**Effect of Reduced Salinity on Feeding Rates of *Haminoea vesicula***

Cody Carlson[1,4], Laura Hanna[2,4], and Michael Montgomery[3,4]

1. Department of Fisheries Biology, California State Polytechnic University, Humboldt, Arcata, CA 95521
2. Department of Biology, Western Washington University, Bellingham, WA 98225
3. Interdepartmental Graduate Program in Marine Science, University of California Santa Barbara, Santa Barbara, CA 93106
4. Friday Harbor Laboratories, Friday Harbor, WA 98520

**Abstract**

Fluctuations in salinity are a characteristic feature of many nearshore marine ecosystems. In regions subject to seasonally extreme freshwater input, such as the Salish Sea, recruitment depends in part upon the ability of planktotrophic larvae to continue growth, feeding and survival during episodes of severely reduced salinity. Previous work has demonstrated a decline in feeding rate for bivalve and gastropod veligers at experimentally reduced salinities. These studies were not conducted through the lens of an environment that experiences wide ranges in salinity, such as the Salish Sea. We tested the effect of reduced salinities on veligers of the cephalaspid gastropod *Haminoea vesicula*, a grazer of epiphytes on eelgrass and soft sediments, by quantifying feeding rates in the laboratory. Feeding rates were not able to be calculated due to issues with data collection. Future research into the impact of reduced salinities on gastropod veliger larvae, especially opisthobranch gastropods, is necessary to understand the complex role salinity could play in larval development and feeding.

## Introduction

In the Salish Sea of northern Washington, U.S.A., and British Columbia, Canada, freshwater influxes are largely seasonal (Riche *et al.*, 2014). Principal inputs are the Fraser and Skagit Rivers, as well as other smaller, glacially fed stream courses and runoff from high seasonal rainfall (Khangaonkar *et al.*, 2017). The Salish Sea is considered a high-salinity environment but is expected to experience an increasing number and altered phenology of low-salinity events as rising temperatures accelerate regional rates of snow- and ice-melt (Bashevkin *et al.*, 2016). It has already been observed by Riche *et al.* (2014) that over the past 50 years, the Salish Sea's contributing watersheds, particularly the Fraser, have experienced a shift in maximum discharge from summer to spring as a result of elevated early-season temperatures. With this phenological shift, severe low-salinity events could have widespread detrimental effects on many marine invertebrate taxa, including their planktonic larvae (George *et al.*, 2021). Larvae with the ability to osmoregulate may be able to better withstand such variations. Osmoregulation could be especially advantageous for intertidal taxa with planktotrophic larvae, such as *H. vesicula*, that may be increasingly exposed to freshwater pulses (George *et al.*, 2021).

Headshield or bubble snails of the family Haminoeidea (Gastropoda: Cephalaspidea) are common herbivores in eelgrass beds worldwide (Oskars *et al.*, 2019). As grazers of epiphytic algae, they likely contribute to significant top-down ecological regulation of eelgrass meadows, supporting high biomass and productivity (Malaquias & Sprung, 2005; Heck & Valentine, 2007). In at least one cephalaspidean species, *Haminoea vesicula* (Gould, 1855), interspecific competition from other grazers (e.g., *Lacuna* spp.) excludes adults from the center of eelgrass patches, restricting them to adjacent mud substrates (Groth, 2021). Other factors, including temperature, affecting this species' distribution and recruitment remain underexplored for early life stages. Such

impacts on these early life stages may lead to higher larval mortality, reduced settlement and metamorphic processes, and therefore suggest implications for broader ecosystem health.

Over the past decade, the eelgrass *Zostera marina* has experienced severe declines along the northeast Pacific due to seagrass wasting disease (SWD) (Renn, 1936; Muehlstein, 1989; Graham *et al.*, 2021). As eelgrass populations decline, organisms whose life histories necessitate the use of eelgrass beds for development may also decline (Graham *et al.*, 2021). Loss of eelgrass habitat to SWD or other stressors like increased sedimentation (Barbier *et al.*, 2011) could negatively impact mesograzers such as *H. vesicula* in the form of reduced food abundance, nursery habitat, and increased exposure to predation and adverse environmental conditions. Successful non-indigenous species are largely more tolerant to a range of environmental conditions compared to native species (Zerebecki & Sorte, 2011), therefore exacerbating the stress experienced by native species. For example, competition with the invasive cephalaspid *Haloa japonica* (Pilsbry, 1895) could further contribute to population declines of *H. vesicula* (Gibson & Chia, 1995). In the face of such competition, the future success of *H. vesicula* may depend on its ability to tolerate, both at larval and adult stages, a wide range of environmental conditions, including episodes of reduced salinity.

Gastropod veligers are known to have a larval kidney that contains an absorptive cell (Gohar & Abul-Ela, 1957; Potts, 1975; Rivest, 1992) and may function as a rudimentary protonephridia. Work by Bayne (1965) and Riisgard et al., (1980) found that particle clearance rate of *M. edulis* veligers was diminished at high algal concentrations when compared to clearance rate at low algal concentrations. While the effects of low salinity on veliger feeding remain poorly understood, prior work has demonstrated a decrease and even cessation of feeding in adults of the suspension-feeding gastropod *Crepipatella dilatata* at salinities equivalent to 20‰ (Chaparro *et*

*al.*, 2008). Laing (2002) found that reduced salinity (< 30‰) led to significantly decreased clearance rates in juveniles of the scallop *Pecten maximus*. This suggests there may be a salinity threshold at which mollusk veligers slow or cease their feeding, with concomitant reductions in experimentally measured rates of filtration and presumed ingestion. We set out to evaluate the effect of exposure to reduced salinity for *H. vesicula* veligers, hypothesizing that a reduction in algal cell ingestion rate would become apparent at a lower salinity compared to the rate in ambient seawater.

## Methods

*Egg mass collection and larval hatching*

Egg masses of *H. vesicula* were collected from sandy flats in False Bay, San Juan Island, WA on 29 June 2023. Veliger larvae naturally hatched from the egg cases and were placed in microalgal-saturated treatments of filtered seawater (FSW, at 0.22 μm) between 2 and 5 days post-hatch (d.p.h.). Prior experiments have indicated that *H. vesicula* has a pelagic larval duration of approximately one month, with depletion of maternal reserves and onset of active feeding 2–3 days post hatching (dph) (Gibson & Chia, 1989). This result conferred confidence in our choice of a multi-day acclimation. One day prior to the beginning of feeding trials, veligers were placed into 100-mL glass containers at one of two salinities and were starved for a period of 24 hours. Each replicate container contained 20 veligers, resulting in a density of 1 larva 5 $mL^{-1}$ FSW.

*Feeding experiment*

We investigated the effects of two salinities on veliger feeding at 30‰ and 15‰ to simulate ambient and freshwater-pulse conditions, respectively. Salinity of the FSW was reduced by adding reverse osmosis water, and the final salinities were confirmed with a hand-held refractometer.

Following Gibson and Chia (1989) and D. Padilla and R. Emlet (pers. comm.), we selected *Isochrysis galbana* as the larval food source. The *I. galbana* was sourced from present static cultures at FHL, raised at ambient temperature and with an unimpeded diel light:dark cycle of 16:8 hours. We calculated the necessary volumes of FSW to add to each flask for a target algal concentration of $10^6$ cells $mL^{-1}$ (*vid.* McFarland *et al.*, 2013). All concentrations were measured using a 0.01-mm improved Neubauer hemocytometer.

We took 1 mL samples to enumerate changes in algal concentration at 0, 15, 30, 45, 60, 900 (15 hr), and 1440 (24 hr) minutes after incubation (Fig. 1). These samples were collected after swirling each container and mixing with a glass pipette. Samples kept in microcentrifuge tube were inspected under a dissecting microscope to ensure no veligers had been entrained. On the few occasions when veligers were present, we returned samples to the replicate containers and collected new ones, repeating the procedure until veliger-free. We added approximately 50 µL of Lugol's solution to each microcentrifuge tube to fix the *I. galbana*, and subsequently quantified cell concentrations with a hemocytometer. Due to constraints of timing, we were unable to assess possible rejection of algal cells as pseudofeces, with the consequence that all measured changes in *I. galbana* concentrations were presumed to be the result of ingestion.

Veliger feeding rate was estimated by computing the change in algal concentration at the end of the incubation in the average of all procedural controls and experimental replicates. To account for algal cell division over the 15-hour study duration, we calculated a "background"

change in *I. galbana* concentrations, in units of algal cells per mL, averaged across the three control flasks at each of the two salinities. Initial and final concentrations in each experimental flask were then assessed relative to this baseline. Any deviation from expected final concentrations was assumed to be a result of veliger feeding. Statistical analyses (unpaired t-tests) were performed on algal concentrations over time and between controls and treatments.

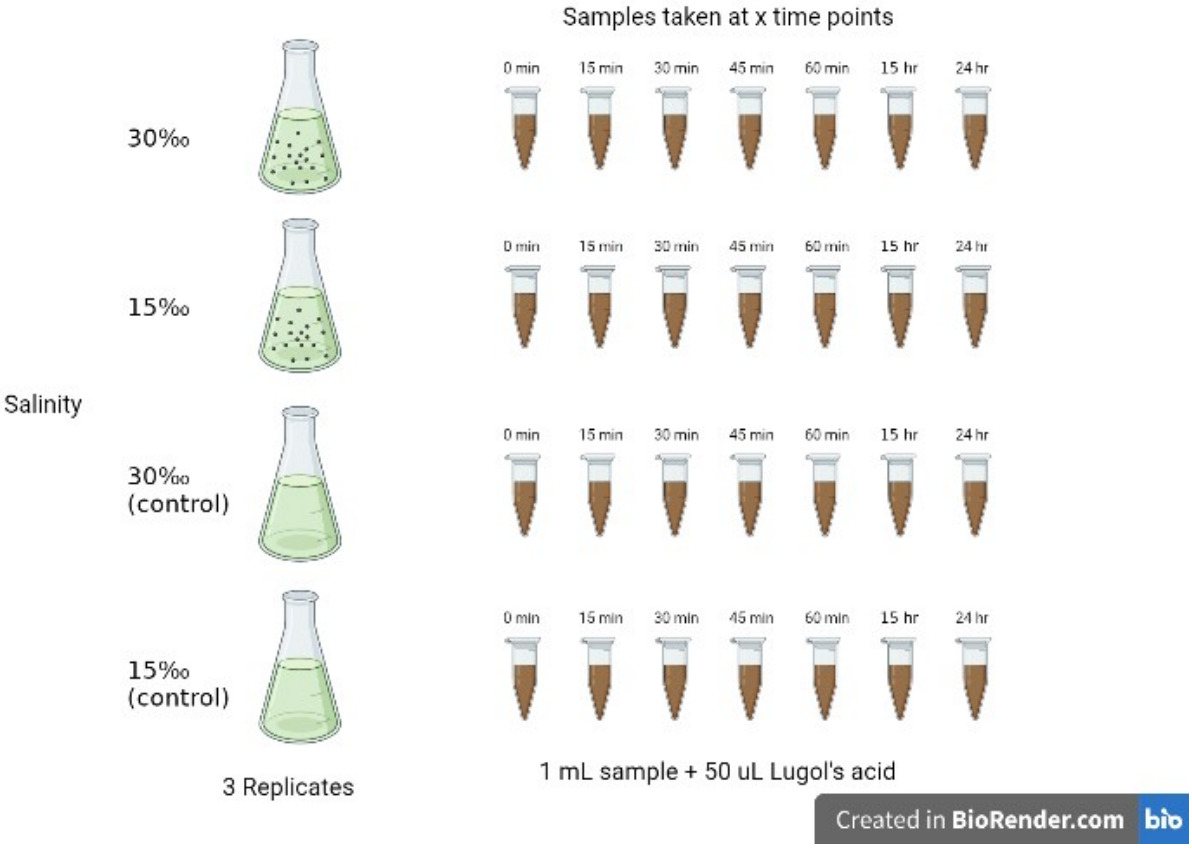


*Figure 1.* Schematic depicting treatment and sample volumes.

**Results**

The concentrations of *I. galbana* in all flasks shared a similar pattern (Fig. 2). The concentrations steadily increased over the course of the first 30 minutes, decreased at 45 minutes,

and then increased again at 60 minutes. The concentrations continued to decrease until the final time point at 24 hours. This pattern was not expected and was the first indication that the concentrations may not have been an accurate representation of what was happening in the flasks.

Control treatments were not significantly different from experimental treatments, and the salinity treatments were not significantly different from one another (Table 1). The lack of a clear reduction in algal cell concentrations meant that with our present methods and number of replicates, accurate estimation of veliger feeding was not possible, therefore precluding any comparisons between ambient and low-salinity conditions.

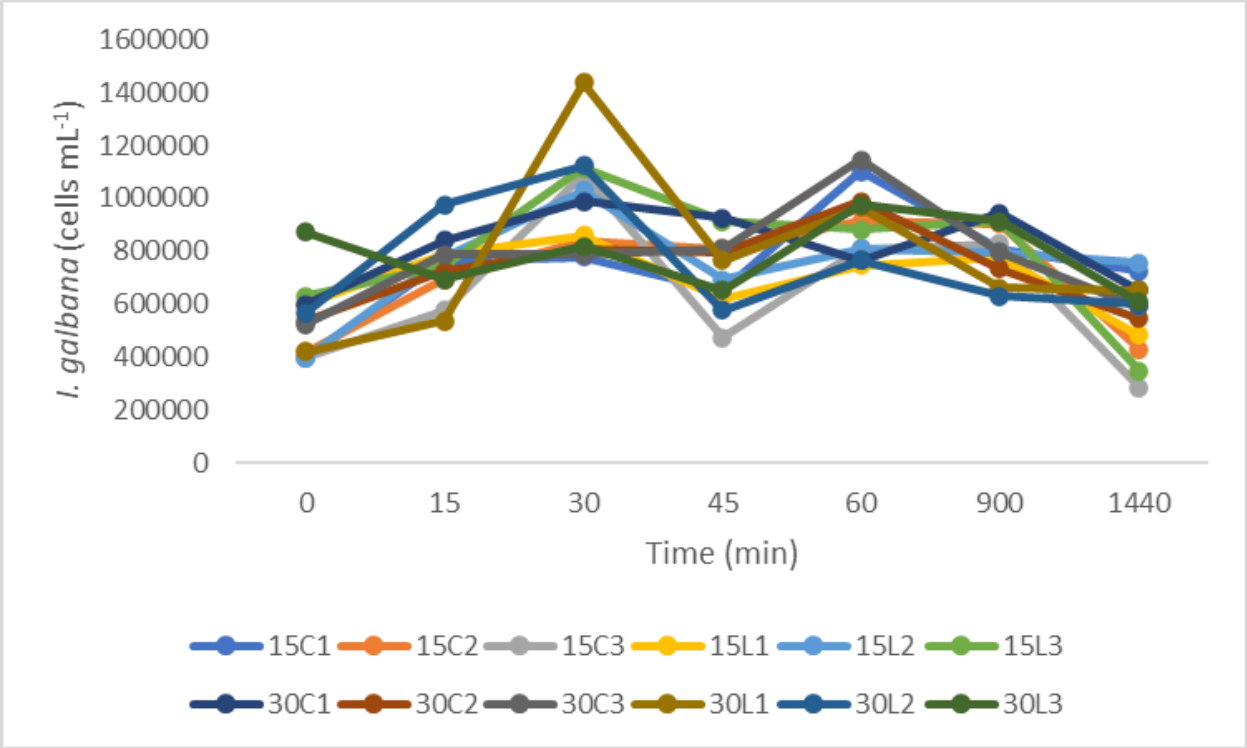


*Figure 2*. Concentration of *I. galbana* over a 24-h incubation period. Samples were taken every 15 minutes over a one hour period and two additional samples at 900 min and 1440 min. Treatments are depicted as separate lines by color.

**Table 1.** Two-tailed t-test of treatment (L) vs. control (C) at selected salinities**.** No significant differences between treatments and controls were detected amongst selected salinities. No effect of salinity or treatment was noted.

| Treatment Comparisons | t-value | p-value |
|---|---|---|
| 30L & 30C | -0.05586 | 0.96 |
| 15C & 15L | -0.71505 | 0.48 |
| 30L & 15L | 0.3872 | 0.35 |
| 30C & 15C | -1.22195 | 0.23 |

**Discussion**

We initially hypothesized that veliger feeding would be negatively impacted by salinities considerably lower than ambient conditions. While our findings do not support this relationship, future work, with an improved and more targeted methodology, may reveal that the lack of correlation is in fact an indication of broad salinity tolerance in this species. Sources of error and recommendations are discussed below.

Algal cell counts were likely inaccurate throughout this study due to the use of a single hemocytometer count per sample. This sampling scheme was not sufficiently sensitive to detect changes in algal concentration over the timescale chosen for this study due to the lack of replicated counts. Evidence for this sampling error was exemplified by the fact that our hemocytometer count showed that the initial treatment concentrations were on the order of $10^5$ cells $mL^{-1}$ in 2-3 replicates, when our initial count and calculation should have yielded the concentration of $10^6$ cells $mL^{-1}$. Furthermore, algal cell counts increased across all treatments and procedural controls after

900 min, but declined at the end of the 24-h incubation. Incorporating more counts per replicate container would ensure more representative algal concentration estimates. Alternatively, employing a tool such as a flow cytometer or Coulter counter automated culture counter could improve algal cell enumeration efficiency (McFarland *et al.*, 2013; Rosa & Padilla, 2022; K.Y.K. Chan, pers. comm.).

The choice to use an initial algal concentration of $10^6$ cells $mL^{-1}$ was made with the knowledge that hemocytometers are inaccurate at dilute cell concentrations. Of note, however, is that larval clearance rates have been shown to decline for *M. edulis* at high algal cell concentrations (1.1-2.0 × $10^5$ cells· $mL^{-1}$) (Bayne, 1965; Riisgard *et al.*, 1980). These studies noted a decrease from 11.4 ± 2.1µL·$h^{-1}$ at low algal cell concentrations (0.03–0.10 mg dry weight·$L^{-1}$) to 3.58–5.42µL·$h^{-1}$ at high algal concentrations (1.1-2.0 × $10^5$ cells· $mL^{-1}$). The decrease in clearance rates at high algal cell concentrations could play a role in the clearance rate of *H. vesicula*.

Additionally, the presence of other algal species, such as diatoms, may have confounded total feeding rates, resulting in an underestimate based on the assumption that veligers were only subsisting on *I. galbana*. Contamination likely occurred due to unsterilized equipment.

Another factor of error in algal cell concentrations is the issue of rearing *I. galbana* at the treatment salinities to ensure no mortality in the algal cells upon placement into the treatments as well as to ensure no salinity-dependent effect on *H. vesicula* feeding preference. Given the alga was cultured in full-strength seawater (30‰), placing the algae into low salinity water could have an effect on cell size and/or exudates chemistry. The lack of difference in ingestion rate could have been due to the lack of salinity uniformity between the algal culture environment and the larval feeding regime.

To quantify the extent to which salinity impacted feeding rates, more replicates over a greater range of salinities are necessary to understand whether there is a "breaking point" for both adults and larval feeding. It is important to understand the tolerances of this species as a means of forecasting the effect reduced salinity and other climate change-driven will have on the species' distribution and recruitment. Previous work on adults of the snail *Crepipatella dilatata* suggests that 26‰ was the point at which feeding rates decreased significantly (Chaparro *et al.*, 2008). Adults of the scallop *Pecten maximus* ceased feeding at salinity below 20‰ (Laing, 2002).

Errors in this study could be remedied by more comprehensive, focused studies into a multitude of areas surrounding *H. vesicula*. These areas include (but are not limited to) physiology in relation to salinity and temperature tolerances of adults and larvae, feeding rates in general but especially at different saturations of *I. galbana*, and general studies into the osmoregulatory processes present in adult and larval cephalaspidean gastropods *H. vesicula*. Should larvae of *H. vesicula* be found to be sensitive to reduced salinity, potential impacts such as population decline and decreases in distribution may be felt in the future. Other areas for future studies include the impact of decline of seagrasses, especially eelgrass due to SWD, on the abundance of *H. vesicula*; the impacts of the arisal of the invasive species *Haloa japonica* on abundance of *H. vesicula*; and how shifts in phytoplankton blooms due to changes in seasonality of maximum river discharge impact the feeding behaviors and growth of *H. vesicula*.

**Conclusion**

The environmental conditions present in the inter- and subtidal ecosystems of the Salish Sea are dynamic and variable in nature. Mesograzers such as *H. vesicula* present in these ecosystems are required to be tolerant to a number of environmental conditions including reduced

salinity events. Our study offers a first attempt at experimentally evaluating the presumably broad tolerance of *H. vesicula* veligers to severe changes in salinity, an attribute that may contribute to this species' future resilience in the face of climate change and other stressors.

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**References**


Bashevkin, S. M., Lee, D., Driver, P., Carrington, E., & George, S. B. (2016). Prior exposure to low salinity affects the vertical distribution of *Pisaster ochraceus* (Echinodermata: Asteroidea) larvae in haloclines. *Marine Ecology Progress Series, 542*, 123–140.

Bayne, B. L. (1965). Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). *Ophelia*, *2*(1), 1–47.

BioRender.com (2023). Retrieved from https://app.biorender.com/illustrations/6499c00e8a33fd224b99e1ff

Chaparro, O. R., Montiel, Y. A., Segura, C. J., Cubillos, V. M., Thompson, R. J., & Navarro, J. M. (2008). The effect of salinity on clearance rate in the suspension-feeding estuarine gastropod *Crepipatella dilatata* under natural and controlled conditions. *Estuarine, Coastal and Shelf Science, 76*(4), 861–868.

Dawkins, P., Eisenlord, M., Yoshioka, R., Fiorenza, E., Fruchter, S., Giammona, F., ... & Harvell, C. D. (2018). Environment, dosage, and pathogen isolate moderate virulence in eelgrass wasting disease. *Diseases of Aquatic Organisms, 130*, 51–63.

George, S. B., Navarro, E., & Kawano, D. (2021). Infrequent fluctuations in temperature and salinity may enhance feeding in *Pisaster ochraceus* (Asteroidea) but not in *Dendraster excentricus* (Echinoidea) larvae. *The Biological Bulletin, 241*(1), 77–91.

Gibson, G. D., & Chia, F. S. (1989). Embryology and larval development of *Haminoea vesicula* Gould (Opisthobranchia: Cephalaspidea). *The Veliger, 32*(4), 409–412.

Gibson, G. D., & Chia, F. S. (1995). Developmental variability in the poecilogonous opisthobranch *Haminaea callidegenita*: life-history traits and effects of environmental parameters. *Marine Ecology Progress Series, 121*, 139–155.

Gohar, H. A. F., & Aboul-Ela, I. A. (1957). The Development of Three Chromodorids (With the Description of a New Species) *Publications of the Marine Biological Station, Al-Ghardaqa, Egypt, 9*, 203–228.

Graham, O. J., Aoki, L. R., Stephens, T., Stokes, J., Dayal, S., Rappazzo, B., & Harvell, C. D. (2021). Effects of seagrass wasting disease on eelgrass growth and belowground sugar in natural meadows. *Frontiers in Marine Science, 8*, 768668.

Groth, M. (2021). Edge of Eden: Epifaunal Biodiversity of Eelgrass Patches in False Bay. http://hdl.handle.net/1773/48619.

Heck, K. L., & Valentine, J. F. (2007). The primacy of top-down effects in shallow benthic ecosystems. *Estuaries and Coasts, 30*, 371–381.

Khangaonkar, T., Nugraha, A., Xu, W., & Balaguru, K. (2019). Salish Sea response to global climate change, sea level rise, and future nutrient loads. *Journal of Geophysical Research: Oceans, 124*(6), 3876–3904.

Laing, I. (2002). Effect of salinity on growth and survival of king scallop spat (*Pecten maximus*). *Aquaculture, 205*(1-2), 171–181.

Little, C. (1981). Osmoregulation and excretion in prosobranch gastropods Part I: physiology and biochemistry. *Journal of Molluscan Studies, 47*(3), 221–247.

Malaquias, M. A. E., Condinho, S., Cervera, J. L., & Sprung, M. (2004). Diet and feeding biology of *Haminoea orbygniana* (Mollusca: Gastropoda: Cephalaspidea). *Journal of the Marine Biological Association of the United Kingdom, 84*(4), 767–772.

Malaquias, M. A. E., & Sprung, M. J. (2005). Population biology of the cephalaspidean mollusc *Haminoea orbygniana* in a temperate coastal lagoon (Ria Formosa, Portugal). *Estuarine, Coastal and Shelf Science, 63*(1-2), 177–185.

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

Muehlstein, L. K. (1989). Perspectives on the wasting disease of eelgrass *Zostera marina*. *Diseases of Aquatic Organisms, 7*, 211–221.

Muehlstein, L. K. (1992). The host-pathogen interaction in the wasting disease of eelgrass (*Zostera marina*). *Canadian Journal of Botany, 70*, 2081–2088.

Orth, R. J., Heck, K. L., & van Montfrans, J. (1984). Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries, 7*, 339–350.

Oskars, T. R., Too, C. C., Rees, D., Mikkelsen, P. M., Willassen, E., & Malaquias, M. A. E. (2019). A molecular phylogeny of the gastropod family Haminoeidae *sensulato* (Heterobranchia: Cephalaspidea): a generic revision. *Invertebrate Systematics, 33*(2), 426–472.

Potts, W. T. W. (1975). Excretion in the gastropods. *Fortschritte der Zoologie, 23*, 76–88.

Rasmussen, E. (1977). The wasting disease of eelgrass (*Zostera marina*) and its effects on environmental factors and fauna. *Seagrass Ecosystems*, 1–52.

Renn, C. (1936). The wasting disease of *Zostera marina*: A phytological investigation of the diseased plant. *The Biological Bulletin, 70*, 148–158.

Riche, O., Johannessen, S. C., & Macdonald, R. W. (2014). Why timing matters in a coastal sea: trends, variability and tipping points in the Strait of Georgia, Canada. *Journal of Marine Systems, 131*, 36–53.

Riisgard, H. U., Randløv, A., & Kristensen, P. S. (1980). Rates of water processing, oxygen

consumption and efficiency of particle retention in veligers and young post-metamorphic *Mytilus edulis*. *Ophelia*, *19*(1), 37–46.

Rivest, B. R. (1992). Studies on the structure and function of the larval kidney complex of prosobranch gastropods. *The Biological Bulletin*, *182*(3), 305-323.

Rosa, M., & Padilla, D. K. (2022). Determinants of food selection by bivalve larvae. *Invertebrate Biology*, *141*(2), e12366.

Zerebecki, R. A., & Sorte, C. J. (2011). Temperature tolerance and stress proteins as mechanisms of invasive species success. *PLOS one, 6*(4), e14806.

**Appendix: Laboratory Hatching of *Lacuna* spp.**

Attempts at collection and hatching of *Lacuna spp.* egg cases were made multiple times from 3 July 2023 to 4 July 2023. The rearing method was adapted from the oyster aquaculture upwelling systems, in which flow-through seawater spills through a covered opening to ensure a steady supply of food and oxygen. To collect newly hatched larvae of *Lacuna spp*., egg cases were placed into "tea-boys" (approx. 100 µm mesh size) within an additional glass container covered in 83µm mesh. Flowing seawater was allowed to overflow into an additional plastic container to ensure redundancy in larval capture and retention as well as to minimize contamination. After a period of two days with no hatching veligers, the choice was made to source larvae from *H. vesicula* instead for the purposes of this study. Additional observations on the *Lacuna spp.* egg masses indicated successful hatching and the appearance of free-swimming *Lacuna spp.* veligers. Methods of rearing *Lacuna spp.* were not previously well-defined and our preliminary attempt may be a method to expand on in the future.