were recorded at each site. For the first few collections, the approximate distance from shore at which each clam species

was found was recorded using transect tape; further collections at the same site always took place in the same general area. It was impossible to determine specific tidal heights at all three sites, so distance to shore was recorded to get relative location estimations for the three species. Shore was self-defined as the apparent high tide line. Average clam depth in the substratum was estimated using a ruler as visual aid. Substratum temperature was recorded when possible by inserting a temperature probe into the substratum at the approximate depth and location of each species at each site. Air temperature was recorded at approximately one meter above ground. General observations about weather conditions and substratum were recorded at every collection site. Finally, two pore water samples were collected from each species' collecting area and were brought back to the lab. They were later centrifuged at 2500 rpm for 6 minutes using an IEC Centra CL2 centrifuge to separate out any solid debris, after which the water samples' salinities (parts per thousand) were determined using a salinity refractometer (RHS-10ATC, Tianjin City Taisite Instrument Co., Ltd).

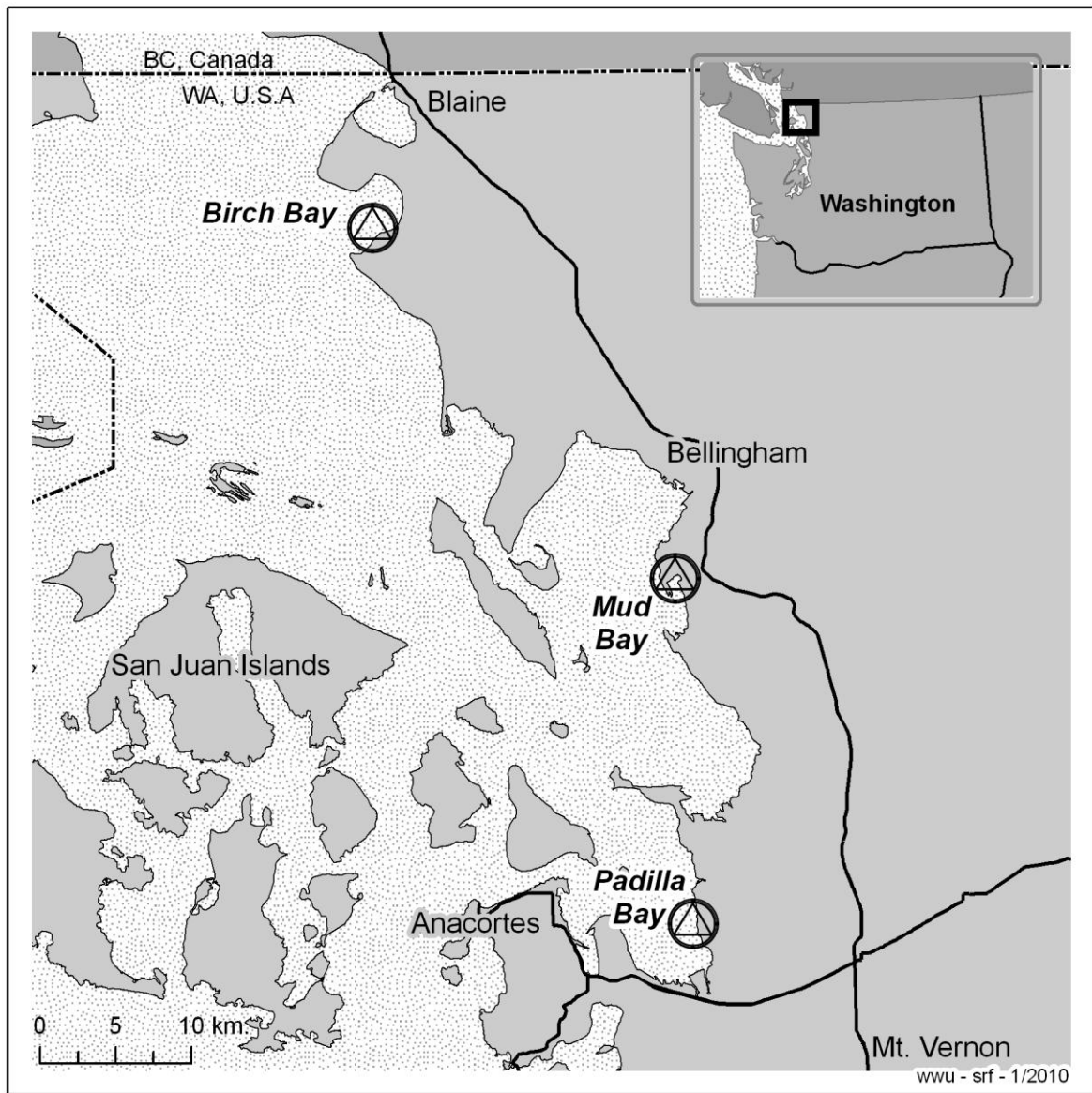


Figure 1: Map of Northwest Washington state displaying all three study sites. From north to south, sites are Birch Bay, Mud Bay and Padilla Bay. Source: Stefan Freelan, Huxley College, WWU.

Gill tissue assay

All clams were maintained in 15 °C temperature-controlled seawater aquaria with pump and filter for 5-7 days, in order to ensure laboratory acclimation. Previous studies with other salt water molluscs have shown that significant acclimation occurs within 3-5 days (Vernberg *et al.*, 1963). Thus, a five day acclimation period was assumed to be enough time to account for any temporal or spatial environmental differences between collection times. Aquaria typically contained 20-60 clams of multiple species, resting on the glass bottom of the tank. Clams were not fed prior to experimental trials.

To determine gill ciliary activity, haphazardly-selected clams were removed from the collected population. Figure 2 provides a diagram of the gill excision procedure used in all experiments. The clams were opened by inserting a blunt scalpel between the valves and cutting both adductor muscles. The gills were removed with scissors and probe, and then placed in individual Petri dishes, one clam per dish. Using a razor blade and scalpel, the gills were cut into ~1-2 mm wide strips, running along the length of the gills, with terminal gill cilia at one end of each strip. This yielded approximately 20-30 total gill pieces per clam, which were then ready for further experimentation. All three species were the same general size and yielded similar sized and shaped gill tissue strips. In addition, the shell length (in millimeters) of each clam was recorded using calipers, where length was considered the longest distance of the shell from anterior to posterior.

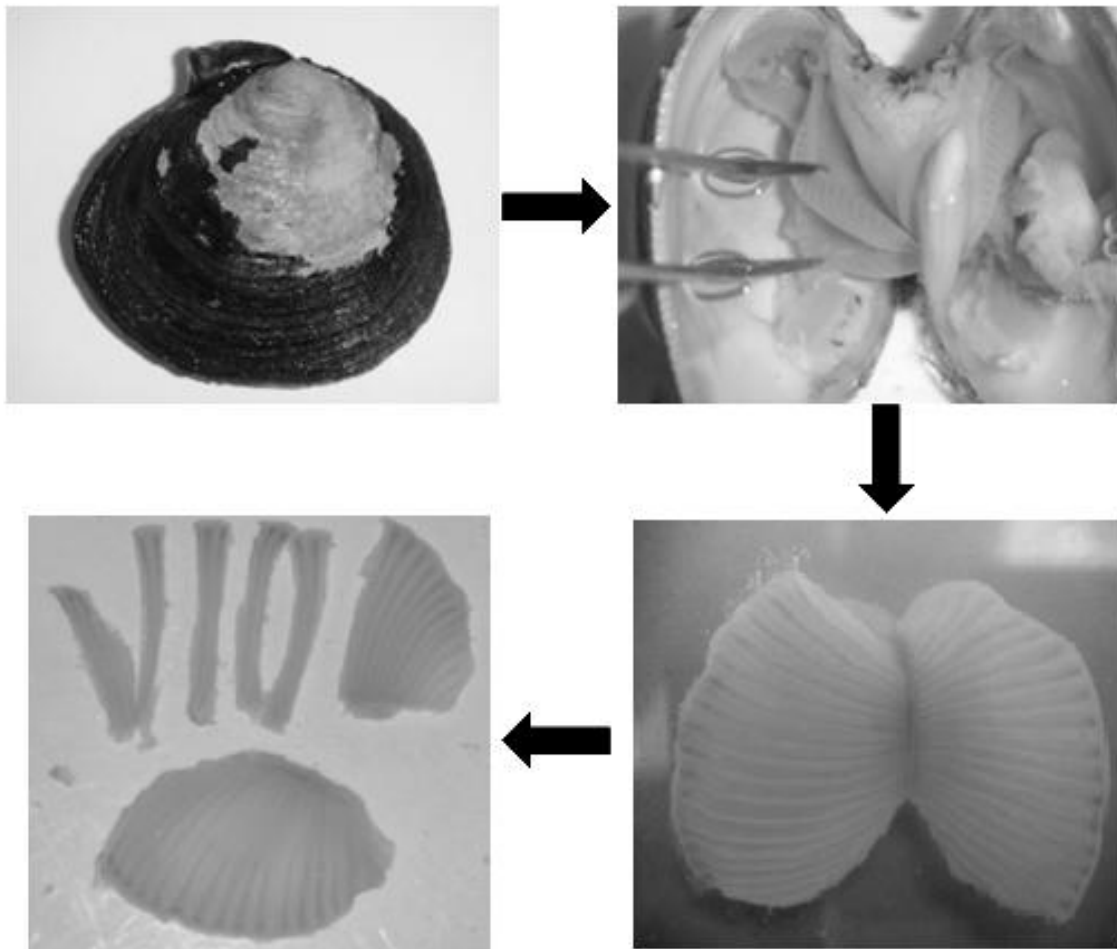


Figure 2: Diagram of gill tissue excision procedure. Clockwise from upper left: 1) Clam removed from acclimation aquaria. 2) Clam cut open with scalpel; tweezers and scissors used to isolate and remove each of four gill lobes per individual. Two lobes are indicated in upper right photo. 3) Gill lobes are spread out in petri dish. 4) Gills cut into strips using razor blade and scalpel. Technique averages 20-30 gill pieces per clam.

After exposure to seawater of varying temperature or salinity (described below), pieces of gill tissue were removed and observed under a compound light microscope at magnifications of 40, 100 and 400x. Terminal cilia activity was observed and rated on a qualitative scale, with 3 being most active and 0 being completely inactive, as described by Vernberg *et al.* (1963).

Temperature tolerance

Appropriate experimental temperature ranges for each species were determined by a preliminary study. Three different temperatures per species were chosen for which the gill cilia activity lasted longer than approximately 15 minutes and less than approximately two hours. The temperatures established for *L. staminea* gill tissue were 35 °C, 35.5 °C and 36 °C; temperatures for *V. philippinarum* gill tissue were 40 °C, 40.5 °C and 41 °C; and temperatures for *N. obscurata* gill tissue were 41 °C, 41.5 °C and 42 °C. These temperatures produced a common time frame for isolated gill cilia to survive, over which the three clam species could be compared.

For the temperature tolerance experiment, five clams from each of the three species collected from each of the three sites were tested at each temperature, with 135 total clams used. Most clams for this experiment were collected in July and August 2008; two *L. staminea* were collected in November 2008. Individual clams were haphazardly selected from the laboratory aquaria and assigned to different temperatures. To determine the temperature tolerance of gill tissue of the three bivalve species, multiple temperature-controlled water baths were used with one or two 500 ml Erlenmeyer flasks containing

approximately 200 ml seawater suspended in each bath (Figure 3). VWR Brand thermometers precise to 0.01 °C were used to monitor the water bath temperature. All gill pieces from each individual clam were placed into an Erlenmeyer flask, with only one clam's gill pieces going into each flask. Flasks were stoppered to prevent evaporative water loss and were submerged into the bath.

At approximately five-minute intervals, one gill strip from a flask was removed via pipette, placed onto a slide and observed under a compound microscope. Ciliary movement was observed and rated on the 0-3 qualitative scale. After observation, the gill piece was discarded. After each time interval, another gill strip from the flask was removed and observed. For temperatures at which the organism was expected to survive for a relatively long period of time, initial time intervals were longer than five minutes in order to maintain enough gill pieces to continue the experiment to its conclusion. When ciliary activity began to decline, the frequency of observations was increased until all cilia were inactive. Upon finding no active cilia, at least two more gill pieces were removed and observed to ensure tissue death across multiple gill pieces. Finally, survival time in minutes was recorded. Multiple water baths with multiple Erlenmeyer flasks were often running at the same time; gill pieces were placed into their respective flasks at staggered start times to facilitate observation and measurement.

Two-way ANOVAs run with the statistical program SPSS, versions 15.0 and 16.0, were used for data analysis. Time to death per water bath was used as the dependent variable; if a water bath contained two separate Erlenmeyer flasks, the data from each flask were averaged to provide one datum per bath. This yielded 28 data points for *L. staminea*, 27

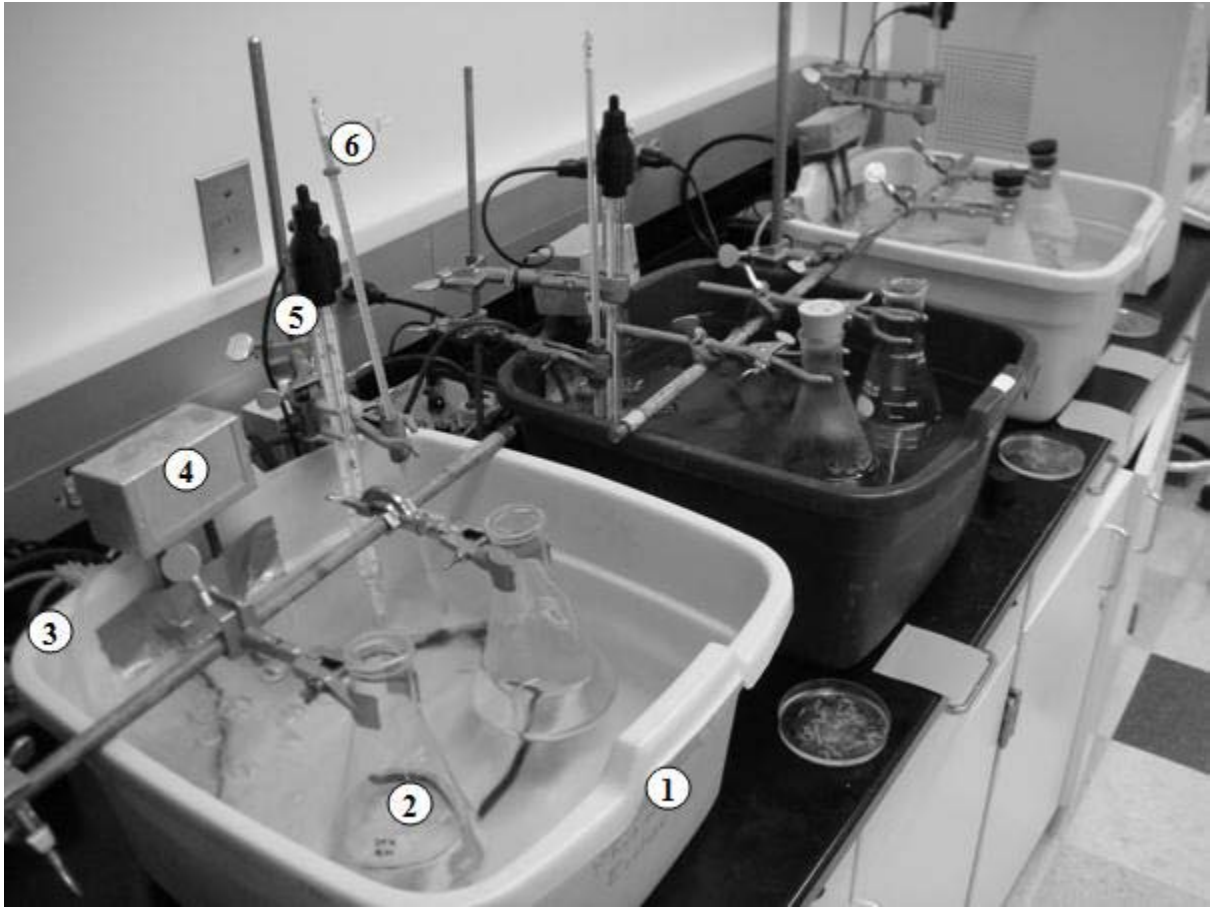


Figure 3: A picture of the temperature-controlled water baths used in the experiment: 1) Water bath; 2) Erlenmeyer flask suspended in water bath; 3) Air stone to circulate water and ensure even temperatures; 4) Water heater with looped heating coil resting on bottom of water bath, linked to 5) Thermoregulator; 6) External thermometer to allow temperature adjustments.

data points for *V. philippinarum* and 27 data points for *N. obscurata*. Site and temperature factors were analyzed, as well as site-temperature interactions. Clam length was also analyzed as a covariate. In addition, the data was natural log transformed and regressions were run to yield linear equations for each species, allowing for direct comparisons between the three species.

Salinity tolerance

To test the tolerance of the three clam species to a range of salinities, baths with different salinities were created by mixing seawater (~30 ppt) with de-ionized water while using a refractometer to attain salinity levels to a precision of ~0.1 ppt. Salinities of 30, 20, 10, 5, 3 and 1 ppt were used. Water was then stored in 2 liter flasks in a 10 °C cold room for at least 24 hours in order to attain temperature equilibrium. Discrepancy between acclimation at 15 °C and experimentation at 10 °C is due to lack of space and inadequate cold room facilities; it was estimated that this should not significantly affect experimental results.

Clams for this experiment were collected in October and November 2008. Eighteen haphazardly-selected clams of each of the three species were removed from the laboratory aquaria after at least five days of acclimation time. Three clams from each of the three species collected at each of the three sites were used in each of the six salinities, with 162 total clams used. Gill pieces were removed from each clam and placed into 18 individual 500 ml Erlenmeyer flasks filled with approximately 200 ml of the temperature-equilibrated water, with three flasks per salinity. Gill pieces were then placed into each flask using a

pipette, with all gill pieces from one individual clam going into an individual flask. All Erlenmeyer flasks were stoppered and held in a 10 °C cold room for 24 hours, after which multiple gill pieces from each flask were removed and observed. Gill ciliary activity levels were recorded on the same qualitative scale (0-3) used in the temperature experiments. Ciliary activity levels of each gill piece observed from one clam were averaged, yielding one value per individual clam at that salinity.

Three-way ANOVAs run with the statistical program SPSS, versions 15.0 and 16.0, were used for data analysis. Site, species and salinity factors were analyzed, as well as site-species, site-salinity, species-salinity and site-species-salinity interactions, with ciliary activity level as the dependent variable. Clam length was also analyzed as a covariate.

Long-term survivorship

To determine the length of time that gill tissue from *N. obscurata* can survive at different salinities, *N. obscurata* (n = 18) were collected from Mud Bay in February 2009 and acclimated for approximately five days. *Nuttallia obscurata* gill tissue was then removed, isolated and placed into Erlenmeyer flasks containing 200 ml of water at a range of salinities (30, 20, 10, 5, 3 and 1 ppt; 3 clams per salinity) as described above. Each week, several gill pieces from each individual were removed and observed, and ciliary activity levels were recorded. Once one gill piece was observed, it was removed from the experiment and discarded. Ciliary activity was checked once per week, with observation of gill pieces in each flask continuing for one week after cessation of ciliary activity in that specific flask;

ciliary cessation was confirmed by checking multiple tissue pieces for any sign of activity.
There was no statistical analysis of the survivorship experiment, only qualitative description.

RESULTS

Site parameters

Ambient temperatures at the three sites differed across time. When the date of collection was included as a covariate in an analysis of sediment temperature across site and species location, no significance of site ($F_{1,36} = 0.934$, $p = 0.340$; ANCOVA) nor species location within a site ($F_{1,36} = 0.011$, $p = 0.917$; ANCOVA) was found. Since collection date was most likely a source of significant variation of on-site temperature sampling, further data analysis involving temperature used a combined data set from all three sites. Within species, no two species were found to significantly differ with respect to sediment temperature recorded (Tukey HSD). General descriptive statistics for each site can be found in Table 1.

Significant differences were found among sites with respect to salinity ($F_{2,39} = 14.616$, $p < 0.001$; ANOVA), but not among species ($F_{2,39} = 1.953$, $p = 0.155$; ANOVA). Including date of collection as a covariate had no effect; site remained significant ($F_{2,38} = 12.783$, $p < 0.001$; ANCOVA) while species location was not significant ($F_{2,38} = 2.083$, $p = 0.139$; ANCOVA). Mean pore water salinity of Padilla Bay was 27.6 ± 0.5 ppt, Mud Bay was 24.9 ± 0.5 ppt, and Birch Bay was 28.9 ± 0.6 ppt (error represents one standard error). More specifically, Mud Bay salinities were significantly lower than those of the other two sites ($p = 0.001$ with Padilla Bay, and $p < 0.001$ with Birch Bay; Tukey HSD), while salinity at the other two sites did not significantly differ from each other ($p = 0.214$).

Table 1: Descriptive measures taken at each study site on multiple collection days. Approximate intertidal locations are self-defined. Temperatures were not taken on every collection trip. Collection depth and salinity measurements were taken on every collection trip. Error represents \pm one standard error.

Birch Bay	Approximate intertidal location	Average collection depth (cm)	Average sediment temperature (°C)	Average pore water salinity (ppt)
<i>L. staminea</i>	mid intertidal	12.7 \pm 2.5	14.5 \pm 0.2	29.8 \pm 0.5
<i>V. philippinarum</i>	mid-high intertidal	8.3 \pm 1.9	16.4 \pm 0.1	28.9 \pm 1.0
<i>N. obscurata</i>	high intertidal	10.8 \pm 0.6	17.5 \pm 0.4	28.0 \pm 1.4
Mud Bay				
<i>L. staminea</i>	mid intertidal	14.8 \pm 1.9	17.3 \pm 1.5	25.8 \pm 2.6
<i>V. philippinarum</i>	mid intertidal	13.6 \pm 2.6	17.3 \pm 1.5	26.0 \pm 2.8
<i>N. obscurata</i>	high intertidal	8.5 \pm 3.2	18.3 \pm 2.5	22.9 \pm 2.7
Padilla Bay				
<i>L. staminea</i>	mid intertidal	6.7 \pm 4.5	17.9 \pm 0.9	27.9 \pm 1.1
<i>V. philippinarum</i>	mid intertidal	10.8 \pm 0.9	17.8 \pm 0.9	26.6 \pm 2.6
<i>N. obscurata</i>	high intertidal	12.1 \pm 2.7	17.8 \pm 0.2	28.2 \pm 1.3

Temperature tolerance

Each species experienced a decline in survival time as temperature increased. Temperature significantly affected time to death of *L. staminea* ($F_{2,19} = 64.580$, $p < 0.001$; ANOVA), *V. philippinarum* ($F_{2,18} = 24.077$, $p < 0.001$; ANOVA) and *N. obscurata* ($F_{2,18} = 46.500$, $p < 0.001$; ANOVA; Figure 4). In addition, within the three-temperature range used for each species, each temperature yielded significantly different times to death ($p \leq 0.025$ for all three species; Tukey HSD).

The temperature tolerances of the three clam species could not be statistically compared to each other due to different temperature ranges for each species. However, Figure 4 allows a visual comparison by plotting results of all species on the same graph. Regressions were used to calculate equations for each species, using the formula $\ln(s) = y_0 + m*t$ (Table 2), where s represents survival time, y_0 represents the y-intercept, m represents the slope and t represents temperature. A single time value can then be input into each equation, yielding the temperature at which each species can survive for that specific length of time (Table 2). Thus, using regression gives a convenient comparison between the three species. Subsequent data interpolation showed that *N. obscurata* can survive for 60 minutes at the highest temperature (41.1 °C), *V. philippinarum* can survive at 40.6 °C for 60 minutes and *L. staminea* can last 60 minutes at 36.1 °C.

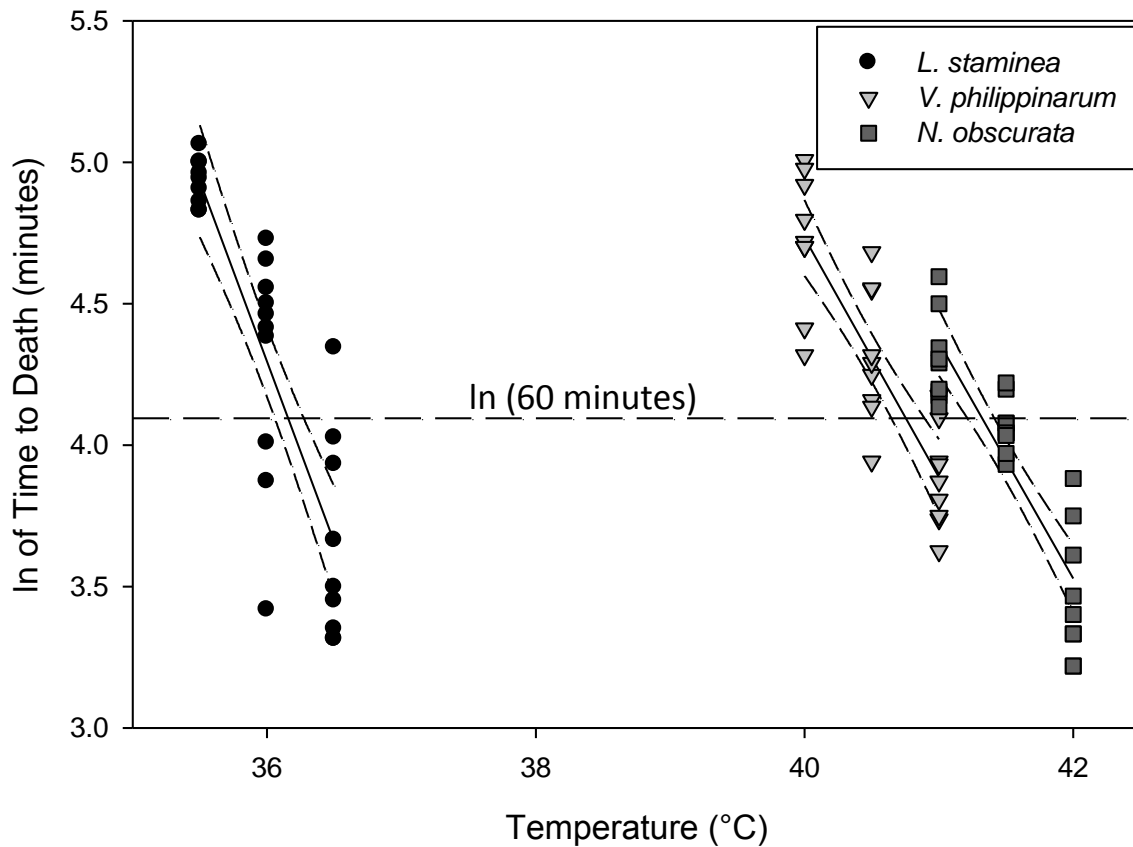


Figure 4: Natural log of survival time in minutes of each species at temperatures ranging from 35.5 °C to 42 °C. Each data point represents the natural log of an average time to death for all gill tissue in a water bath, not per individual clam. The results from all sites were combined. Regression lines (Table 2) are surrounded by 95% confidence intervals. The ln(60 minutes) line provides a visual representation of the interpolations in Table 2.

Table 2: Regressions calculated for each study species, where s represents survival time in minutes and t represents temperature in degrees Celsius. The column at farthest right shows the interpolated temperature at which gill tissue of each species can survive for 60 minutes.

Species	Equation	r^2	Temperature at a survival time of 60 min
<i>L. staminea</i>	$\ln(s) = 50.3 - 1.28*t$	0.74	36.1
<i>V. philippinarum</i>	$\ln(s) = 38.6 - 0.85*t$	0.74	40.6
<i>N. obscurata</i>	$\ln(s) = 38.6 - 0.84*t$	0.78	41.1

Figure 5 shows the correlations between clam length and time to death; clam length was examined as a covariate and found to be non-significant for each species (*L. staminea*: $F_{1,18} = 0.988$, $p = 0.333$; *V. philippinarum*: $F_{1,17} = 1.385$, $p = 0.255$; *N. obscurata*: $F_{1,17} = 0.166$, $p = 0.689$; ANCOVA). Clam lengths of *L. staminea* averaged 45.0 ± 6.0 mm, lengths of *V. philippinarum* averaged 47.3 ± 4.8 mm and lengths of *N. obscurata* averaged 49.2 ± 4.9 mm (error represents one standard deviation).

Collection site was also found to be non-significant with respect to temperature tolerance of each species (*L. staminea*: $F_{2,19} = 1.870$, $p = 0.181$; *V. philippinarum*: $F_{2,18} = 0.685$, $p = 0.517$; *N. obscurata*: $F_{2,18} = 1.658$, $p = 0.218$; ANOVA).

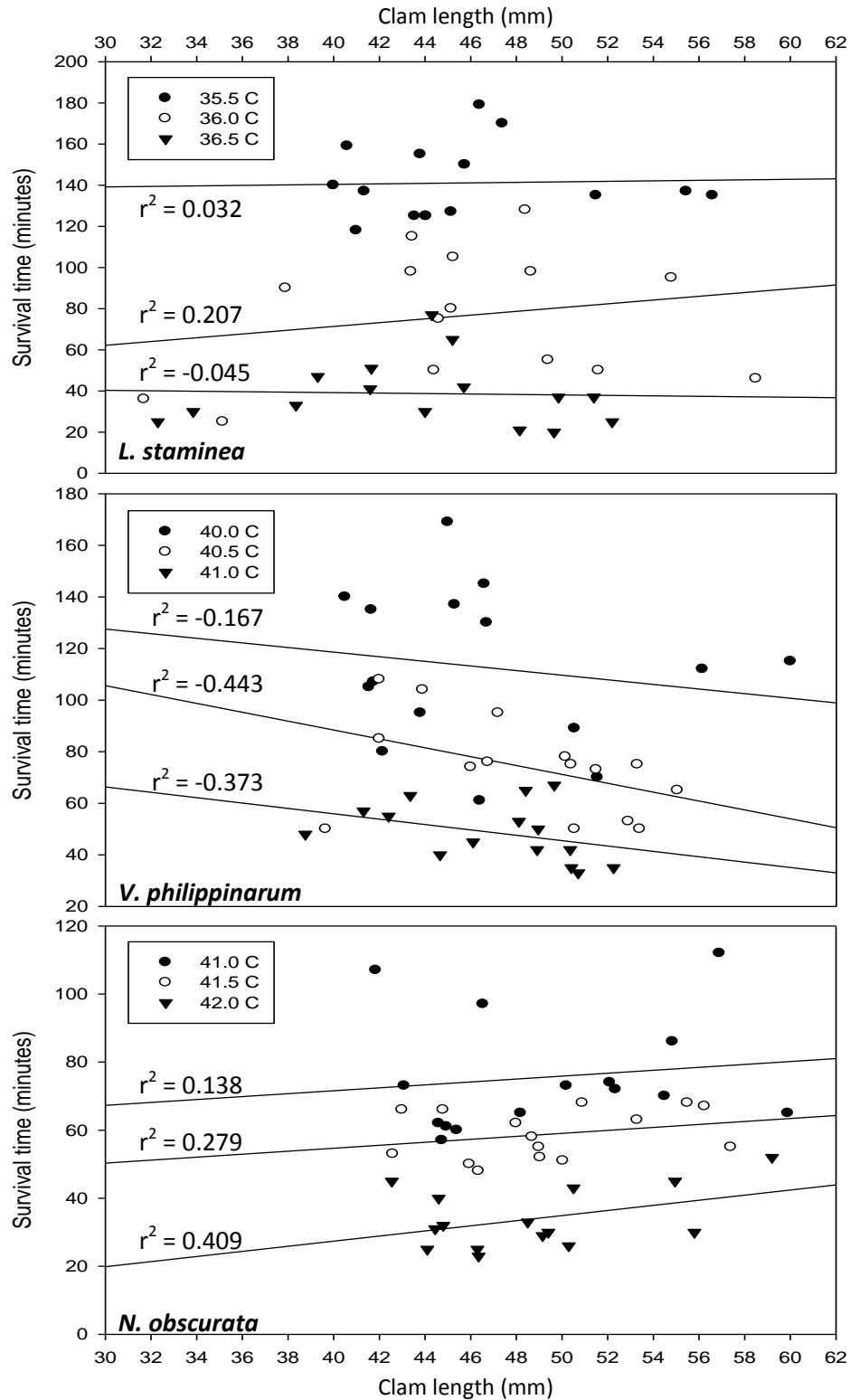


Figure 5: Correlations between time to death and clam length. Each data point represents one individual. Correlation coefficients listed at right of corresponding lines.

Salinity tolerance

Salinity significantly affected gill ciliary activity levels after 24 h of exposure to different salinities ($F_{5,108} = 85.705$, $p < 0.001$; ANOVA; Figure 6), with activity levels dropping consistently with salinity decline. Differences among collection sites ($F_{2,108} = 4.749$, $p = 0.011$; ANOVA) and species ($F_{2,108} = 243.122$, $p < 0.001$; ANOVA) were also significant. There were also significant interactions between species and salinity levels ($F_{10,108} = 22.038$, $p < 0.001$; ANOVA), site and salinity levels ($F_{10,108} = 2.549$, $p = 0.008$; ANOVA) and site and species ($F_{4,108} = 3.770$, $p = 0.007$; ANOVA).

Species-salinity interactions were supported by Tukey HSD post-hoc tests, which revealed that all three species significantly differed from each other with respect to gill ciliary activity in the different salinities ($p < 0.001$ for all three species). Padilla Bay individuals yielded significantly higher ciliary activity levels at lower salinities than individuals taken from the other two sites. Ciliary activity levels from Padilla Bay specimens differed from those of Birch Bay ($p = 0.038$) and those of Mud Bay ($p = 0.016$); Birch Bay and Mud Bay individuals did not have significantly different ciliary activity levels with respect to salinity ($p = 0.944$). Figure 7 shows the gill ciliary activity levels of each species (*L. staminea*, *V. philippinarum* and *N. obscurata*, respectively) by site and salinity.

Figure 6 shows that on the whole, *L. staminea* gill tissue was least able to survive in lower salinities, followed by tissue of *V. philippinarum*. *Leukoma staminea* gill cilia begin dropping in activity level at salinities of 20 ppt and below, while *V. philippinarum* gill cilia

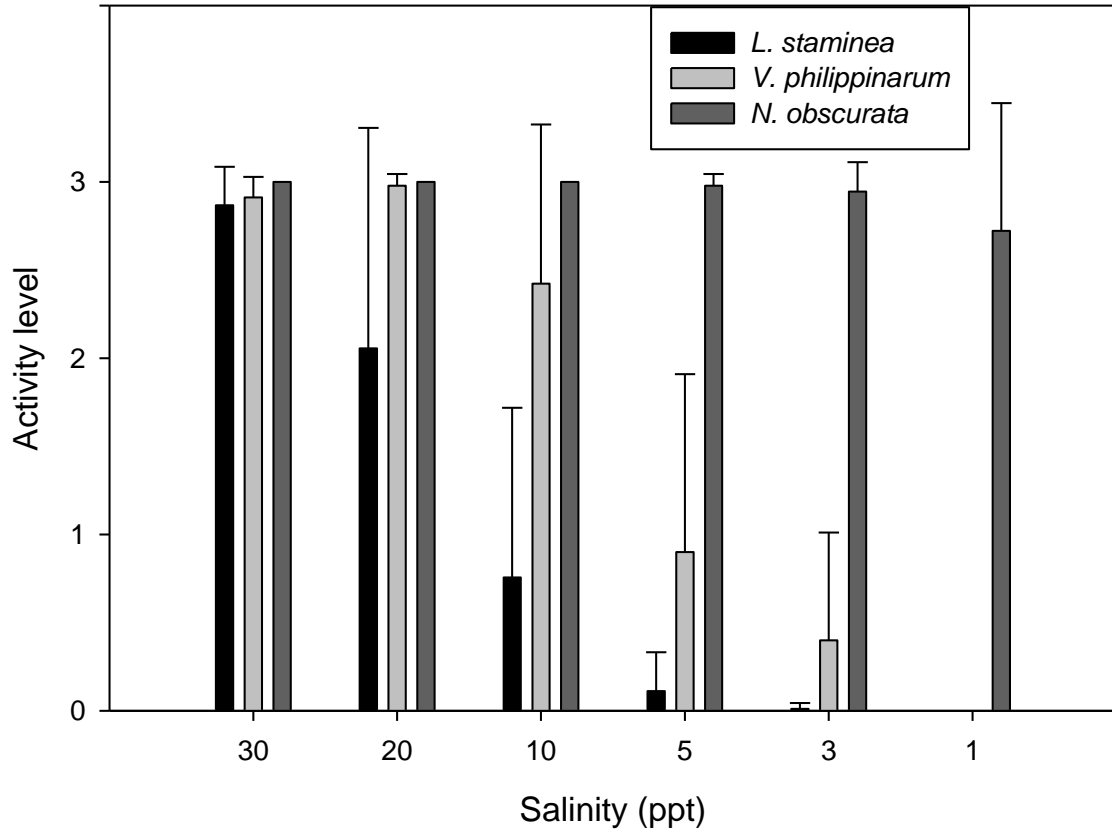


Figure 6: Gill ciliary activity levels after 24 h of exposure to environments of varying salinity. Activity level is from 0-3, where 3 is fully active and 0 is total death. Error bars represent \pm one standard deviation (n = 9 per bar; 3 per salinity level per site per species). Data from all sites have been combined in this figure.

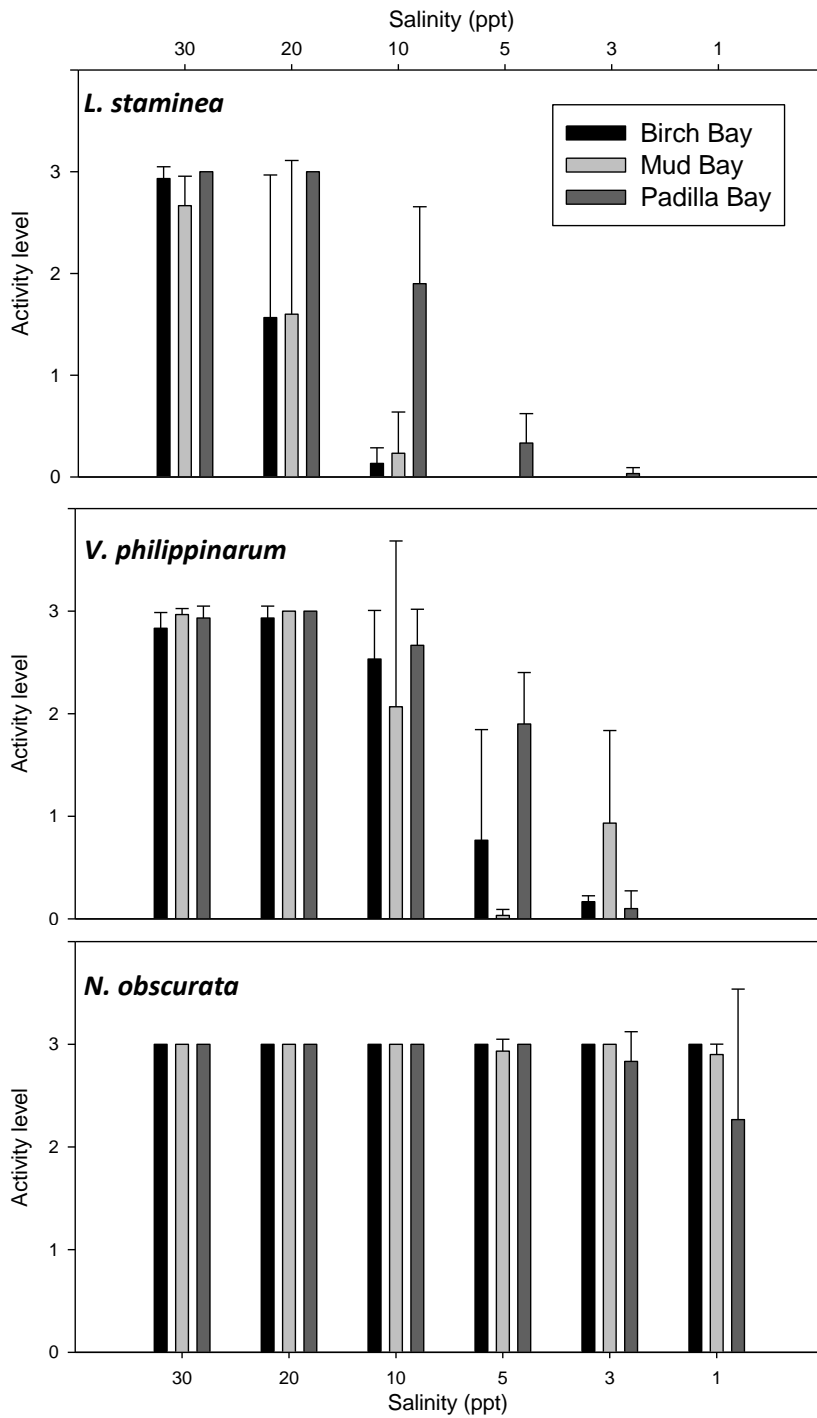


Figure 7: Gill ciliary activity levels after 24 h exposure to environments of varying salinity, with distinct results from each site shown (n = 3 per bar). Error bars represent \pm one standard deviation.

activity decreases starting at approximately 10 ppt salinity. This is in stark contrast to *N. obscurata* gill tissue, which consistently survived with little or no reduction in ciliary activity levels at all salinities tested.

Clam length was examined as a covariate and found to be non-significant for all three species (*L. staminea*: $F_{1,35} = 1.314$, $p = 0.259$; *V. philippinarum*: $F_{1,35} = 2.825$, $p = 0.856$; *N. obscurata*: $F_{1,35} = 0.922$, $p = 0.344$; ANCOVA). Clam lengths of *L. staminea* used in the salinity studies averaged 42.1 ± 5.3 mm, lengths of *V. philippinarum* averaged 45.1 ± 4.3 mm and lengths of *N. obscurata* averaged 44.9 ± 3.9 mm (error represents one standard deviation).

Survivorship of gill tissue over time

Preliminary studies found that gill tissue of *L. staminea* and *V. philippinarum* consistently died within 48 hours of exposure to the salinities used in these experiments. All gill pieces from *N. obscurata*, however, survived with no decline in activity level for at least two weeks in each salinity treatment (1-30 ppt). Many gill pieces survived for a month or more. Gill tissue death in this experiment was due to invasion by ciliated microorganisms, which eventually consumed most of the gill pieces. All but one sample died or were consumed by ciliates by the seventh week of the experiment. The remaining sample remained at the highest activity level for nine weeks at 5 ppt salinity before being consumed by ciliates. This ciliate invasion and consumption of the gill pieces allows no statistically significant analysis of the data; however, observations through the course of the experiment

suggest no real difference in gill ciliary survivorship or activity level due to salinity variation, at least within the time span of this experiment.

DISCUSSION

Broad physiological tolerances

Nuttallia obscurata gill tissue had significantly greater tolerance to higher temperatures and to lower salinities than did the two other bivalves, *Venerupis philippinarum* and *Leukoma staminea*. Because gill ciliary activity is an accepted correlate of whole organismal tolerance (Karpenko and Ivanovsky, 1993; Stevens, 1987; Vernberg *et al.*, 1963), it is apparent that *N. obscurata* can survive in a much wider range of temperature and salinity conditions. Likewise, the remarkable long-term survivorship of *N. obscurata* gill tissue was demonstrated by its ability to survive in a wide range of salinities for a period of time tenfold that of the other two study species. This extreme hardiness in conditions of high temperature as well as low salinity most likely contributes to *N. obscurata*'s great success as a marine invasive species in the Northeastern Pacific.

Temperature

Results from the temperature experiment support the hypothesis that *N. obscurata* gill tissue would have higher temperature tolerance than gill tissue of *V. philippinarum* and of *L. staminea*. In addition, *V. philippinarum* gill tissue did indeed have a higher temperature tolerance than that of *L. staminea*. Interpolations generated with the experimental data that yield a temperature at which each study species dies after 60 minutes of temperature exposure suggest that *N. obscurata* can survive higher temperatures than can *V.*

philippinarum and *L. staminea*. Within a species, temperature increases lead to significant decreases in gill tissue survivorship.

Past studies have shown both that gill ciliary activity can be considered a good correlate for whole organismal tolerance (Vernberg *et al.*, 1963), as well as that the lethal temperatures of a marine organism can directly correlate to normal environmental conditions (Hodgson, 1999; Kennedy and Mihursky, 1971; Somero, 2002). Vernberg *et al.* (1963) found that three North Carolina marine bivalves have a high positive correlation between whole animal and gill ciliary response to different salinities. Kennedy and Mihursky (1971) found that lethal temperatures of several Chesapeake Bay bivalves corresponded to vertical distribution in the benthos. A review by Hodgson (1999) reports on evidence that some siphonariid limpets living in higher, more exposed areas are more tolerant of higher temperatures than species in the lower intertidal. Finally, a review by Somero (2002) discusses evidence from a range of studies demonstrating correlation between resistance to high temperature and vertical distribution. Thus, clams that reside in shallower substrata are likely exposed to more heat and consequently have a higher temperature tolerance than those which live in deeper, cooler layers.

A similar effect may be occurring with the three bivalve species in the present study. Although there were no obvious differences between the recorded depths of each species in the substratum in this study, the three species were often located in different areas of the intertidal zone. The invasive *N. obscurata* was typically found higher up in the intertidal, the native *L. staminea* was generally found the lowest, and *V. philippinarum* was usually either mixed in with *L. staminea* or slightly higher in the intertidal. Those individuals living at

lower areas in the intertidal zone are consequently covered by water for a greater percentage of time than those at higher levels, and would therefore not have to tolerate as much temperature fluctuation. This corresponds directly to results showing that *N. obscurata*, which lives at the highest levels, has the highest temperature tolerance, while the lower-dwelling *L. staminea* has the lowest temperature tolerance.

The three study species also often differed in the types of sediment in which they were found. At the study sites located in Mud Bay and Padilla Bay, *L. staminea* and *V. philippinarum* were both found living directly in the intertidal mud flats, while most of the *N. obscurata* were living in the gravel-sand substratum located at the upper edge of the intertidal. These different environments may also have different temperature fluctuations – the thicker, denser mud may maintain a more constant temperature than the more porous gravel substrate.

In Birch Bay, all three species were collected in gravel-sand substrate; however, again most *N. obscurata* were found higher up in the intertidal zone than were the other two species. Despite the environmental differences of Birch Bay compared to Mud Bay and Padilla Bay, there were no significant site-to-site differences in temperature tolerance of the three species. This is likely due to the fact that water temperature is fairly standard throughout the Georgia basin, and that the pre-experimentation lab acclimation period was sufficient to remove any site-based temperature tolerance differences.

In addition, Kolpakov and Kolpakov (2005) found that a population of *N. obscurata* living in a brackish lake responded to seasonal variations in temperature by moving to different depths within the substratum. During cooler winter temperatures, the purple varnish

clams burrowed deeper within the ground, while during the warmer summer, they moved to the surface. My study did not focus on seasonal changes in burrowing depth; however, personal observation during seven months' worth of collecting suggests that some burrowing variation was present. Populations of *N. obscurata*, especially individuals from Mud Bay, which was used as a collection site in both summer and winter months, tended to be deeper during colder, winter temperatures. In addition, Byers (2002) found that the ability of *N. obscurata* to burrow deep in the intertidal substratum helped the clams withstand crab predation pressure. This ability may very well aid in withstanding a range of temperatures, as well.

It is likely that temperature thus plays an important role in the spatial habitat location of *N. obscurata*, as well as that of *L. staminea* and *V. philippinarum*. What's more, temperature strongly affects different bivalve physiological processes, such as oxygen consumption (Shumway and Koehn, 1982), reproduction (Philippart *et al.*, 2003), growth (Dudas, 2005), and respiration (Kennedy and Mihursky, 1972; Newell, 1969). Bivalve respiration is largely controlled by the gill cilia: they provide the power that controls water circulation through the organism, allowing for a constant influx of oxygenated water while expelling waste water (Dame, 1996). The fact that the gill cilia of the three study species are directly affected by temperature suggests that, at least in these three species, temperature does indeed have an effect on respiratory processes. Further studies examining whole-organism responses to temperature, rather than responses of isolated gill tissue, would be necessary to pinpoint other effects of temperature. Likewise, additional experiments are

required to determine the cellular and physiological effects of increased temperature on gill tissue of these three bivalves.

The higher lethal temperature of *N. obscurata* gill tissue compared to that of the other two clams may contribute to the purple varnish clam's success as an invasive species. There were few other clams found in the same areas as *N. obscurata*, suggesting that its higher temperature tolerance allowed *N. obscurata* to both colonize a new or underused niche in the area, and survive well once established. Species with higher environmental plasticity – such as the greater temperature tolerance of the invasive *N. obscurata* compared to those of the already-established *V. philippinarum* and *L. staminea* – can more easily colonize new areas, and have less difficulty surviving once established, than can species with lower environmental plasticity (Sakai *et al.*, 2001). Indeed, Mack *et al.* (2000) suggest that environmental forces are some of the most important factors in determining both initial and long-term survival of invasive species. This clearly supports the idea that *N. obscurata*'s rapid colonization and spread throughout the coastal Northeastern Pacific is at least in part due to its relatively high temperature tolerance. Likewise, the ability to withstand higher temperatures may explain why *N. obscurata* was often found higher in the intertidal zone.

Salinity

The results support the hypothesis that *N. obscurata* gill tissue has the highest tolerance for low salinities, followed by tissue of *V. philippinarum* and lastly that of *L. staminea* having the least tolerance for low salinities. All three species were significantly different from each other, with the gill tissue of the invasive *N. obscurata* having the highest

tolerances, and gill tissue of the native *L. staminea* least able to withstand low salinity environments. Overall, for each study species, decreases in salinity led to decreases in gill ciliary activity. Interestingly, *N. obscurata* gill cilia had a far higher tolerance to lower salinities than did cilia of the other two clams – all *N. obscurata* samples lived for at least two weeks at all salinities, with several samples surviving for a much longer duration. This is in stark contrast to gill tissue of *V. philippinarum* and *L. staminea*, which died within 48 hours at all salinities.

Like temperature, salinity affects a variety of organismal properties, including osmoregulation, relative solute proportions, gas absorption and saturation, density and viscosity, dispersal, establishment and habitat selection (Gunter, 1961; Jassby *et al.*, 1995; Kinne, 1964; Mann and Harding, 2003). It is often difficult to determine the precise physiological effects of salinity on an organism, as it is often not one specific effect, but a combination of effects, which may be in turn influenced by other factors such as temperature (Gunter, 1961; Shumway and Koehn, 1982; Vernberg *et al.* 1963). However, osmoregulation is one of the more important factors affected by salinity, and is especially crucial in intertidal organisms, which are exposed to potentially wide-ranging and rapidly-changing osmotic pressures (Gilles, 1972; Kinne, 1964).

It is currently unknown to what extent *N. obscurata* can osmoregulate, and how it does so, but it is clear that either its gill tissue can osmoregulate to a much greater degree than can tissue of *V. philippinarum* and *L. staminea*, or that the tissue can simply osmoconform and cope with a much greater range of salinities. Both osmoregulation and osmoconformity may in fact play some role in *N. obscurata* survivorship at low salinities, as

both can be important factors in other estuarine bivalves. Neufeld and Wright (1996) determined that gill tissue of the estuarine mussel *Geukensia demissa* participated in both osmoconforming and osmoregulating activities, depending on external environmental conditions. In *G. demissa* gill tissue, short-term regulation of cell volume is typically absent, allowing the organism to more efficiently survive environments of fluctuating salinities. However, during long-term exposure to lowered salinities, gill tissue cells do eventually experience loss of solutes and decrease in volume. It is possible that *N. obscurata* tissue undergoes similar activities, and can combine both osmoregulation and osmoconformity depending on the circumstances. Research is currently underway in our lab to further investigate the extent of *N. obscurata* osmoregulation; work with isolated gill tissue as well as with whole organisms is being conducted.

In other marine bivalves, it has been determined that temporary hyperosmotic states observed within the organism are often due to a tightly-closed shell (Gilles, 1972). Of course, this state cannot be sustained indefinitely, as the organism in question will eventually need oxygen input as well as food from the outside environment. Thus, the shell-closing mechanism is typically a short-term response. Based on personal observation, it appears that this strategy is not frequently used by *N. obscurata*: seemingly healthy varnish clam individuals were seen with siphons extended outside of the shell at salinities significantly lower than that of normal seawater. This is further supported by Kolpakov and Kolpakov (2005), who observed and collected purple varnish clam specimens living and reproducing in sand and silt-sand bottoms of a brackish lake with salinities ranging from 8 to 22 ppt. It is possible that this population of *N. obscurata* could use the shell-closing mechanism to

survive at the low end of this range. However, it is clear that, when living in an environment with a maximum salinity of 22 ppt, the varnish clam must use methods other than shell-closing in order to cope with reduced salinities.

Although the results clearly demonstrate significant ability of *N. obscurata* gill tissue to withstand lower salinities, the presence of several significant interactions must also be explained. The significant interaction between site and salinity shows that the site at which individuals are collected has an effect on salinity tolerance. This could be due to differences in salinity fluctuation or observed salinity levels across sites, which may convey more or less ability for a clam to survive in different salinities. The significant interaction between site and species indicates that to some degree, species from different sites have different 24-hour survivorships, no matter the salinity. This is likely due to any of a number of possible environmental variations at each site, which could convey greater or lesser tolerance of different salinities in this experiment. It may also result from possible hybridization, which will further be examined later. Finally, interaction between species and salinity simply shows that each study species can tolerate different salinities to different degrees.

One important difference between the temperature and salinity results was the presence of a significant site-to-site difference in the salinity results, but not in the temperature studies. In contrast to the initial on-site measurements that demonstrated a significantly lower average pore water salinity at Mud Bay than at Padilla Bay or Birch Bay, it was Padilla Bay clams that yielded significantly different ciliary activity levels than from those collected at Birch Bay and Mud Bay, while Birch Bay and Mud Bay clam cilia did not have significantly different activity levels with respect to salinity. Overall, Padilla Bay *V.*

philippinarum and *L. staminea* gill tissue demonstrated significantly higher ciliary activity levels at lower salinities than individuals of the same species collected from different sites. Ciliary activity of *N. obscurata* did not display any site-to-site differences, as almost all gill tissue samples maintained the highest ciliary activity levels for the duration of the experiment, no matter the salinity levels or the site.

This significant difference found in Padilla Bay specimens may stem from one or more factors. It is possible that Padilla Bay has lower average salinities than does Mud Bay or Birch Bay, or that it experiences greater fluctuations in salinity. This is in contrast with the on-site measurements that show the lowest salinities at Mud Bay, which are supported by the fact that Mud Bay is very shallow, quite small and has considerable freshwater input via Chuckanut Creek. However, there are also several reasons why Padilla Bay may have overall lower salinities. It is entirely possible that the few on-site measurements taken are not an appropriate representative of the overall estuarine system at each site. Padilla Bay is a large estuary, with significant freshwater input from the Skagit River. The large amount of freshwater entering Padilla Bay could result in lower average salinities and/or greater fluctuation. Data taken from Bayview Channel in Padilla Bay in 2008 show annual salinity fluctuations from 25.6 to 31.1 ppt (National Oceanic and Atmospheric Administration, 2004). However, these data cannot be used to compare Padilla Bay with the other study sites, as comparable salinity data were not available. In addition, this reasoning may not account for observed differences among sites. All three bays are interconnected as part of the Georgia Strait, so average salinities in each bay should be fairly similar. More research

should certainly be conducted to further investigate possible salinity differences between each site.

One other factor may also help explain the differences between Padilla Bay and the other two sites. Padilla Bay was unique among study sites in that suitable specimens of *V. philippinarum* and *L. staminea* were more difficult to find. Many of the target clams located in Padilla Bay appeared to be hybrids, possessing physical characteristics representing a blend of both *V. philippinarum* and *L. staminea*. These potential hybrids were consistently rejected for more definitive specimens; however, it is possible that even some of these samples that were ultimately brought back and used in the experiments were of hybrid form. In the last several decades, it has become increasingly apparent that hybridization may confer significant evolutionary advantages, including additive genetic and environmental variances and rapid adaptation (Grant and Grant, 1994; Rieseberg *et al.*, 1999). It is certainly possible that hybridized specimens may possess greater tolerances for lower salinities, or a larger range of salinities. Indeed, hybridization has been previously observed in a wide range of marine invertebrates, including various genera of mussels and clams, some commercially-important (Menzel and Menzel, 1965; Perry *et al.*, 2002; Pfenninger *et al.*, 2002; Skibinski *et al.*, 1978). Many of these hybrid bivalves possessed greater abiotic tolerances, growth rates and other evolutionary advantages. It has also been suggested that *V. philippinarum* and *L. staminea* may hybridize (Cowles, 2007). Why this potential hybridization may be occurring in Padilla Bay is unknown. It is clear that further research should be done in this area.

Survivorship

Nuttallia obscurata gill tissue all survived for at least two weeks in a wide range of salinity, with many pieces of tissue lasting for many weeks longer. This is in stark contrast to gill tissue of *V. philippinarum* and *L. staminea*, which consistently died within 48 hours of excision, no matter the salinity to which they were exposed, in preliminary studies. Other bivalves display similar trends to *V. philippinarum* and *L. staminea*: in another study exposing excised gill tissue to varying salinities, Vernberg *et al.* (1963) found that three species of bivalve (*Aequipecten irradians*, *Modiolus demissus* and *Crassostrea virginica*) living on the East coast of North America also typically survived for only 24 hours or less at low salinities.

Due to experimental problems, the long-term survivorship experiment failed to yield a maximum survival time for *N. obscurata*. All samples used were eventually infected with ciliated microorganisms which ultimately consumed or destroyed all pieces of gill tissue in each Erlenmeyer flask. It is likely that either the unfiltered seawater used as a base solution in the experiment, or the collected clams themselves, were vectors for ciliate invasion. However, important information can still be gleaned from the survivorship experiment. The fact that tissue death was apparently due to ciliate invasion rather than exposure to varying salinities gives us no clear correlation between salinity and survivorship. Even the gill samples surviving for the longest amount of time followed no clear pattern with respect to salinity. In fact, the longest-lived sample, which survived for nine weeks with no noticeable decline in activity level, was exposed to an environment of only 5 ppt salinity. Thus, this

experiment demonstrated an incredible survivorship of the invasive *N. obscurata* compared to *V. philippinarum* and *L. staminea*, as well as compared to other bivalves.

***Nuttallia obscurata* as an invasive species**

Within the last two decades, *N. obscurata* has become one of the dominant bivalve species in the intertidal Northeastern Pacific (Dudas and Dower, 2006; Ray, 2005). Like many other marine invaders of note, it has spread rapidly and has established itself with great efficiency throughout the region. It is clear that *N. obscurata* has a greater environmental plasticity than *V. philippinarum* and *L. staminea*. In addition to surviving at higher temperatures, it can survive at lower salinities for much longer periods of time. It has been found that species distribution in soft-substrate intertidal zones is often principally determined by temperature and salinity (Gunter, 1961; Vernberg *et al.*, 1963). Because these soft-substrate zones are also often exposed to extreme abiotic variation (Kneib, 1984), it is no wonder that organisms with higher environmental plasticity – those that can tolerate greater fluctuations in abiotic conditions like temperature and salinity – can survive better than species with less tolerance (Boesch *et al.*, 1976; Jassby *et al.*, 1995; Peck *et al.*, 2004). This is true even on a local level: in Padilla Bay, Harley (1998) found that large scale environmental gradients determine both the strength of interactions and the spatial overlap of different species. The higher temperature tolerance of *N. obscurata* likely allows it to live higher in the intertidal than *V. philippinarum* and *L. staminea*.

This ability to live in areas not populated by other clam species lets the purple varnish clam more easily establish itself and grow in population size. However, anecdotal evidence,

combined with personal observation and other recent studies indicate that in addition to living in habitat not frequented by other clams like *V. philippinarum* and *L. staminea*, *N. obscurata* has been found on Manila clam farms (Dudas, 2005). Furthermore, studies have shown that that *N. obscurata* has a competitive advantage over *V. philippinarum* in the high intertidal. Such characteristics as burrowing preference, reproductive capability and abiotic tolerances may all contribute to this competitive advantage (Ray, 2005; Dudas, 2005). It is clear that *N. obscurata* does indeed have higher abiotic tolerances than *V. philippinarum*, as well as *L. staminea*, which could very well contribute to its success as an invasive species.

The invasive success of *N. obscurata* is one shared by many other marine organisms. Like most other marine invasives, the purple varnish clam likely arrived to its new home in the Northeastern Pacific via ballast water discharge from international shipping (Mills, 2002; Molnar *et al.*, 2008). And like many other invasives, the high abiotic tolerances of *N. obscurata* compared to clams like *V. philippinarum* and *L. staminea* likely contribute to its invasive success. Although *N. obscurata* is a marine organism, it can easily survive in brackish and estuarine waters (Kolpakov and Kolpakov, 2005). What's more, Lee and Bell (1999) suggest that temperature and salinity often interact when affecting tolerance. Organisms possessing a broad temperature tolerance as well as a broad salinity tolerance may have an easier time invading freshwater systems. It is not often that a marine species can successfully invade freshwater systems, but special attention should be paid to *N. obscurata*, considering its high tolerance for low salinities and for high temperatures, as well as the results indicating that even its isolated gill tissue can live for weeks in almost fresh water. It has become increasingly obvious that potential marine to fresh water transitions have not

been adequately studied. Despite this, there are records of a number of species, including cnidarians, annelids, molluscs, arthropods and a host of fish species, that have succeeded in doing so (Lee and Bell, 1999). One bivalve invader of note is *Dreissena polymorpha*, the zebra mussel. Introduced to North America from Europe in 1986, it has since spread throughout the Great Lakes and is rapidly moving westward, while creating immense ecological and economical damage (May *et al.*, 2006; McMahon, 1996). The purple varnish clam has not yet colonized areas of fresh water, but further research into long-term survivability of *N. obscurata* in freshwater systems is recommended.

It is necessary to look to the future when considering the place that *N. obscurata* will have in North America. As projected by the Intergovernmental Panel on Climate Change, global surface warming is predicted to increase between one to four degrees Celsius during the next century (Pachauri and Reisinger, 2007). Marine and terrestrial species alike will be affected by warmer temperatures, and it has been proposed by many that increases in temperature will lead to increased biological invasions, even shifting dominance to non-indigenous species (Stachowicz *et al.*, 2002; Vitousek, 1994). Global warming will provide even more aid for species with higher temperature tolerances to thrive and spread. As Kolpakov and Kolpakov (2005) suggest, *N. obscurata* has potential to become increasingly dominant in the sublittoral as temperatures increase.

It seems that the purple varnish clam is here to stay in the Northeastern Pacific, and will likely continue to spread, given its high abiotic tolerances as well as other physiological characteristics such as burrowing ability and reproductive factors (Dudas, 2005; Ray, 2005). Although it has so far only invaded the Northeastern Pacific, there is the ever-present

possibility that it will invade other regions, either by direct shipping from Asia, or from secondary shipping vectors stemming from the Northeastern Pacific. Other notable marine invaders have also posed threats to multiple regions across the globe, including the green crab, *Carcinus maenas*, which has invaded a wide variety of areas including Australia, the Northeast Pacific, the Northwest Pacific and South Africa (Grosholz and Ruiz, 1996). To prevent further invasions by *N. obscurata*, which would likely occur via international shipping, increased care must be taken to regulate and control ballast water contents (Molnar *et al.*, 2008). Other regions rendered vulnerable due to high shipping volume should also maintain watch for any sign of *N. obscurata* invasion. If an introduction is caught early enough, there may be some possibility of halting the spread of *N. obscurata* before it has a chance to establish itself.

Further investigations and recommendations

In the Northeastern Pacific, there is still much to learn about the purple varnish clam. Further research should be done to explore the cellular mechanisms of *N. obscurata* that allow its gill tissue to survive at low salinities for extremely long periods of time. Osmoregulation would be an ideal topic to investigate. In addition, work should be done with both *N. obscurata* gill tissue, as well as whole organism studies, looking at interactions between temperature and salinity. There has been much previous research investigating the effects of temperature and salinity combined on marine organisms. These abiotic factors often have an additive effect: organisms exposed to higher temperatures have less tolerance of lower salinities (Kinne, 1964). Information about *N. obscurata* response to varying

temperature-salinity combinations may prove useful in understanding its capacity for biological invasion.

Further investigation into the potential for *N. obscurata* as a commercially-marketable species is also recommended. Like the now commercially-important Manila clam, which was accidentally introduced to the Northeastern Pacific in the 1930s, the purple varnish clam still has some potential as a commercial asset (Washington Department of Fish and Wildlife, 2000). Although previous studies have found it unlikely that *N. obscurata* will be commercially harvested in Washington in the near future, it is currently being sold in some markets in Vancouver, British Columbia and elsewhere (Figure 8) (Gillespie *et al.*, 2001; Meacham, 2008; Ray, 2005). It has also been observed on the menu of at least one Seattle, Washington seafood restaurant, marketed under the name mahogany clam. Recreational harvesting in the state of Washington is currently allowed, but *N. obscurata* has not proved to be a popular choice. Nonetheless, the purple varnish clam has been reported as similar to *V. philippinarum* and *L. staminea* in taste and texture, and has an extremely long shelf life of at least four weeks when refrigerated, suggesting that there is still potential for a commercial fishery at some point in the future (Yates, 1999). Despite initial results to the contrary, further investigation into the possibility of *N. obscurata* as a commercial resource is recommended, as the purple varnish clam is likely here to stay in the Northeastern Pacific.



Figure 8: Commercially-sold *N. obscurata* mixed with *V. philippinarum* in a market on Granville Island, Vancouver, British Columbia.

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