THE ROLE OF ENERGY RESERVES ON MORTALITY AND STRESS-TOLERANCE THRESHOLDS DURING THE EARLY BENTHIC PHASE IN INTERTIDAL INVERTEBRATES

by

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ABSTRACT

For many intertidal marine invertebrate species, mortality rates during the early benthic phase are often very high and highly variable, which can lead to fluctuations in the abundance and distribution of adult invertebrate populations and the structure of the intertidal community. One of the major factors hypothesized to contribute to this mortality is insufficient energy reserves at the onset of the early benthic phase. Yet the role of energy reserves in regulating early survivorship has not been directly tested, and so remains speculative. This thesis explores both the direct and indirect impacts of energy reserves on mortality during the early benthic phase.

Recently settled or hatched individuals of six invertebrate species were collected from natural populations, maintained without food, and their survivorship was monitored. Contrary to expectations, starved individuals of all six species had high survivorship through the critical first 10 d of the early benthic phase, with half of the species experiencing <2% mortality, and the remaining three species experiencing only 6 – 12% mortality, and no difference in short-term survivorship was detected among starved individuals of three different size classes (a proxy for energy reserves) of *Nucella. ostrina* hatchlings. This study is the first to reveal that depleted energy reserves are not a primary direct cause of high mortality at the start of the early benthic phase, as had previously been hypothesized.

Indirect impacts of initial energy reserves were then examined by determining the extent to which energy reserves affect acute tolerance thresholds of early benthic phase individuals to two of the most challenging intertidal environmental stressors: desiccation and high emersion temperature. Levels of energy reserve were controlled by maintaining early benthic phase individuals of two invertebrate species without food. These were then exposed to an experimental range of emersion temperatures and desiccation periods. Duration of starvation, an indirect measure of energy levels, had no effect on tolerance to emersion temperature but did significantly impact tolerance to desiccation. This suggests that desiccation is likely a more important selective pressure on intertidal invertebrates and could favor the evolution of greater energy reserves at the onset of the early benthic phase.

Interestingly, acute tolerance thresholds to both stressors were significantly reduced for smaller individuals relative to those with greater body mass, suggesting that body size – and particularly the surface area: volume ratio may be a better indicator of vulnerability to abiotic stressors than energy content. Together, these findings reveal that depleted energy reserves at the onset of the early benthic phase can affect rates of early benthic phase mortality through indirect effects, by making individuals more vulnerable to certain stressors encountered in early benthic life. These findings are important because they reveal that although insufficient energy reserves are not a major source of early mortality, they can still impact recruitment and community structure in the intertidal zone.

KEYWORDS: Early benthic phase, mortality factors, recruitment, tolerance thresholds, starvation, energy reserves, emersion temperature, desiccation

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CHAPTER 1: General Introduction

Recruitment rates for many species of intertidal invertebrates are highly variable, which leads to significant spatial and temporal fluctuations in adult populations. Recruitment rates are determined by both the supply of larvae colonizing the habitat, and their success in reaching adulthood, and thus recruiting to the adult population (Keough and Downes 1982; Hadfield 1986). Larval (i.e. pre-settlement) mortality is high for many intertidal species (Jablonski and Lutz 1983; Gaines and Roughgarden 1985), and can impact recruitment when larval supply is low. However, larval supply is generally not limiting, and so its impact on population dynamics is often small (Hunt and Scheibling 1997; Fraschetti et al. 2003). Conversely, post-settlement mortality, or mortality during the early benthic phase, has been shown to heavily affect adult population abundance and distribution for many intertidal species (Connell 1985; Hughes 1990; Stoner 1990; Miller and Waldbusser 2016).

The early benthic phase (EBP) is the period immediately following settlement or hatching into the benthic environment, and it can encompass both pre- and post-metamorphic life stages (Sandee et al. 2016). The beginning of the EBP marks a major transition from the larval stage, that is either pelagic or contained within a protective egg capsule, to independent benthic life. This transition to the benthic intertidal zone is also associated with many biotic and abiotic challenges. For this reason, the EBP is characterized by very high mortality, with the most critical period occurring in the first 24 – 48 h (Guillou and Tartu 1994; Gosselin and Qian 1997; Hunt and Scheibling 1997). Many new cohorts experience 30 – 100% mortality in the first few days or weeks following settling or hatching into the benthic environment (Gosselin and Qian 1997; Hunt and Scheibling 1997). Mortality rates during this time frame are also highly variable, not only among species, but also among and within cohorts of the same species (Jarrett 2000; Phillips 2017). For example, mortality rates of newly settled barnacles range from 22% to 87% in the first 48 hours after settlement (Hunt and Scheibling 1997). Some of this variability may be attributed to different magnitudes of mortality factors, such as predation, competition, and abiotic stress, being experienced at different times, or in different locations (Gosselin and Qian 1997; Dahlhoff et al. 2001; Helmuth and Hofmann 2001). However, the specific causes of variability in mortality rates among and within cohorts are not fully understood. These findings suggest that individuals are most vulnerable at the very beginning of independent benthic life, and that understanding the rates and variability in mortality during the EBP may be critical for determining fluctuations in adult populations.

The beginning of independent benthic life is associated with many challenges that contribute to the high rates of EBP mortality. The most significant of these challenges are the abiotic factors linked to periods of emersion during low tide including elevated temperatures and desiccation stress (Gosselin and Chia 1995; Helmuth and Hofmann 2001; Miller et al. 2009; Shanks 2009). Benthic invertebrates possess many morphological, physiological, and behavioural adaptations to combat these abiotic stressors, but their natural tolerances can be exceeded by the environmental conditions they experience, resulting in extensive mortality (Foster 1971; Gosselin and Chia 1995; Miller et al. 2009; Jenewein and Gosselin 2013a; Jenewein and Gosselin 2013b). This is especially true for EBP individuals, as their small body size and lack, or underdevelopment, of protective structures makes them more vulnerable (Foster 1971; Gosselin 1997, Hamilton and Gosselin 2020).

Initial energy reserves held by the individual at the onset of EBP have been proposed as another major factor in determining success and survival through the critical first days (Gosselin and Qian 1997; Hunt and Scheibling 1997; Jarrett and Pechenik 1997), as individuals with greater energy reserves tend to grow faster and have lower mortality than their lower energy counterparts (Goulden et al. 1987; Phillips 2002; Emlet and Sadro 2006). It has been suggested that many benthic invertebrates may begin the EBP with relatively low energy reserves, as many species transition through energy intensive stages, such as metamorphosis, before beginning independent benthic life (Lucas et al. 1979; Wendt 2000; Thiyagarajan et al. 2003; Bryan 2004; Bennett and Marshall 2005). Many species also have a non-feeding phase directly before or following metamorphosis, during which they are entirely dependent upon their stored energy to complete metamorphosis and support metabolism throughout the first hours or days of early benthic life (Lucas et al. 1979; Pechenik et al. 1993; Gosselin and Chia 1994). Additionally, there is evidence that levels of energy reserves, as measured by organic content or size at hatching, at the onset of the EBP may vary considerably among individuals, and that energy stores are variable both among and within cohorts (Jarrett and Pechenik 1997; Jarrett 2003; Lloyd and Gosselin 2011). Beginning the EBP with low energy reserves greatly impacts the size and growth rates of individuals (Miller 1993; Pechenik et al. 1996; Thiyagarajan et al. 2005; Emlet and Sadro 2006), which can in turn have implications for survivorship (Moran 1999; Phillips 2002; Thiyagarajan et al. 2002; Phillips 2017). It is possible that some portion of individuals may begin the EBP with such critically low energy stores that they are near a minimum threshold necessary to survive and would either inevitably die of energy depletion very soon after metamorphosis, or need to quickly feed to replenish energy stores in order to survive. These direct effects of low energy on mortality remain largely untested but have the potential to play a vital role in determining which individuals survive through the first few days of the EBP.

The amount of energy held by the individual at the onset of the EBP has a variety of other effects on the individual's performance. Individuals with low energy reserves, as estimated by low organic content, small larval size, or poor feeding conditions, often grow more slowly and have a longer time to maturity than individuals with greater energy stores (Moran and Emlet 2001; Marshall et al. 2003; Thiyagarajan et al. 2003; Torres et al. 2016). Stored energy may also play an essential role in stress tolerance, as energy demands can increase during exposure to environmental stress (Sokolova et al. 2012), such that limited energy reserves may inhibit the physiological mechanisms utilized by intertidal invertebrates to tolerate adverse conditions in the intertidal zone. These physiological mechanisms include a reduced metabolic rate, the production of protective proteins such as heat shock proteins, and the synthesis of new proteins to replace those that have been denatured (Sokolova and Pörtner 2001; Somero 2002; Berger and Emlet 2007). Each of these responses have an associated energetic cost, and some portion of individuals may possess energy stores low enough that their physiological tolerances to these environmental stressors are impacted. In this way, low energy reserves could indirectly lead to increased likelihood of mortality. However, the influence of initial energy reserves on tolerance to environmental stressors in EBP invertebrates has been mostly speculative.

The overall goal of this thesis was to examine the extent to which initial energy reserves at the onset of the EBP impact the rates of EBP mortality in intertidal invertebrates. I examined both the direct effect of energy content in EBP individuals on their survival as well as the indirect effects of energy content, by determining the relationship between initial energy reserves and vulnerability to physiological stress. The specific objectives of this project were (1) to determine the proportion of individuals that begin the EBP with energy reserves that are close to or below a minimum energy threshold, (2) examine the effect of delayed feeding on the performance of EBP invertebrates, and (3) examine how initial energy content affects acute tolerance thresholds to two major environmental stressors: (a) high emersion temperature and (b) desiccation. Exploring these direct and indirect impacts of initial energy content on EBP mortality will aid our understanding of the factors that contribute to the high rates of EBP mortality among intertidal invertebrates, and thus the mechanisms that drive the variable population dynamics of many intertidal species.

Chapter 2 of this thesis focuses on the direct effects of initial energy content on mortality and performance during the first days and weeks of the EBP. To assess what proportion of individuals begin the EBP close to or below a minimum energy threshold, individuals of six invertebrate species were maintained in the laboratory without food for up to 100 d and their survivorship throughout this period was monitored. To examine the effect of a delay of feeding on performance, groups of three intertidal species were exposed to different durations of starvation (0 – 50 d) before providing individuals with food. Each individual was measured at the end of the starvation period and again after a 30 d feeding period, and the impact of different durations of feeding delay on survivorship and growth was determined.

Chapter 3 examines the indirect effects of energy on EBP mortality by determining the relationship between initial energy content and physiological tolerance to the two most important environmental stressors in the intertidal zone: desiccation and elevated temperature. EBP individuals of two species were collected and groups with different quantities of energy reserve were established. Since direct measurement of energy content in live individuals was not possible, two proxy methods were used to quantify energy reserves: duration of starvation prior to experimentation, and initial body mass. The various energy treatment groups were then exposed to a range of temperatures and durations in a low humidity environment in a series of controlled laboratory experiments. Acute tolerance thresholds to each stressor were then determined and compared between the different energy treatment groups.

Chapter 4 provides a summary of the major findings of this thesis, implications of the findings for policy, and possible directions for future study.

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CHAPTER 2: Role of depleted initial energy reserves in early benthic phase mortality of six marine invertebrate species[1]

INTRODUCTION

Recruitment rates of benthic marine invertebrates are highly variable and can heavily impact the abundance and distribution of adult populations (Connell 1985; Stoner 1990; Hunt and Scheibling 1998; Miller and Waldbusser 2016). This is largely due to high rates of mortality during the first few days of independent benthic life (Thorson 1966; Keough and Downes 1982; Hadfield 1986; Osman et al. 1989; Guillou and Tartu 1994; Gosselin and Qian 1997; Hunt and Scheibling 1997; Ellien et al. 2000), a period identified as the early benthic phase (EBP) that encompasses early juvenile life and may also include pre-metamorphic larval life (Sandee et al. 2016). In addition, EBP mortality rates are highly variable not only among species, but also among and within cohorts of the same species (Gosselin and Qian 1996; Jarrett and Pechenik 1997; Jarrett 2000; Phillips 2017). The mechanisms responsible for the high rates and extensive variability in EBP mortality are not yet fully understood and remain an area of active research.

A factor that has been proposed as a major cause of mortality during early benthic life is depleted energy reserves, when individuals begin the EBP with minimal energy stores (Gosselin and Qian 1997; Hunt and Scheibling 1997; Jarrett and Pechenik 1997). Energy reserves at the start of independent benthic life are known to affect other relevant aspects of EBP performance, including the subsequent size and growth rate of individuals throughout juvenile life (Miller 1993; Pechenik et al. 1996; Miller and Emlet 1999; Pechenik et al. 2002; Thiyagarajan et al. 2003; Thiyagarajan et al. 2005; Emlet and Sadro 2006). Previous studies have also revealed a correlation between initial body size and mortality, with smaller individuals experiencing higher rates of mortality than larger EBP individuals (Moran 1999; Phillips 2002; Thiyagarajan et al. 2002; Phillips 2017). Those studies have suggested the relationship between body size and mortality may be due to increased vulnerability to predation, competition, or abiotic stressors, but it might also be due to insufficient initial

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energy reserves directly causing EBP mortality. While these indirect lines of evidence are consistent with the hypothesis that depleted initial energy reserves directly cause EBP mortality, the hypothesis remains untested.

For the depleted initial energy hypothesis to be correct, there must be variation in initial energy reserves, with some individuals having relatively low energy levels. Accordingly, variation in organic content, a proxy for energy reserves, at the onset of the EBP among individuals does occur in at least some species, with some individuals beginning the EBP with relatively low organic content (Jarrett and Pechenik 1997; Moran and Emlet 2001). This variation is largely due to differences in maternal provisioning or in food availability to the larvae, as well as competent larvae of many species transitioning through energetically demanding periods of development prior to the onset of the EBP (Lucas et al. 1979; Thiyagarajan et al. 2003). The most energetically demanding of these developmental periods is metamorphosis, which involves extensive rearrangement of tissues and loss or replacement of numerous body structures (Wendt 2000; Thiyagarajan et al. 2002, 2003; Bryan 2004; Bennett and Marshall 2005). Additionally, development in some species includes a substantial non-feeding phase immediately before, during, or following the onset of the EBP (Anderson 1994; Gosselin and Chia 1994). During these non-feeding periods, and until metamorphosis is completed and feeding begins, individuals are solely reliant upon their internal energy stores for resting and active metabolism (Lucas et al. 1979; Pechenik et al. 1993; Gosselin and Chia 1994). Accordingly, it has been suggested that some portion of individuals may begin the EBP with such critically low energy stores that they are near a minimum threshold necessary to survive (Jarrett and Pechenik 1997); those individuals would either inevitably die of energy depletion very soon after metamorphosis, or need to quickly feed to replenish energy stores to survive. We illustrate this hypothesis in Figure 2.1, representing two hypothetical frequency distributions of energy reserves among EBP individuals as well as a theoretical minimum threshold of energy reserves represented by the vertical line. Curve A represents the null hypothesis, whereby all individuals have sufficient energy to survive, in which case the depletion of energy reserves would not be a direct cause of EBP mortality. Conversely, curve B represents the current hypothesis, that a proportion of individuals begin the EBP below the minimum energy reserve threshold, and thus inevitably die during or shortly after metamorphosis. In addition, if the hypothesis represented by curve

B is correct, then a substantial proportion of EBP individuals would have energy reserves only slightly larger than the minimum threshold; these individuals would need to feed immediately to survive or would otherwise drop below the threshold and die. Thus, a goal of the present study was to test these two hypotheses to establish the role of energy reserves as a direct cause of EBP mortality.

While EBP mortality rates are often very high, they are also highly variable among and within populations (Gosselin and Qian 1997; Hunt and Scheibling 1997; Jarrett 2000; Phillips 2017). The hypothesis that variation in energy reserves (bell curves in Figure 2.1) is a cause of the variability in EBP mortality is partly supported by evidence (Jarrett and Pechenik 1997; Phillips 2006) of natural variation among individuals in the amount of organic content or lipid stores present at the onset of the EBP. Internal energy reserves can vary depending on the source of energy, and in some cases upon the quantity of food available prior to the beginning of the EBP. For species with planktotrophic larvae, EBP energy reserves originate primarily from particulate food consumed during their larval phase. For these species, energy reserves can vary among individuals depending on the quantity and quality of food they encounter (Jarrett 2003; Moran and Manahan 2004), whether they transition through a non-feeding phase, and whether they encounter conditions that delay settlement (Pechenik et al. 1993; Phillips 2002; Thiyagarajan 2003, 2007). For direct developing species, EBP energy reserves originate entirely from maternal provisioning, and thus may vary with the condition of the maternal parent (Moran and McAlister 2009; van der Sman et al. 2009) or with the genetic programming of the population or species (Rivest 1983; Allen and Marshall 2013). In some cases, unfertilized nurse eggs are provided to the embryos to consume as they develop; for these species, energy levels can further vary among individuals of the same cohort depending on the quantity of nurse eggs consumed before hatching (Jablonski and Lutz 1983; Lloyd and Gosselin 2011; Marko et al. 2014). All these differences, both interspecific and intraspecific, result in individuals beginning the EBP with vastly different amounts of internal energy reserves, and this might play a substantial role in determining which individuals survive through the first few days of independent benthic life. Additionally, any of these factors has the potential to shift the frequency distribution of energy reserves to the left or right, as depicted by the bell curves A and B in Figure 2.1, or even alter the shape of these curves and thus also affect the proportion of the individuals falling below the minimum threshold for survival.

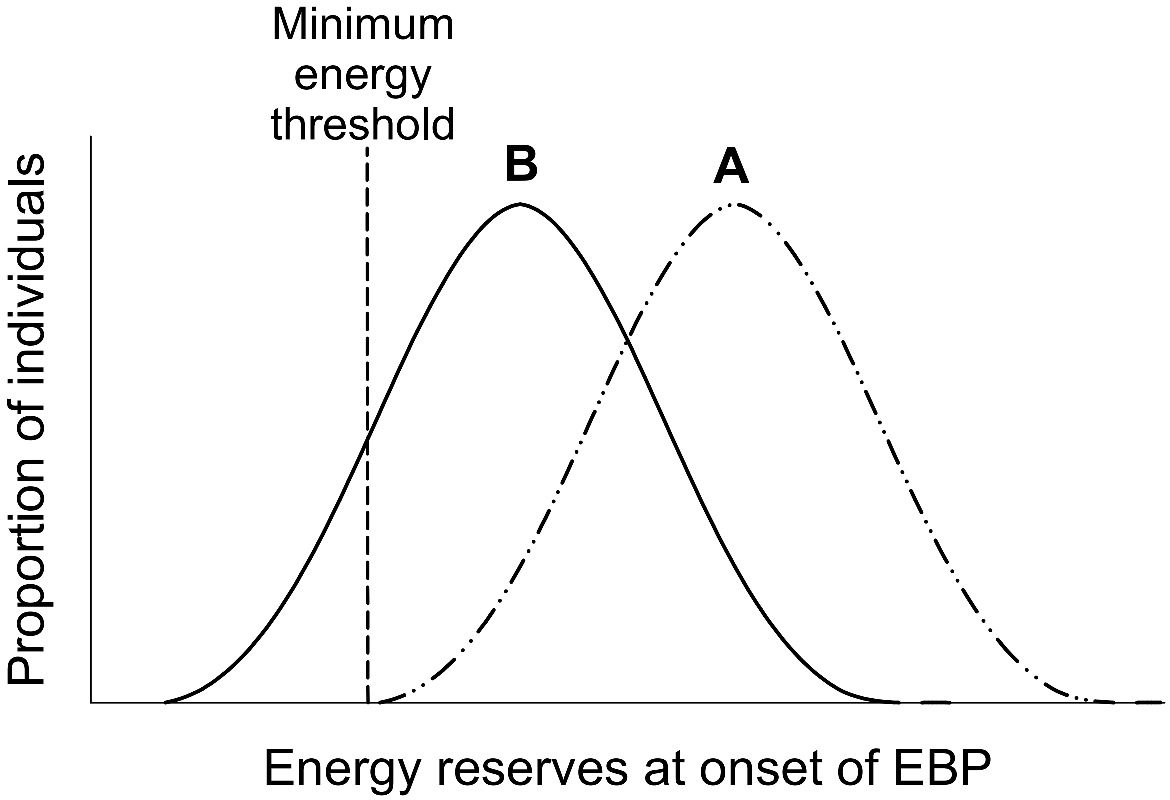

Figure 2.1. Two hypothetical frequency distributions (bell curves) of energy reserves among individuals at the onset of the EBP. The dashed vertical line represents a minimum energy threshold required for EBP survival.

For individuals starting the EBP with only slightly more internal energy than the minimum threshold, rapidly accessing a new food source to replenish their energy reserves could be vital to their survival. A delay in feeding at this point might drop them below the threshold, and thus push them beyond their "point of no return" (commonly referred to as PNR in other publications), a pivotal point beyond which animals are unable to recover even if provided with food (Blaxter and Hempel 1963; Moran and Manahan 2004). For individuals that do not feed before this point, both their survivorship and ability to grow are likely to be impaired (Takami et al. 2000; Roberts et al. 2001; Chen et al. 2005). The existence and timing of a point of no return has been studied for the larval phase of several species of intertidal invertebrates (Moran and Manahan 2004; Yan et al. 2009; Gebauer et al. 2010; Espinoza et al. 2017) but has not been well established for EBP individuals of most species. Determining the starvation point at which an individual's energy reserves are so heavily depleted that they are unable to recover may be useful for understanding how energy reserves impact EBP survivorship and growth.

The main purpose of this study was to determine the role of energy content in regulating survivorship during the critical first days of independent benthic life, thus helping to better understand the mechanisms controlling EBP mortality, and thus recruitment, of benthic invertebrates. The first specific objective of the study was (1) to establish whether energy reserves of EBP individuals could be experimentally controlled by starvation; this was addressed by determining the relationship between total organic content of EBP individuals and the duration of starvation they had experienced. This was followed by two additional specific objectives: (2) to test the hypothesis (Figure 2.1, curve B) that a large proportion of individuals begin the EBP with critically low energy stores such that they either die or require rapid access to food to survive the first days of independent benthic life, and (3) to examine the effect of delayed feeding on the performance (growth and survival) of EBP invertebrates over a period of several weeks.

METHODS

Study species

This study examined the role of initial energy reserves on survivorship in six species of benthic intertidal invertebrates. All animals used in this study were from wild populations and had developed through the embryonic and planktonic larval phases in the field. Species were selected based on sufficient availability of early benthic phase (EBP) specimens in the field. All six species are abundant on the west coast of Vancouver Island, Canada. The barnacles *Balanus glandula* Darwin 1854 and *Chthamalus dalli* Pilsbry 1916, as well as the mussel *Mytilus trossulus* Gould 1850, colonize exposed hard substrata in the mid to high intertidal zone. *Nucella ostrina* Gould 1852 and *Nucella lamellosa* Gmelin 1791 are predatory snails that inhabit the mid-intertidal zone. Finally, *Petrolisthes cinctipes* Randall 1840 and *P. eriomerus* Stimpson 1871 are small (~2 cm) porcellanid crabs that inhabit cryptic habitats, primarily the undersides of large rocks (Jensen 1989), in the low to mid intertidal zone; EBP individuals of these two species could not be readily distinguished, so these were grouped together and designated as *Petrolisthes* spp.

These species have different sources and quantities of energy reserves at the beginning of the EBP. *Nucella ostrina* and *N. lamellosa* are lecithotrophic, and thus depend entirely on maternal provisioning for EBP energy reserves. The four other species, however, have planktotrophic larvae, and a large portion of their EBP energy reserves come from the food they consume during the larval stage. Additionally, *B. glandula* and *C. dalli* have a non-feeding cyprid larval stage prior to metamorphosis, and thus use up some energy stores prior to the EBP, whereas *M. trossulus* and *Petrolisthes* spp. are able to feed up to and throughout metamorphosis. By including species with different developmental histories, this study explored how EBP survivorship is impacted by energy reserves across species with different modes of energy acquisition prior to entering the EBP.

Study site and collection of animals

Research was conducted at the Bamfield Marine Sciences Centre (BMSC) on the west coast of Vancouver Island from May to September of 2019. Animals were collected from five rocky intertidal shore sites located in Barkley Sound (Figure 2.2). Sites were selected based on the availability of EBP animals of each species. Collected animals were brought to and maintained at BMSC, where all experiments were conducted.

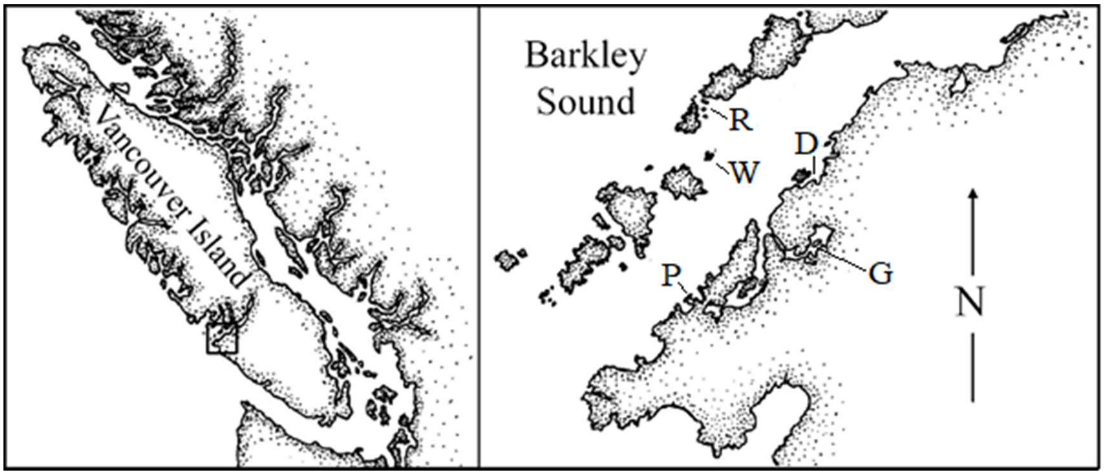

Figure 2.2. Map of field sites in Barkley Sound near Bamfield, British Columbia: Ross Islets (R), Wizard Islet (W), Prasiola Point (P), Dixon Island (D), and Grappler Inlet (G). Map modified from Gosselin and Chia (1995).

Juvenile barnacles (*B. glandula* and *C. dalli*) were collected as described by Sandee et al. (2016) and Hamilton & Gosselin (2020). Small rocks (5 – 10 cm diameter) were gathered from the intertidal zone and brought to the laboratory where all small settlers were dislodged under a dissecting microscope, and a perimeter was marked on the rock around the cleared area with nail polish. Marked rocks were then returned to the field and left for 48-72 h (4-6 tidal cycles) to allow for new settlement, after which rocks were recovered and examined for newly settled barnacles. At that age, barnacles would still be reliant upon energy reserves obtained during the larval phase, as juvenile barnacles do not begin to feed until 2 – 5 d post-metamorphosis (Rainbow and Walker 1977). Ripe egg capsules of both *Nucella* species (*N. ostrina* and *N. lamellosa*) were collected from intertidal substrata using fine-tipped forceps as in Gosselin & Chia (1995). Egg capsules were returned to the laboratory and held in small cages in aerated seawater for 72 h, after which all newly hatched juvenile snails were removed and retained for experiments. EBP *M. trossulus* were extracted from tufts of the filamentous algae *Cladophora columbiana*. Extracted mussels were measured and only recently settled juveniles measuring 250 – 600 µm shell length (Phillips 2017) were retained for experiments. *Petrolisthes spp.* megalopae were collected from the underside of large rocks in the intertidal zone. Megalopae were carefully removed with a pair of soft insect forceps and transported to BMSC in small containers. All animals were brought to the laboratory within 3 h of collection.

Procedures for rearing EBP individuals without food

To experimentally control the energy reserves of EBP individuals, most experiments in this study involved rearing EBP individuals without food. In all experiments described below, the following procedures were used to rear EBP individuals of each species while ensuring they did not have access to food. All animals were held in seawater filtered to 1.0 µm to ensure no food particles were available for feeding. *M. trossulus*, however, can capture particles < 1.0 µm (Strohmeier et al. 2012), so filtered water used for this species was also autoclaved and then vacuum-filtered to 0.45 µm. Rocks containing newly-settled barnacles were kept in 1 L containers, each with an air stone for aeration. All other animals were kept in small cages (1.5 – 600 mL) with mesh-covered cut-out windows to allow for water flow while preventing escapement. Water was maintained at 15 – 17°C, which approximates average sea surface temperatures during the summer months (Iwabuchi and Gosselin 2019). Filtered water was replaced every second day and holding containers and cages were rinsed and scrubbed to prevent the growth of algae.

Relationship between organic matter content in EBP individuals and duration of starvation

To determine if starvation could be used as a method for predictably depleting energy reserves in EBP individuals, we tested the hypothesis that organic content decreases as a function of the duration of starvation. This experiment was carried out with *N. ostrina* hatchlings, and total organic matter (OM) was measured by ash-free dry weight (AFDW). AFDW is an effective, albeit simple, indicator of energy content (Moran and McAlister 2009). It does not, however, reflect the energetic value of any single type of organic matter, such as triacyglycerols (Podolsky 2002), which are the primary source of metabolizable energy for marine invertebrates (Sewell 2005; Whitehill and Moran 2012). Thus, it is assumed that the first OM to be depleted by starved EBP individuals will be these energetic lipids, followed by other forms of OM, resulting in a progressive decrease in AFDW.

This experiment was carried out twice, using two different batches of hatchlings. Batch 1 was collected from Ross Islets on July 17, 2019 and Batch 2 was collected from Prasiola Point on July 26, 2019. Only intermediate-sized *N. ostrina* hatchlings (1.11 – 1.40 mm shell length) were used. Hatchlings from each batch were kept in 50 mL cages, with 100 – 200 individuals in each cage and maintained without food following the procedure stated above. Every 10 d, beginning immediately after hatching, samples of 52 – 60 unfed *N. ostrina* hatchlings from each batch were removed from their respective holding cages. These were briefly rinsed in distilled water to remove adhering salts and then stored at -80°C. AFDW of each sample was obtained using a procedure adapted from Pechenik et al. (1993). For each sample of 52-60 frozen snails, the sample (all snails together) was placed in a pre-weighed foil pan and dried at 65°C for 48 h. After drying, the pan was weighed on a Fisher Scientific accuSeries balance with an instrument precision of ±0.01 mg to quantify total dry weight and then placed in a muffle furnace at 500°C for 5 h. The pan was then placed in a sealed desiccator containing silica gel desiccant for 20 min to cool, and then reweighed to determine ash weight (i.e. the weight of the sample without the OM). AFDW, a measure of OM content of each sample, was calculated by subtracting the ash weight from the initial dry weight. Ash weight consisted almost entirely of the shell, and shell weight was not expected to decline with starvation, so ash weight was used as an indicator of initial snail body mass prior to starvation. The OM estimate (AFDW) was therefore divided by the ash weight to obtain the OM:ash weight ratio; this ratio standardized results for all snails, accounting for any minor differences in initial body size among individuals.

Ability of EBP individuals to survive exclusively on their initial energy reserves

Survivorship of EBP individuals unable to replenish energy reserves

This experiment determined, for each of the six species, the proportion of individuals that begin the EBP with energy reserves close to the minimum threshold required for survival. EBP individuals were collected on several dates throughout the spring and summer, and each batch of individuals was tested separately as a replicate trial. For each species, 2-8 batches of individuals were tested, each batch consisting of 9-200 individuals (Appendix A). The number of batches collected for each species, as well as the number of individuals in each batch, were dependent on the availability of EBP individuals in the field. All animals were held in 50 mL cages without food, as described above, for up to 100 d starting immediately after the onset of the EBP. Every 10 d, survival was assessed by examining all individuals for movement under a dissecting microscope. If an animal did not show any signs of movement within 2 min, their tissues were gently contacted with a probe and they were observed for an additional 2 min. If no movement was observed at this point, the individual was recorded as dead and removed from the experiment. The proportion of individuals surviving each 10 d period was recorded.

Influence of initial body mass on survivorship of EBP individuals unable to replenish energy reserves

There is a strong correlation between body mass and quantity of organic matter among EBP invertebrates (Moran and Emlet 2001). Specifically, larger individuals have larger stores of lipids (Phillips 2002; Emlet and Sadro 2006), which are the primary metabolizable energy source for many intertidal species (Thiyagarajan et al. 2002; Sewell 2005; Whitehill and Moran 2012). Thus, body mass was used as a proxy for energy reserves to determine their influence on survivorship. To determine how the ability to survive periods of starvation is influenced by body mass at the start of the EBP, the survivorship of three size classes of newly hatched *N. ostrina* were examined. Most *N. ostrina* egg capsules contain 7-20 fertilized embryos that will hatch into juvenile snails, as well as 500 – 600 unfertilized nurse eggs that the embryos consume as they develop inside the capsule (Lloyd and Gosselin 2011; Marko et al. 2014). *N. ostrina* hatchlings consume different numbers of nurse eggs while in the capsule, and therefore hatchlings range considerably in size upon emergence, with larger hatchlings having consumed more nurse eggs and thus having greater energy reserves than smaller hatchlings. Newly hatched *N. ostrina* were sorted into three shell length (SL) categories: small (0.81 – 1.10 mm SL), medium (1.11 – 1.40 mm SL), and large (1.41 – 1.80 mm SL). These SL measurements were then converted to body mass (wet weight, WW) using a regression equation for *N. ostrina* from Hamilton & Gosselin (2020) (Table 2.1). Eight batches of hatchlings were collected throughout the spring and summer; each batch was sorted into the three size classes, and each size class of a given batch contained 11-217 hatchlings (Appendix A). In some batches, certain size classes were not included in this experiment because too few individuals of the size class were available. Hatchlings were held in 50 mL cages without food for up to 70 d, beginning at the time of hatching (i.e. the onset of EBP). Every 10 d, survival was assessed by examining all individuals under a dissecting microscope, as described above, and the number of survivors of each size class was recorded.

Table 2.1. Shell lengths (SL) and corresponding body masses (wet weight, WW) for *N. ostrina* hatchlings. The conversion equation is: WW = 2.98 * log(SL) - 3.84, from Hamilton & Gosselin (2020).

Size class	Shell length (SL) in mm	Body mass (WW) in mg
Small	0.81 – 1.10	0.074 – 0.192
Medium	1.11 – 1.40	0.197 – 0.394
Large	1.41 – 1.80	0.402 – 0.833

Ability to recover after delayed feeding in EBP invertebrates

The ability of EBP individuals to recover from periods of starvation was examined in three species: *B. glandula*, *C. dalli*, and *N. ostrina*. EBP individuals of each species were reared without food, beginning at the onset of EBP, for various durations (0, 10, 20, 30, 40 or 50 d) before being fed for 30 d. Growth during the 30 d feeding period was determined by measuring the size (rostro-carinal diameter for barnacles, SL for *N. ostrina*) of each individual at the end of the starvation period (i.e. just before feeding began), and then again at the end of the 30 d feeding period. The number of individuals that died during the 30 d feeding period was also recorded for each group. All individuals of the same species were within a narrow size range at the beginning of the experiment. The experimental design for each of the three species was as follows: 6 duration of starvation treatments per species X 10 – 15 individuals per starvation treatment.

During the feeding period, *B. glandula* and *C. dalli* were held in 1 L aerated containers and fed a mixture of diatoms (*Chaetoceros muelleri* and *C. gracilis*) and flagellates (*Tertaselmis* spp.) that had been reared in the laboratory. Algae were provided at a total concentration of $1x10^6$ cells/mL, determined by counting algal cells with a hemocytometer. Water and algae were changed every second day. To feed *N. ostrina* hatchlings, each individual was placed in a small (1.5 mL) cage with 10 juvenile mussels (*M. trossulus*), a preferred prey species for EBP *N. ostrina* (Gosselin and Chia 1994). *N. ostrina* feed by drilling a small hole through the shell of their prey with their radula, making it possible to determine whether the hatchling had fed.

To assess the effect of delayed feeding on the performance of EBP invertebrates, individuals were categorized as either recovering (i.e. alive and showing growth) or not recovering (i.e. dead, or alive but with no growth) following the 30 d feeding period. The percent of individuals that were recovering in each starvation treatment was then calculated for each species. Additionally, the growth (i.e. change in size) of each EBP individual during the feeding period was calculated to assess the effects of delayed feeding on the ability to resume growth when food becomes available.

Data analysis

In all experiments, survivorship data followed a binomial distribution. All survivorship data were analyzed using generalized linear models (GLM) with a binomial link function to determine the starvation LD_{50} for each species, and the % survivorship after 5 d starvation and the starvation LD_{20} for each size class of *N. ostrina*. The fit of each GLM model was tested by comparing the residual deviance to the residual degrees of freedom, which indicated goodness of fit for each model (i.e. residual deviance / residual df < 1). One-way ANOVA and the Tukey HSD multiple comparisons test were used to compare the % survivorship after 5 d starvation and the starvation LD_{20} among the three size classes of *N. ostrina* hatchlings. The Shapiro-Wilk normality test and the Bartlett test of homogeneity of variances were used to assess the assumptions of ANOVA. All statistical analyses were conducted using R statistical software (version 3.5.0) (R Core Team 2018).

RESULTS

Relationship between organic matter content in EBP individuals and duration of starvation

The relationship between organic matter (OM):ash weight ratio and duration of starvation starting at the onset of the early benthic phase (EBP)(Figure 2.3) was effectively described by an exponential decay model: $y = y0 + a * e^{-bx}$, where *y0* is the intercept, a is the lower limit reached for y, x is the duration of starvation, and b is the decay coefficient. The OM:ash weight ratio of *Nucella ostrina* was significantly related to the duration of starvation in both batches of *N. ostrina* hatchlings (Batch 1: F = 583.95, df = 6, $R^2 = 0.996$, $p < 0.001$; Batch 2: F = 175.45, df = 5, $R^2 = 0.989$, $p = 0.006$). Based on this analysis, approximately half the OM content of an individual was utilized by day 10 of starvation (Batch 1: 51.05%, Batch 2: 49.35%).

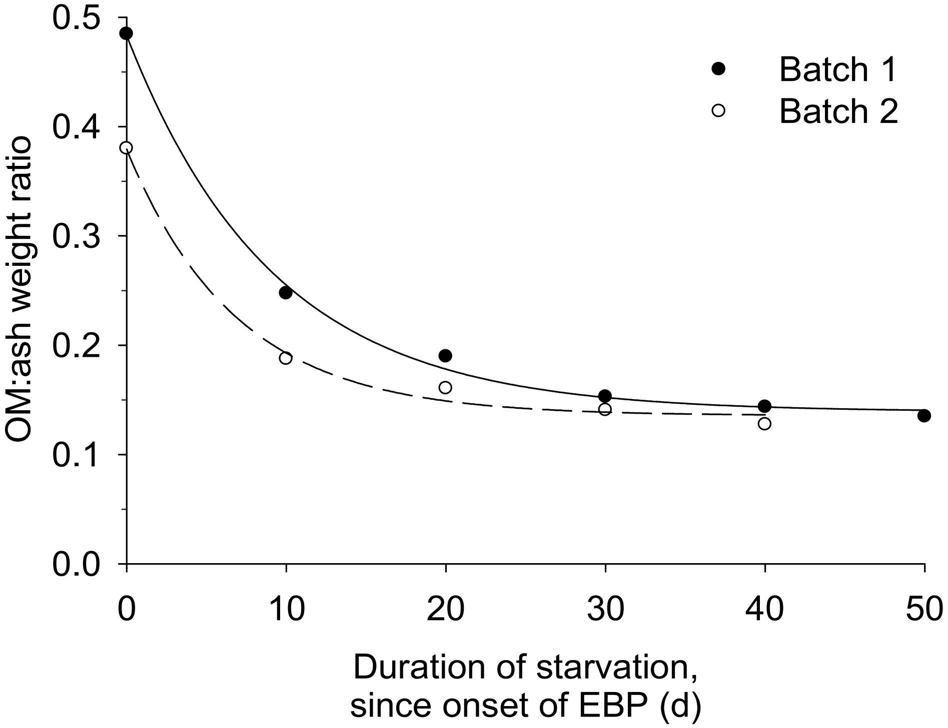

Figure 2.3. Relationship between OM:ash weight ratio and the duration of starvation, starting at the onset of the EBP, for *Nucella ostrina* hatchlings. OM is used as an indicator of total energy content and ash weight is used as an index of initial total body mass. Each value represents a combined sample of 52 - 60 individuals. Each batch represents a single collection of hatchlings from the field.

Ability of EBP individuals to survive exclusively on their initial energy reserves

Survivorship of EBP invertebrates unable to replenish energy reserves

Survivorship was impacted by the duration of starvation experienced by the individuals (e.g. *Balanus glandula*, Figure 2.4). The relationship, assessed using generalized linear model (GLM) with a binomial link function, was significant in four of the six species (Table 2.2). For *Mytilus trossulus* and *Petrolisthes spp.* the relationship was not quite significant, likely due to the modest sample sizes, as only two batches of individuals were collected for each of these species, whereas 5 – 9 replicate batches were examined in each of the other four species. The trends of decreasing survivorship as a function of duration of starvation for *M. trossulus* and *Petrolisthes spp.* were nevertheless similar to those of the other four species (Appendix A).

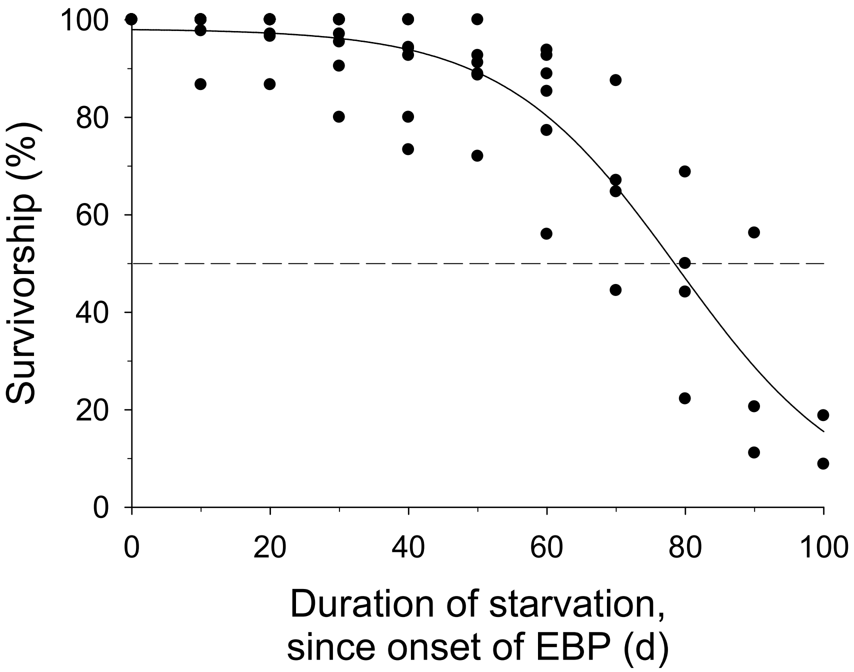

Figure 2.4. Percent survivorship of starved *B. glandula* as a function of the duration of starvation, starting at the onset of the EBP. The point where the curve intersects the dashed line represents the starvation LD50.

The duration of starvation that is lethal to 50% of individuals (starvation LD_{50}), as determined from the above GLM analyses, varied substantially among species (Table 2.2). Starvation LD_{50} ranged from 27.9 ± 4.75 d (average ± SE) for *Nucella lamellosa* to 78.4 ± 7.59 d for *B. glandula*. When survivorship of all six species during the first 10 d of starvation was combined in a single regression analysis, the percent survivorship declined significantly, but moderately (Linear regression: $F = 7.589$, $n = 6$, $R^2 = 0.26$, $p = 0.012$) (Figure 2.5). As a result, survivorship remained high throughout these first 10 d. After 5 d of starvation, average survivorship was >90% in all species; after 10 d starvation average survivorship was still >90% in four of the six species (*B. glandula*, *C. dalli*, *N. ostrina*, and *Petrolisthes spp*), and >85% for the remaining two (*N. lamellosa* and *M. trossulus*).

Table 2.2. Starvation LD50 and associated standard error (SE) from generalized linear model (GLM) analysis of survival as a function of duration of starvation for each of the 6 species. The z statistic represents the strength of the relationship between the % survivorship and the duration of starvation; df = degrees of freedom.

Species	Starvation LD_{50} (d)	SE	z statistic	df	p
B. glandula	78.4	7.59	-3.40	62	< 0.001
C. dalli	69.3	7.20	-3.23	42	0.001
N. ostrina	55.9	9.66	-2.56	51	< 0.001
N. lamellosa	27.9	4.75	-2.99	30	0.003
M. trossulus	58.3	14.28	-1.76	16	0.079
Petrolisthes spp.	50.8	5.70	-1.59	12	0.113

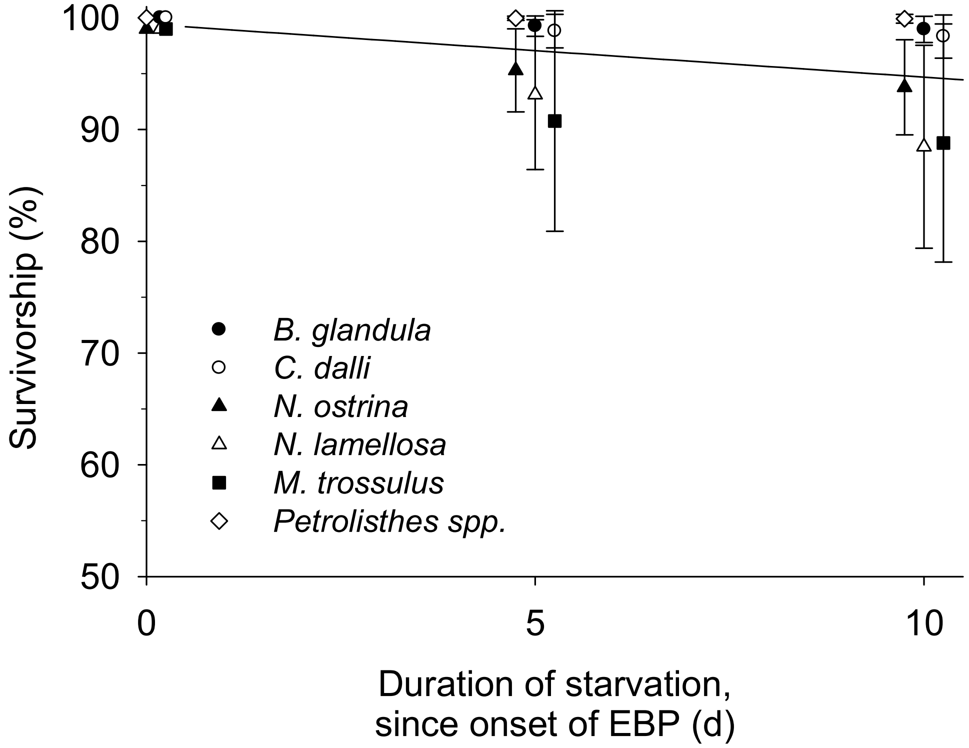

Figure 2.5. Survivorship of individuals starved for the first 10 d since the onset of EBP for six benthic intertidal species. Each point represents the average % survivorship and associated standard errors calculated from GLMs for each species (Table 2.1).

Influence of initial body mass on survivorship of EBP individuals unable to replenish energy reserves

Survivorship was significantly affected by the duration of starvation for each batch of all three size classes of *N. ostrina* hatchlings (Table 2.3). The shape of this relationship, however, differed between size classes. The impact of starvation on survivorship of *N. ostrina* in each size class during the critical first days of the EBP was examined using two different approaches: (1) determining the proportion of individuals that survived 5 d of starvation after hatching, and (2) determining the number of days of starvation required to cause 20% mortality of hatchlings (starvation LD_{20}). The starvation LD_{20} was used instead of the LD_{50} because some of the Medium and Large size class batches did not reach 50% mortality during the time frame of the experiment.

Table 2.3. Results of the GLM analysis of percent survivorship as a function of duration of starvation for each size class from each batch of *N. ostrina* hatchlings. Z statistics indicate the fit of the relationship between duration of starvation and % survivorship; df = degrees of freedom. The binomial link function was used in all GLMs.

Batch	Size class	n	Estimate	SE	z value	df	p
1	Small	13	-0.056	0.010	-5.37	8	<0.001
	Medium	29	-0.034	0.015	-2.34	5	0.020
2	Small	106	-0.092	0.006	-15.78	7	<0.001
	Medium	90	-0.078	0.007	-11.20	7	<0.001
	Large	217	-0.036	0.004	-8.22	7	<0.001
3	Medium	37	-0.088	0.012	-7.14	4	<0.001
	Large	56	-0.045	0.006	-7.16	7	<0.001
4	Large	59	-0.050	0.007	-6.87	6	<0.001
5	Small	11	-0.108	0.028	-3.87	5	<0.001
	Medium	25	-0.033	0.012	-2.67	5	0.008
	Large	15	-0.036	0.018	-2.03	5	0.042
6	Small	57	-0.088	0.009	-9.05	5	<0.001
	Medium	152	-0.058	0.006	-10.17	5	<0.001
	Large	76	-0.051	0.009	-5.99	5	<0.001
7	Small	34	-0.102	0.014	-7.57	5	<0.001
	Medium	115	-0.042	0.008	-5.33	5	<0.001
	Large	85	-0.080	0.016	-5.12	5	<0.001
8	Small	68	-0.077	0.014	-5.38	4	<0.001
	Large	34	-0.105	0.034	-3.05	4	0.002

The three size classes survived equally well without food in the short-term, but their fate eventually differed as time without food became extended. Survivorship after 5 d of starvation did not differ significantly among the three size classes (one-way ANOVA: F = 1.207, df = 16, $p = 0.325$), with survivorship ranging from 93 - 96 % (Figure 2.6A). The starvation LD_{20}, however, did differ significantly among size classes (one-way ANOVA: F = 5.718, df = 16, $p = 0.013$) (Figure 2.6B). The LD_{20} for the Small size class was significantly shorter than for the Large size class (Tukey HSD: $p = 0.010$), whereas the LD_{20} for the Medium size class was not significantly different from either the Small ($p = 0.132$) or the Large ($p = 0.453$) size classes.

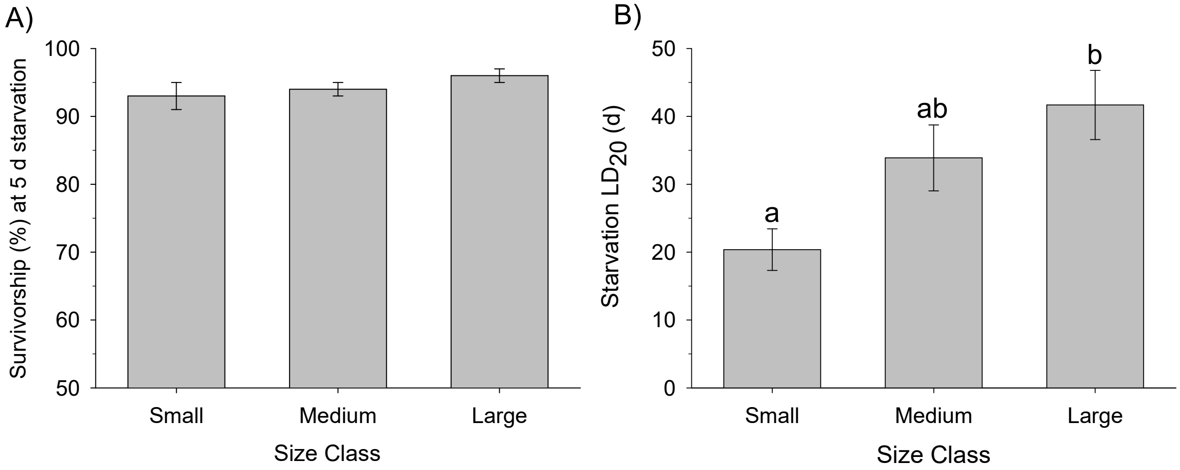

Figure 2.6. Effect of initial body size of *Nucella ostrina* hatchlings on (A) the survivorship at 5 d and (B) the starvation LD20. Small = 0.80 – 1.10 mm shell length; Medium = 1.11 – 1.40 mm shell length; Large = 1.41 – 1.80 mm shell length. Each mean value and associated standard error are calculated from generalized linear models (GLM) for 6 – 7 batches of each size class. Different letters above the bars indicate values that were significantly different based on Tukey HSD tests ($p < 0.05$).

Ability to recover after delayed feeding for EBP invertebrates

The duration of starvation experienced before being offered food had a significant or nearly significant effect on the percent of individuals able to recover (i.e. survive and grow during the 30 d feeding period) for *C. dalli* (Linear regression: F = 17.56, n = 6, $R^2 = 0.81$, $p = 0.014$) and *N. ostrina (*F = 7.08, n = 6, $R^2 = 0.64$, $p = 0.056$) (Figure 2.7). The duration of starvation resulting in only 50% of individuals being able to recover (i.e. the recovery LD_{50})

was >20 d in both species, but was somewhat longer in *C. dalli* than in *N. ostrina* (*C. dalli*: 28.6 ± 4.2 d (SE); *N. ostrina*: 20.9 ± 6.6 d).

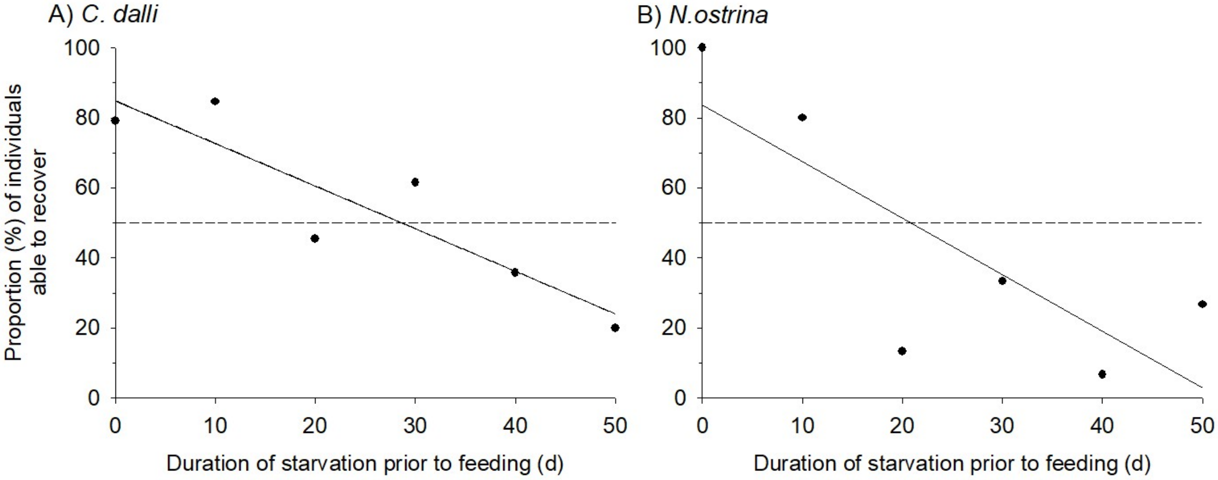

Figure 2.7. Percent of individuals able to recover, as a function of duration of starvation in (A) *C. dalli* and (B) *N. ostrina*. Equations of the regression lines: (A) $y = 84.8758-1.2184x$, and (B) $y = 83.8095-1.6190x$. The point where the model intersects the dashed line indicates the duration of starvation resulting in only 50% of individuals being able to recover when fed.

In *B. glandula,* the ability of individuals to recover was not significantly affected by the duration of starvation they experience (Linear regression: $F = 0.512$, $n = 6$, $R^2 = 0.11$, $p = 0.512$). Once *B. glandula* individuals were provided with food after any period of starvation, very few deaths were observed (3 deaths, each from a different starvation group, out of a total of 88 individuals), and all surviving individuals exhibited some growth during the 30 d feeding period. Thus, to analyze the effect of starvation on recovery of *B. glandula*, the average growth throughout the 30 d feeding period was calculated for each treatment (i.e. each duration of starvation) in order to assess the effect of starvation on their ability to grow once food becomes available. Additionally, each individual was measured at the end of the starvation period just prior to feeding, and an average shell diameter was calculated for each treatment group to assess if any growth occurred during the starvation period. The three individuals that died during the 30 d feeding period were excluded from this analysis.

Shell diameter at the end of the starvation period differed among treatment groups (Figure 2.8A) (one-way ANOVA: $F = 7.90$, $df = 74$, $p < 0.001$). This indicates that even in the absence of particulate food, EBP *B. glandula* deposited some new calcium carbonate into their shells, thus increasing their shell diameter. However, the increase in shell diameter was moderate (up to 0.15 mm, or 22%, increase) and significant increases did not occur beyond 20 d of starvation. In contrast, growth during the 30 d feeding period was significantly and strongly affected by the duration of starvation experienced (Figure 2.8B) (Linear regression: $F = 65.93$, $n = 79$, $R^2 = 0.46$, $p < 0.001$). Individuals starved for longer periods of time grew at a substantially slower rate than those starved for a shorter period once particulate food was introduced.

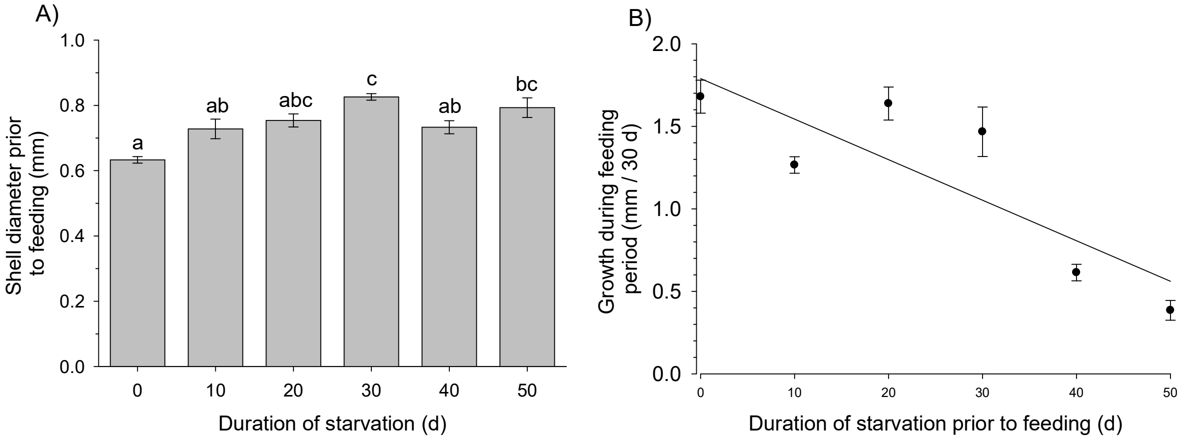

Figure 2.8. Effect of duration of starvation on (A) shell diameter prior to feeding, and (B) subsequent growth of EBP *B. glandula*. Values represent average shell diameter ± SE at the end of the period of starvation (A) and average growth ± SE during the 30 d feeding period (B). Different letters above error bars on the bar graph indicate values that were significantly different. Equation of the regression line in (B): $y = 1.76033 - 0.023276x$.

DISCUSSION

Relationship between organic matter content in EBP individuals and duration of starvation

Energy content, as estimated by organic matter (OM), in early benthic phase (EBP) *Nucella ostrina* declined rapidly, and in a predictable way, as a function of duration of starvation. The OM:ash weight ratio, a proportional estimate of energy content (relative to initial body size), significantly decreased as the period of starvation increased. The depletion of energy content was particularly rapid in the first weeks of starvation; the OM:ash weight ratio dropped by half after only 10 d of starvation, and decreased by an additional 19% by day 20. In both batches of hatchlings, the OM:ash weight ratio levelled off by day 40 at approximately 0.14, in other words when the mass of remaining organic matter in the body was 14% of the mass of the inorganic material, the latter mostly consisting of the shell. This continuous reduction in OM content is consistent with hatchlings progressively using up their available energy reserves when not able to feed, and confirms the assumption that ash-free dry weight (AFDW) is an effective measure of energetic lipids. The minimum OM:ash weight ratio of 0.14 is likely determined by the amount of organic matter that is difficult to use as an energy source by the hatchlings. Almost all organic compounds that can readily be metabolized to provide usable energy, mainly energetic lipids such as triacylglycerols (Thiyagarajan et al. 2002; Sewell 2005; Lee et al. 2006; Whitehill and Moran 2012; Gosselin et al. 2019), would thus be utilized within 40-50 d of the onset of the EBP if starved, leaving compounds such as structural lipids and proteins as the bulk of the remaining organic matter. Since OM predictably decreases as a function of the duration of starvation, this finding confirmed that duration of starvation is a useful proxy for metabolizable energy content at the onset of the EBP.

Ability of EBP individuals to survive exclusively on their initial energy reserves

Survivorship of EBP invertebrates unable to replenish energy reserves

Short-term starvation of individuals from the onset of the EBP did not have the same implications for survivorship as longer-term starvation. Unsurprisingly, for all six species in this study, starvation eventually caused high mortality. However, a large proportion of individuals of all species were capable of surviving prolonged periods of starvation; in 5 of the 6 species, more than 50% of individuals were still alive after 50 d of starvation, and some individuals survived starvation for more than 100 d. In addition, survivorship during starvation decreased gradually within each species, suggesting considerable variation in either the amount of initial energy reserves or in metabolic rate among individuals of a given species at the onset of early benthic life. Together, these results indicate that a large proportion of EBP individuals can survive for remarkably extended periods of time solely on the internal energy reserves obtained either by larval feeding in planktotrophic species or from maternal provisioning in direct-developing species.

The most significant finding of this study was that all six species of invertebrates were highly tolerant of starvation in the short-term, i.e. the first few days of the EBP. Survivorship was minimally impacted by starvation during the first 10 d of the EBP, with three species experiencing < 2% mortality, and the remaining three species experiencing only 6 – 12% mortality through this time period. This finding is not consistent with the starting hypothesis that a substantial proportion of individuals would die in the first few days of starvation due to depleted energy stores (Figure 2.1, curve B). Rather, these low rates of mortality suggest that for each of the examined species, very few individuals, if any, enter the EBP with such critically low energy reserves that they are below or only slightly above the minimum threshold needed to survive.

This finding of very low mortality of starved individuals during the first days of early benthic life is surprising. Many species of intertidal invertebrates have extremely high mortality rates in the field, especially during the first 10 d of the EBP, often ranging from 30-100% (Gosselin and Qian 1997; Hunt and Scheibling 1997). More specifically, high mortality during the first 3-10 d of early benthic life have been reported for wild populations of a variety of intertidal taxa, some of which were included in the present study: barnacles (Gosselin and Qian 1996; Shanks 2009; Gosselin and Jones 2010; Jenewein and Gosselin 2013a), gastropods (Spight 1975; Moran and Emlet 2001), mussels (Bownes and McQuaid 2009; Von Der Meden et al. 2012; Phillips 2017), and crabs (Spitzer et al. 2003). Several studies have hypothesized that the high rate of mortality during the first few days of the EBP might be due in part to a substantial portion of individuals having insufficient internal energy reserves at the beginning of the EBP (Gosselin and Qian 1997; Jarrett and Pechenik 1997; Phillips 2002; Thiyagarajan et al. 2002; Thiyagarajan et al. 2007; Phillips 2017). The present study, however, demonstrated that under controlled conditions, where individuals were isolated from other mortality factors (e.g. predation, competition, environmental stressors), survivorship remained high during the first weeks of the EBP even when animals were starved and thus relied solely on existing energy reserves present at the onset of the EBP. Interestingly, the impact of starvation on early survivorship was similar regardless of mode of larval development or source of EBP energy reserves. The four species with planktotrophic larvae (*B. glandula*, *C. dalli*, *M. trossulus*, and *Petrolisthes* spp.) as well as the two direct developing, lecithotrophic species (*N. ostrina* and *N. lamellosa*) all had remarkably high survivorship throughout the first 10 d of EBP when starved. This pattern was also unaffected by whether the larvae continue to feed through to competence and settlement or experience some period of pre-EBP starvation as a result of having a non-feeding phase. Together, these findings fail to reject the theoretical null hypothesis depicted by curve A in Figure 2.1, revealing that depleted energy reserves are not a significant cause of the high mortality rates observed during the first weeks of the EBP.

Influence of initial body mass on survivorship of EBP individuals unable to replenish energy reserves

Differences in starvation tolerance among the three size classes of newly hatched *N. ostrina* depended on the temporal scale at which these were examined. When considering longer-term impacts, the time required to reach 20% mortality (i.e. starvation LD_{20}) differed among size classes, with small individuals reaching this point an average of 23 d earlier than their larger counterparts. This is consistent with the idea that smaller individuals have a smaller reserve of metabolizable energy and reach a minimum energy threshold more quickly during periods of starvation than larger individuals. However, the impact of size in determining starvation tolerance was subtle, as a difference was only observed between the largest and smallest size classes; the intermediate size class did not differ from either of the other size classes in their ability to tolerate starvation. The advantage bestowed by the larger energy reserve of large hatchlings was also moderate; even small individuals were able to survive for extended periods of time without replenishing energy stores.

The modest long-term energetic advantage of a larger body size was not apparent on a shorter time scale: there was no significant effect of hatchling size on survivorship during the first 5 d of the EBP. This indicates that even the smallest size class of *N. ostrina* hatchlings have sufficient energy upon hatching to survive through the first 5 d without replenishing their energy stores, consistent with the null hypothesis that nearly all individuals enter the EBP with sufficient energy reserves (Figure 2.1, curve A). This is not entirely surprising for *N. ostrina*, as hatchlings of this species do not begin feeding until 3 – 10 d after hatching (Gosselin and Chia 1994). These findings nevertheless further demonstrate that depletion of energy stores is not a significant cause of EBP mortality.

Offspring size is often a good indicator of offspring fitness; indeed, this is a central tenet of life history theory (Sinervo 1990, Stearns 1992, Roff 1992). For benthic invertebrates, larger larval size often translates into larger EBP size, which in turn leads to greater performance (survival and growth) during the EBP (Jarrett and Pechenik 1997; Phillips 2002; Thiyagarajan et al. 2003; Marshall and Keough 2004; Emlet and Sadro 2006). While many of these studies have focused on the larval stage, very few studies have examined the relationship between offspring size and offspring performance after the onset of EBP. The present study found no significant difference in short-term survivorship among starved individuals of different initial body sizes, and only modest differences in long-term survivorship. These findings are not dissimilar to those of Moran and Emlet (2001), who found increased survivorship for larger hatchlings in longer outplant experiments (36 – 54 d), but no significant difference in shorter duration outplants (9 d). This indicates that the short-term response to starvation and energy depletion is independent of the size of recently hatched individuals.

In populations of many benthic species, larger offspring do tend to outperform their smaller counterparts; larger offspring grow more quickly (Moran and Emlet 2001; Marshall et al. 2003; Thiyagarajan et al. 2003; Torres et al. 2016), reach maturity earlier (Moran and Emlet 2001; Marshall et al. 2003), and often have lower rates of mortality (Phillips 2002; Thiyagarajan et al. 2003; Emlet and Sadro 2006) than smaller offspring. The results of the present study, however, indicate that the larger energy reserve of a larger body size does not lead to greater intrinsic performance during the first days of the EBP. Rather, having a larger body size may provide other benefits that allow for greater success. A larger body size may allow individuals to handle and consume prey more efficiently and thus grow at a faster rate (Palmer 1990; Moran and Emlet 2001). Being larger, or being able to grow quickly to reach larger body sizes, also decreases risk of predation (Paine 1976; Rumrill 1990; Bashevkin and Pechenik 2015) and vulnerability to desiccation (Gosselin 1997) by reaching a size refuge (i.e. growing large enough to considerably reduce vulnerability to stressors in their environment).

Ability to recover after delayed feeding for EBP invertebrates

Although EBP individuals could survive extended periods of starvation, the ability of *Chthamalus dalli, Nucella ostrina,* and *Balanus glandula* to recover once access to food was regained was negatively affected by the duration of starvation to which they were exposed at the onset of the EBP. The effect, however, was expressed differently among species. In *C. dalli* and *N. ostrina*, the proportion of individuals able to survive and begin to grow once food was introduced decreased with increasing duration of starvation. Conversely, in *B. glandula* the proportion of individuals able to recover was not impacted by the duration of starvation, but the rate of growth throughout the 30 d feeding period decreased significantly as the duration of starvation increased.

In *C. dalli* and *N. ostrina*, recovery was impacted by starvation, and in both species the duration of starvation that resulted in only 50% of individuals able to recover was considerably shorter than the starvation LD_{50}, found in the previous experiment. Thus, although individuals are able to survive on internal energy stores for extended periods of time, they may still be destined to die if unable to replenish those energy stores much sooner than the LD_{50}. One possible explanation for the difference between the starvation LD_{50} and the starvation period resulting in 50% recovery is that extended periods of starvation may cause EBP individuals to lose the capacity to feed by inducing a reabsorption or atrophy of digestive structures (Takami et al. 2000; Espinoza et al. 2017). From that point on, individuals would then continue to deplete all remaining available internal energy reserves but be incapable of efficiently feeding and thus replenishing those energy stores if food were to become available again. We did note that *C. dalli* exhibited behaviours associated with feeding, such as extending and beating their cirri, throughout the 30 d feeding period even in individuals that did not grow; cirral beating in those individuals most likely served primarily for gas exchange. Similarly, each *N. ostrina*, including those that did not grow, attacked at least one juvenile mussel during the 30 d feeding period, as evidenced by drill marks observed on the valves of dead mussels. This suggests that although EBP individuals are still capable of exhibiting behaviours associated with feeding, extended periods of starvation may have reduced their ability to digest or absorb nutrients, even if they are able to access food items. Another possible explanation for the inability of individuals to recover from prolonged periods of starvation is that they were unable to extract sufficient energy from consumed food to both replenish the energy they had spent during the starvation period and also begin to grow. In this case more food, or a longer feeding period, may be required before these animals can fully recover.

The recovery of starved *B. glandula* after regaining access to food differed relative to the other species in this study. Regardless of the duration of starvation experienced in this study, virtually all *B. glandula* individuals showed some ability to recover. The rate of growth throughout the 30 d feeding period, however, was significantly impacted by the duration of starvation. The 0 d group, which did not experience any starvation, increased in size five times more during the 30 d feeding period than the group starved for 50 d. This considerable reduction in growth rate following starvation may have further implications for survivorship, as slower growing individuals will take longer to reach a size refuge, and thus remain susceptible to factors such as competition (Connell 1961; Pechenik 1990), predation (Osman and Whitlach 1995; Gosselin and Rehak 2007), and desiccation (Hamilton and Gosselin 2020) for a longer period of time.

B. glandula also differed from the other species in terms of growth of shell material while being starved (i.e. prior to feeding). During the first 20 d of starvation, some growth was apparent in all groups of EBP *B. glandula*. While growth throughout the starvation period was modest, it indicates that EBP *B. glandula* are capable of depositing new shell material and thus increasing their shell diameter for the first few weeks of the EBP even without feeding, and thus without replenishing their energy stores. This suggests that increasing shell size during the EBP is not entirely reliant on obtaining food, nor is it exclusively stimulated by the growth of body tissues. However, the increase in shell diameter in newly settled *B. glandula* that have been without food for any duration was greatly reduced when compared to individuals that had fed.

Finally, in all three species studied in this experiment, an extended period of starvation following the onset of the EBP eventually had a negative impact on the ability to recover, either by directly causing mortality or by impacting growth. Although depletion of energy was not found to be a direct, major source of early mortality among EBP individuals, these findings suggest that EBP individuals with reduced energy reserves would still be at a marked disadvantage for longer-term survival in the intertidal zone.

Ecological implications

Cohorts of benthic invertebrates in the natural environment suffer extremely high mortality rates during the first days and weeks of the EBP. This study revealed that insufficient energy reserves are not likely a direct cause of this EBP mortality, as had previously been hypothesized (Gosselin and Qian 1997; Hunt and Scheibling 1997; Jarrett and Pechenik 1997; Phillips 2017). For those individuals that survive through the larval phase and make it to the start of the EBP, energy reserves obtained through larval feeding or maternal provisioning thus appear to be sufficient to sustain the metabolism of the individual through the critical first days of early benthic life. This further indicates that low food availability during the first 5-10 days of the EBP would not be a direct cause of mortality during that time. EBP survivorship in wild populations might nevertheless be impacted by initial energy reserves through indirect effects. Low energy reserves may cause EBP individuals to have low tolerance thresholds to environmental stressors, such as extreme temperatures (Miller et al. 2009; Jenewein and Gosselin 2013b; Hamilton and Gosselin 2020), desiccation (Foster 1971; Gosselin and Chia 1995; Miller et al. 2009; Jenewein and Gosselin 2013a; Jenewein and Gosselin 2013b), and low salinity (Qiu and Qian 1999; Thiyagarajan et al. 2007). If so, this could explain previously reported associations between low energy reserves and high EBP mortality rates in some species (Phillips 2002; Thiyagarajan et al. 2003; Emlet and Sadro 2006). The role of energy levels on stress tolerance thresholds, and the possible indirect effects of initial energy reserves on EBP mortality, are not well understood and require further investigation.

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CHAPTER 3: The role of initial energy reserves on stress tolerance thresholds during the early benthic phase for intertidal invertebrates

INTRODUCTION

Intertidal invertebrates are extremely vulnerable during their early benthic phase (EBP), the period immediately following settlement or hatching into the benthic environment, and which can encompass both pre- and post-metamorphic life stages. Most new cohorts of intertidal invertebrates suffer mortality rates of 30 - 100% in the first few days of the EBP (Gosselin and Qian 1997; Hunt and Scheibling 1997). This high level of mortality can drastically impact recruitment rates, and thus influence overall population abundance and distribution (Connell 1985; Stoner 1990; Hunt and Scheibling 1997). Though generally high, EBP mortality is also highly variable not only among species, but also among cohorts of a single species, and even among individuals within a cohort (Jarrett 2000; Phillips 2002). Many factors can cause mortality during the EBP, however the mechanisms responsible for the high degree of variation in mortality are still not fully understood.

Among the most important factors contributing to EBP mortality are those associated with aerial exposure at low tide and with the unique challenges associated with these periods of emersion. Two of the most significant of these environmental stressors are desiccation and elevated emersion temperature, both of which have been shown to cause significant mortality in many species of intertidal invertebrates (Foster 1971; Gosselin and Chia 1995; Helmuth and Hofmann 2001; Miller et al. 2009; Jenewein and Gosselin 2013a). Indeed, these two stressors define the upper limit of the vertical range for many intertidal species (Connell 1961; Foster 1971). Loss of moisture from body tissues (i.e. desiccation) and elevated temperatures both have the potential to disrupt a range of physiological processes including protein stability, organ function, and gas exchange (Buckley et al. 2001; Sokolova and Pörtner 2001; Miller et al. 2009). EBP individuals are particularly vulnerable to these stressors owing to their very small body size, and thus large surface-to-volume ratio and small reservoir of water in their tissues, which greatly reduces their ability to tolerate adverse environmental conditions (Lowell 1984; Gosselin 1997).

Individuals fed reduced rations throughout the larval phase often have lower success rates through the EBP than those reared on full rations (Phillips 2002; Thiyagarajan et al.

2003; Emlet and Sadro 2006). This suggests that low initial energy reserves might lead to higher EBP mortality, and thus energy reserves at the onset of the EBP have previously been proposed as a factor that may play a major role in determining whether an individual will survive the critical first days of early benthic life (Gosselin and Qian 1997; Hunt and Scheibling 1997; Jarrett and Pechenik 1997). This hypothesis is supported by evidence that individuals within a cohort differ considerably in size and amount of organic content held at the start of the EBP, both of which are proxies for energy reserves (Marshall et al. 2003, Emlet and Sadro 2006, Phillips 2006), with some individuals possessing relatively low energy reserves at the onset of the EBP (Lucas et al. 1979; Jarrett and Pechenik 1997; Thiyagarajan et al. 2003). Additionally, limiting larval diet can negatively impact growth rate during juvenile life (Woodcock and Benkendorff 2008; Pechenik and Tyrell 2015; Gilman and Rognstad 2018), leaving individuals more vulnerable to mortality factors for a longer period of time. In wild populations, differences in energy reserves among EBP individuals could be due to different rates of feeding in the larval stage, or delayed metamorphosis (Thiyagarajan et al. 2003). Many species also transition through energy intensive stages or non-feeding periods prior to entering the EBP, during which initial energy reserves can become depleted (Lucas et al. 1979; Wendt 2000; Bryan 2004; Bennett and Marshall 2005). Recent research, however, has revealed that the vast majority of individuals entering the EBP likely have adequate energy reserves to survive the critical first days to weeks of early benthic life, and that depleted energy reserves are not an important direct cause of EBP mortality (Chapter 2). Initial energy reserves might nevertheless have indirect effects on EBP mortality by altering tolerance thresholds to environmental stressors; these indirect effects of initial energy reserves are therefore the focus of the present study.

The physiological mechanisms and adaptations utilized by EBP individuals to tolerate adverse conditions are thought to be energy dependent, as energy demand increases during exposure to environmental stress (Sokolova et al. 2012). These mechanisms can include increasing the production of protective proteins such as heat shock proteins, synthesizing new proteins to replace those that have been damaged, restructuring cellular membranes, and shifting gene expression to accommodate these adaptive mechanisms (Sokolova and Pörtner 2001; Hofmann et al. 2002; Somero 2002; Berger and Emlet 2007). Many of these mechanisms have an associated energetic cost, and thus individuals with limited energy reserves may be unable to adequately respond to the physiological stressors they encounter. In this way, low energy reserves could indirectly lead to increased EBP mortality by reducing the ability of the individual to respond to environmental stressors such as desiccation and high emersion temperature.

Consequently, the purpose of this study was to assess the indirect impacts of depleted energy reserves on EBP mortality by testing the hypothesis that depleted initial energy content affects vulnerability to physiological stress during the EBP. Two species of intertidal invertebrates, the barnacle *Balanus glandula* and the snail *Nucella ostrina*, were used as model organisms. The specific goals of this study were to examine the extent to which initial energy content affects acute tolerance thresholds of EBP individuals to the two most challenging environmental stressors in the intertidal zone: (1) desiccation and (2) high emersion temperature. Since direct measurement of energy content in live individuals was not possible, two proxy methods were used to estimate energy reserves: duration of starvation prior to experimentation, and initial body mass.

METHODS

Study site and species collection

This research was conducted at the Bamfield Marine Sciences Centre from May to September, in 2018 and 2019. Specimens of two species of intertidal invertebrates were collected from rocky intertidal shore sites in Barkley Sound, on the west coast of Vancouver Island, Canada: *Balanus glandula* were collected from Wizard Islet (N 48°51.19, W 125°09.56) and Dixon Island (N 48°51.06, W 125°07.20), and *Nucella ostrina* were collected from Dixon Island, Prasiola Point (N 48°49.05, W 125°10.12), and Ross Islets (N 48°52.23, W 125°09.67). Sites were selected based on availability of early benthic phase (EBP) individuals of these two species.

The two species examined in this study differ in their source and quantity of initial energy reserves at the beginning of the EBP. *N. ostrina* are lecithotrophic, and thus depend entirely on maternal provisioning, primarily via ingestion of nurse eggs in the egg capsule. Uneven division of nurse eggs among capsule mates results in some individuals having greater energy reserves and larger body size at hatching (Moran and Emlet 2001; Lloyd and Gosselin 2011; Marko et al. 2014). *B. glandula* have planktotrophic larvae so the majority of their EBP energy reserves are obtained from the food they consume during the larval stage. They also transition through a non-feeding cyprid stage and undergo an energy intensive metamorphosis, such that some individuals may have more depleted energy reserves at the onset of the EBP (Wendt 2000; Thiyagarajan et al. 2003).

EBP *B. glandula* were collected as described by Sandee et al. (2016). Small (5 – 10 cm diameter) rocks were collected from the intertidal zone, cleared of all small barnacles, and a perimeter was marked around the cleared area with nail polish before being returned to the intertidal zone. Rocks were then recovered 48 – 72 h later and examined for newly settled barnacles; rocks containing new settlers were retained for experiments. EBP *N. ostrina* were collected by removing ripe egg capsules from intertidal substrata using fine-tipped forceps, as in Gosselin & Chia (1995). Egg capsules were returned to the laboratory and held in small cages in aerated seawater for 72 h; all newly hatched individuals were then collected and retained for experiments.

Establishing treatment levels of energy reserves

To assess how the vulnerability of EBP invertebrates to physiological stressors is affected by the individual's energy reserves, the first step was to obtain groups of individuals with different levels of energy content. The measurement of energy content, for example by quantifying organic content, in such small animals is a destructive process, such that individuals in which energy content had been quantified could not then be used for experiments. Consequently, two proxy methods were used to obtain relative estimates of energy reserves in live EBP individuals: duration of starvation and body mass. Energy reserves were estimated by duration of starvation by rearing groups of individuals of a similar body size without food for different periods of time. Organic content, an effective measure of energy content (Moran and McAlister 2009), predictably decreases as a function of the duration of starvation (*N. ostrina*: Chapter 2; Moran and Emlet 2001). All newly metamorphosed *B. glandula* obtained for these experiments were 0.60 – 0.68 mm shell diameter, a very narrow range of body sizes.. *N. ostrina*, however, varied considerably in size at hatching (Lloyd and Gosselin 2011; Marko et al. 2014). All newly-hatched snails were thus measured and sorted into three size classes based on shell length (SL): small (0.8 – 1.10 mm SL), medium (1.11 - 1.40 mm SL), and large (1.41 – 1.80 mm SL). Only medium size class *N. ostrina* (1.11 – 1.40 mm SL). Only medium size class *N. ostrina* (1.11 – 1.40 mm SL) were used when testing the influence of duration of starvation on tolerance thresholds. All EBP individuals were held in water that was filtered to 1.0 µm, aerated, and maintained at 15-17 °C. The water was replaced every second day and containers and cages were rinsed and scrubbed to prevent the growth of biofilm, thus ensuring no food particles were available. A sample of individuals were removed from the starvation holding cages every 10 d, beginning on day 0 and continuing to day 60, and used in tolerance experiments. Accordingly, groups of individuals representing seven distinct energy level treatments were obtained: 0, 10, 20, 30, 40, 50, and 60 d durations of starvation.

The second approach used to establish distinct energy reserve treatments involved sorting individuals by initial body size, and using body mass as a proxy for energy reserves. Body mass and quantity of organic matter are strongly correlated among EBP invertebrates (Moran and Emlet 2001) with larger individuals having greater stores of lipids, which are the primary source of metabolizable energy for intertidal species (Phillips 2002; Thiyagarajan et al. 2002; Emlet and Sadro 2006; Whitehill and Moran 2012). This method was only feasible for *N. ostrina*, as there was insufficient variation in body size (0.60 – 0.68 mm shell diameter) of newly metamorphosed *B. glandula* at our field sites. As previously mentioned, *N. ostrina*, had considerable variation in size at hatching and individuals in two shell length (SL) categories were used in stress experiments: small (0.8 – 1.10 mm SL) and large (1.41 – 1.80 mm SL). These SL measurements were then converted to wet weight (WW) body mass (Table 3.1) using a regression equation for *N. ostrina* from Hamilton & Gosselin (2020). All individuals were held without food in the laboratory for a 10 d acclimation period before tolerance experiments were conducted.

Table 3.1. Shell lengths (SL) and corresponding body masses (wet weight, WW) for *N. ostrina* hatchlings. The conversion equation is: WW = 2.98 * log(SL) - 3.84, from Hamilton & Gosselin (2020).

Size class	Shell length (SL) in mm	Body mass (WW) in mg
Small	0.8 – 1.10	0.074 – 0.192
Large	1.41 – 1.80	0.402 – 0.833

Emersion temperature tolerance

To determine if emersion temperature tolerance varies as a function of energy reserve, individuals in each energy level treatment (i.e. duration of starvation or size class) were exposed to five temperature treatments (Table 3.2). The range of temperatures to which each species was exposed was determined in preliminary trials and included temperatures that resulted in 0 - 100% mortality; the low end of the temperature range resulting in no mortality served as a control for the experiment. Immediately before each experiment, all individuals were examined under a dissecting microscope to ensure they were alive, and also that there were no cracks in their shells, as this might have affected their tolerance. Rocks containing newly-settled *B. glandula* were then blot dried and transferred to sealed 591 ml plastic Ziplock® containers. *N. ostrina* hatchlings were first placed in small cages made of plastic microcentrifuge tubes with the lower half removed and covered with 600 μm mesh to prevent their escape. These small cages were then placed within separate, sealed 591 ml containers, one small cage per container. A sheet of paper towel saturated with seawater was also placed in each container to maintain a high humidity level (>90% RH), as well as a data logger (Hygrochron iButton® model DS1923) that recorded temperature and relative humidity every 15 min. All replicate containers were then placed in an incubator set at the lowest temperature in the experimental range for 6 h. Following the 6 h temperature treatment, all rocks and small cages were removed from containers and submerged in 15 - 17°C seawater for a 6 h recovery period, and then examined again under a dissecting microscope to assess survivorship. The 6 h treatment and recovery intervals were chosen to approximate a natural tidal cycle, so that animals were exposed to emersion conditions for a duration similar to that experienced in the intertidal zone. Dead individuals were recorded and removed, then rocks containing the surviving *B. glandula* were blot dried and surviving *N. ostrina* were placed back into small cages. All rocks and small cages were transferred back into their respective containers and placed into the incubator set to the next experimental temperature (i.e. increased by 2 °C). This process was repeated until the final experimental temperature was reached. Temperature within the incubators was stable throughout all experiments and stayed within ±0.5 degrees of the intended temperature for each treatment. It took up to 30 min for the internal temperature of each container to reach equilibrium with the incubator, but once the target temperature was reached it remained stable for the remainder of the 6 h treatment. Relative humidity remained above 90% for the duration of the experiment in all treatments. The temperature at which each individual died was recorded and an average temperature at death was calculated for all the individuals in each replicate container.

Table 3.2. Experimental range of emersion temperatures and desiccation periods used for each species. Ranges were set to include treatments that result in 0 – 100% mortality.

Species	Experimental emersion temperature treatments (°C)	Experimental desiccation duration treatment (h)
B. glandula	36, 38, 40, 42, 44	4, 6, 8, 10, 12, 14, 16, 18, 20
N. ostrina	26, 28, 30, 32, 34	0.25, 0.5, 1.0, 1.5, 2.0, 3.0

For the experiments using duration of starvation as a proxy for energy reserves, a single collection of at least 200 individuals of each species was used, and animals were maintained as stated above. All individuals in this experiment were collected from Dixon Island; *B. glandula* and *N. ostrina* were collected on 21 May 2019 and 14 June 2019, respectively. Using only individuals from a single collection for each species ensured that they had all experienced similar environmental conditions prior to collection. At the start of the experiment (day 0), and then again after each 10 d interval, 30 individuals were removed from the starvation holding tanks and used in the emersion temperature experiments as described above. The experimental design for each of the two species was: x 7 durations of starvation x 3 replicate groups of individuals per duration of starvation x 10 individuals per replicate group.

The experiment using body mass as a proxy for energy reserves was designed slightly differently. *N. ostrina* were collected from the field on three separate dates, twice from Dixon Island and once from Prasiola Point, and each of these three batches of individuals was used in a different trial. Comparisons of tolerance thresholds between size classes were only carried out between groups from the same trial, thus accounting for temporal and spatial differences among groups. Following the 10 d acclimation period, 30 - 50 individuals of each size class from each trial were used in the emersion temperature experiments as described above. The experimental design for this experiment was: 3 trials x 2 size classes per trial x 3 – 5 replicate groups of individuals per size class x 10 individuals per replicate group.

Desiccation tolerance

The effect of energy reserves on tolerance to desiccation stress was determined by exposing the various energy treatment groups to a low humidity environment for set periods of time. The durations of exposure to desiccation used in this experiment were determined in preliminary trials and set to include treatment periods that result in 0 – 100% mortality (Table 3.2). Desiccation conditions were quantified using vapour pressure deficit (VPD) as in Jenewein and Gosselin (2013). VPD accounts for both temperature and relative humidity (RH) and is an effective measure of desiccation potential. Given that *B. glandula* and *N. ostrina* occupy different microhabitats and levels in the intertidal zone, they normally experience different degrees of desiccation stress. Additionally, EBP *B. glandula* are known to have a higher desiccation tolerance threshold than EBP *N. ostrina* (Hamilton and Gosselin 2020). Thus, the two species were exposed to different VPD levels in the present experiment; *B. glandula* were exposed to a VPD of 2.0 kPa, whereas *N. ostrina* were exposed to the less stressful VPD of 1.6 kPa. Both VPD measurements are common in the intertidal zone during low tide throughout the summer months (Jenewein and Gosselin 2013b). To ensure this experiment tested desiccation tolerance and not temperature stress, desiccation trials were performed in a temperature-controlled incubator set well below the temperature tolerance threshold for each species, as determined in the emersion temperature experiment described above: *B. glandula* trials were carried out at 22°C, and *N. ostrina* trials were carried out at 19°C. The RH needed to obtain the above VPD measurements (approximately 20 – 28% RH for *B. glandula* and 25 - 31% RH for *N. ostrina*) was then achieved by placing a large pan of silica desiccant in the bottom of the incubator as well as two small fans to create a continuous air flow. A data logger (Lascar Electronics, model EL-USB-2) was also placed in the incubator to record temperature and RH every 10 min, allowing the calculation of VPD. For all desiccation tolerance experiments, VPD remained stable and close to the target set for each species, although there were slight fluctuations among trials. Additionally, it took up to 10 min for the internal RH of the incubator to stabilize after the door had been closed, thus the first RH reading was omitted from the average VPD calculation. Throughout experiments involving *B. glandula* and *N. ostrina,* VPD ranged from 1.91 – 2.10 kPa and 1.56 – 1.69 kPa, respectively.

Before each experiment, all individuals were examined under a dissecting microscope to ensure they were alive, and that there were no cracks in their shells, as this may have impacted their ability to tolerate desiccation conditions. Rocks containing *B. glandula* were blot dried and then placed in a small desiccator with silica desiccant crystals for 10 min to remove any remaining water on the rock surface. *N. ostrina* were placed in small dry cages using a pair of flexible insect forceps. The rocks or small cages were then transferred into open 591 mL containers and placed into the prepared incubator for a set period of time. Following the desiccation treatment, animals were removed from the incubator and immediately submerged in seawater for 12 h to recover, after which they were checked for survival and the number of live and dead individuals in each treatment was recorded. Contrary to the emersion temperature experiments, individuals that survived a desiccation treatment were not used again in further treatments; a different set of individuals were used for each desiccation duration treatment.

For the experiment using duration of starvation as a proxy for energy reserves, more individuals were required than could be obtained from a single field collection. Accordingly, the experiment involved 2 - 4 trials per desiccation period; each trial used EBP individuals from one field site collected on a same day, but the collection sites and days differed among trials. Data from all trials were combined for analysis, and trial was included as a fixed factor. At 10 d intervals, beginning on day 0 and ending on day 30 for *B. glandula* and day 50 for *N. ostrina*, groups of 7-12 individuals were removed from holding tanks and assigned to one of the desiccation duration treatments. The experimental design for each of the two species was as follows: x 4 – 6 duration of starvation treatments per species x 6 – 9 desiccation duration treatments per starvation treatment x 2 – 4 replicate groups of individuals per desiccation treatment x 7 – 12 individuals per replicate group.

For experiments using body mass as a proxy for energy reserves, three replicate trials were conducted, each using animals from a separate collection (i.e. collected from different locations on different days). Immediately prior to a desiccation trial, six groups of 5 – 10 individuals of each size class were removed from holding tanks and assigned to one of the six desiccation duration treatments. Thus, the experimental design was: 2 size classes x 6 desiccation duration treatments per size class x 3 replicate groups of individuals per desiccation treatment x 5 – 10 individuals per replicate group.

Data analysis

Data from the emersion temperature experiments were not normally distributed, and so did not meet the requirements for ANOVA. Thus, the Kruskal-Wallis non-parametric analysis of variance was used to compare temperature at death among the various energy reserve treatments (i.e. durations of starvation). When the Kruskal-Wallis test revealed a significant difference, a Dunn's multiple comparisons test was then used to compare each starvation treatment to the 0 d control to assess how tolerance was impacted by starvation. To compare emersion temperature tolerance between size classes, a randomized block ANOVA was used, with trial as the blocking factor. Although the data did not meet the requirements for ANOVA, the p-value was deemed sufficiently low as to be unaffected by using a parametric test (Blanca et al. 2017). A significance value of 0.05 was used for both the Kruskal-Wallis and ANOVA statistical tests.

Survivorship data from all desiccation experiments followed a binomial distribution and were thus analysed using generalized linear models (GLM) with a binomial link function. Each energy reserve treatment (i.e. duration of starvation or size class) was analyzed separately, and their desiccation LD_{50} was determined from the resulting curve. To identify differences in acute desiccation tolerance among the various energy reserve treatments, all survivorship data for a given species were fit to a single GLM. In all cases, trial was included as a fixed factor to account for potential differences between batches collected from different sites or at different times and a significance value of 0.05 was used. All statistical analyses were conducted using R statistical software (version 3.5.0) (R Core Team 2018).

RESULTS

Emersion temperature tolerance

Effect of starvation duration on emersion temperature tolerance

Although there was significant variation in average temperature at death among the duration of starvation treatments in both species (Kruskal-Wallis test; *Balanus glandula*: χ^2 = 17.85, df = 6, p = 0.007; *Nucella ostrina*: χ^2 = 15.55, df = 5, p = 0.008), results for the non-starved control treatment were not significantly different from any of the starved treatments (Figure 3.1). Duration of starvation for up to 60 d had no apparent effect on acute emersion temperature tolerance.

Results from the 30 d starvation treatment for *N. ostrina* were removed from the analysis due to uncharacteristically low temperature tolerance; 100% of individuals in that group died at 30°C, a temperature that was only lethal to a few individuals in any other starvation treatment. In addition, very high mortality also occurred among other individuals, remaining in the same holding cage, that were not used in the temperature tolerance experiment. It was apparent that the individuals in this holding cage had become unhealthy and their ability to tolerate emersion conditions was impacted. The 40, 50, and 60 d starvation treatments involved hatchlings from the same batch of animals, collected at the same time from the same location, as those in the 0, 10, 20 and 30 d starvation treatment, but those in the 40, 50 and 60 d treatments were not held in the same holding cage as the ones used in the 30 d treatment. In addition, individuals remaining in the holding cage for the 40, 50 and 60 treatments did not experience high mortality.

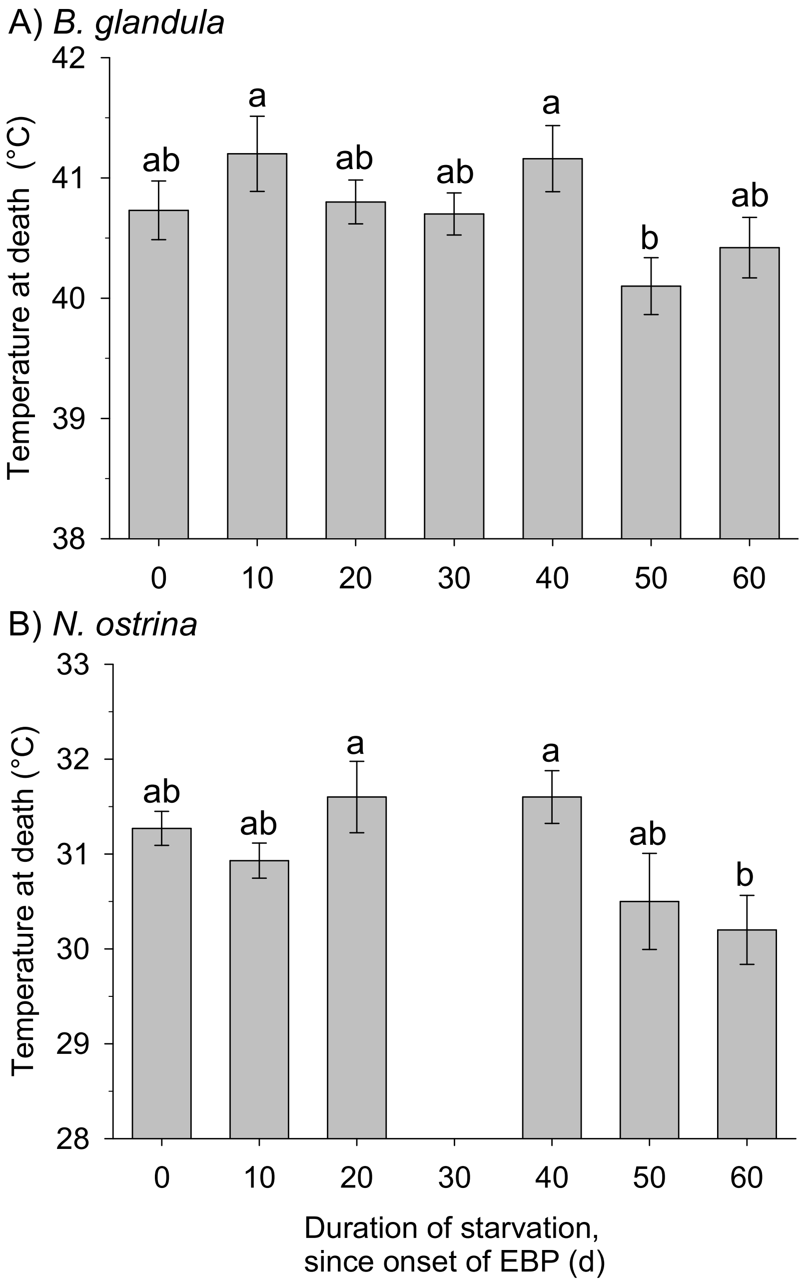

Figure 3.1. Acute temperature tolerance thresholds, measured as average temperature at death, as a function of duration of starvation in EBP. (A) *B. glandula* and (B) *N. ostrina.* The 30 d *N. ostrina* group was removed from this analysis due to inconsistent results for this batch of hatchlings and mass mortality among the remaining hatchlings of the same batch that were held in the same cage. In both species, starvation was initiated at the onset of the EBP. Error bars represent SE. Bars identified with different letters were significantly different based on Dunn's multiple comparison test.

Effect of body mass on emersion temperature tolerance

Acute temperature tolerance in early benthic phase (EBP) *N. ostrina*, as measured by temperature at death (Figure 3.2), differed significantly between the two size classes (Randomized block ANOVA using trial as the blocking factor: $F = 71.02,_{2,250}$, $p < 0.001$). In each trial, the large size class had significantly higher tolerance thresholds than the small size class; the differences between the size classes were 1.47°C, 0.89°C, and 2.12°C in trials 1, 2, and 3 respectively. This indicates that a greater initial body mass provides better tolerance to increased emersion temperatures. Additionally, within each size class (large or small), temperature at death varied significantly among trials ($p < 0.001$) (i.e. among groups collected from different sites or on different dates). This suggests that temperature tolerance thresholds can vary among similarly-sized hatchlings depending on where or when they completed encapsulated development.

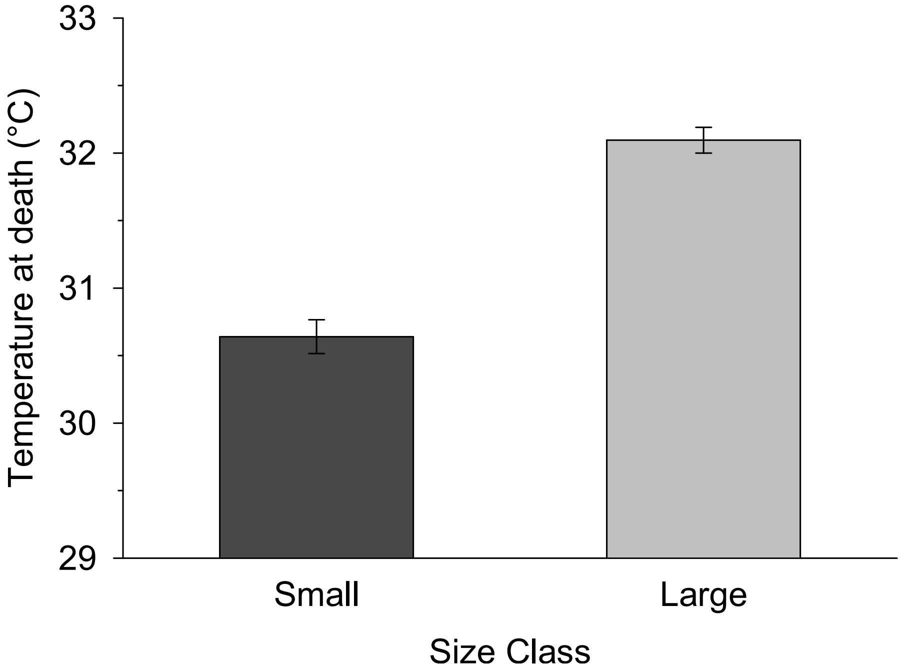

Figure 3.2. Effect of initial body size on acute temperature tolerance, as measured by average temperature at death, for EBP *N. ostrina*. Error bars represent SE. These means are significantly different based on a randomized block ANOVA ($p <0.001$).

Desiccation tolerance

Effect of starvation duration on desiccation tolerance

Within each starvation treatment, survivorship was significantly impacted by the duration of the desiccation treatment in both species (Figure 3.3 and Appendix B), as determined by separate generalized linear model (GLM) analyses of each treatment (Table 3.3). The duration of desiccation resulting in 50% mortality (i.e. the desiccation LD_{50}) decreased with increasing duration of starvation (Table 3.3).

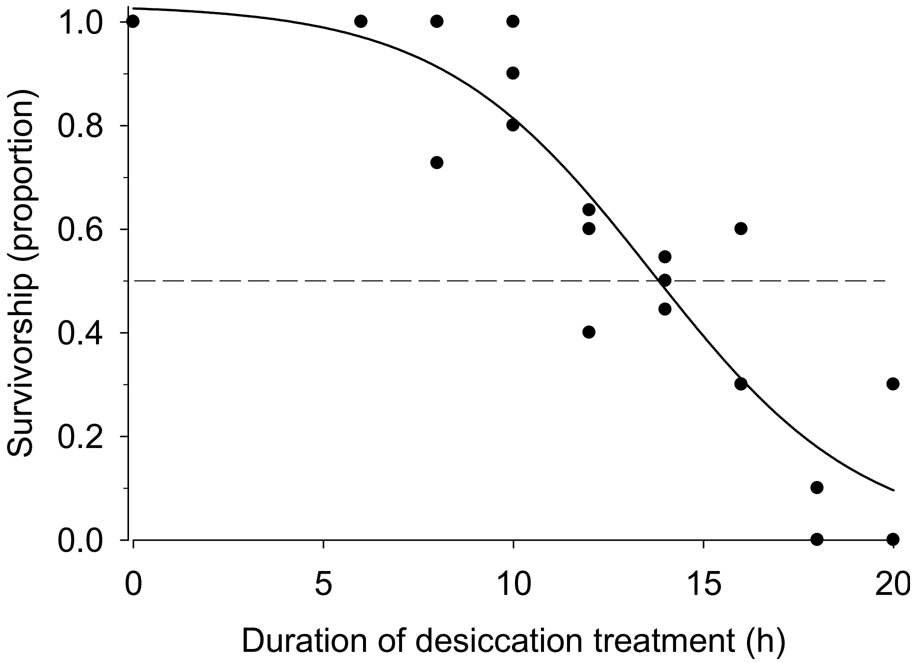

Figure 3.3. Effect of exposure to desiccation conditions on the survivorship of newly metamorphosed (not starved) EBP *B. glandula*. Each point represents the proportion of individuals from a group of 10 that survived the desiccation treatment for a given duration (h). The point where the curve intersects the dashed line represents the desiccation LD_{50}; in this case, LD_{50} = 13.98 h.

Table 3.3. Desiccation LD50 and associated standard error (SE) for groups of *B. glandula* and *N. ostrina* starved for different durations of time since the onset of EBP. LD50 values were calculated from generalized linear models (GLM) of the relationship between the proportion of individuals surviving and the duration of exposure to the desiccation treatment. The z statistic represents the strength of this relationship; df = degrees of freedom; p values represent the probability that survivorship varied as a function of duration of exposure to the desiccation treatment.

Species	Duration of starvation (d)	Desiccation LD_{50} (h)	SE	z statistic	df	p
B. glandula	0	13.98	0.45	-7.60	22	< 0.001
	10	11.94	0.37	-7.97	23	< 0.001
	20	8.14	0.43	-8.56	27	< 0.001
	30	5.87	0.53	-4.37	13	< 0.001
N. ostrina	0	1.22	0.07	-7.02	18	< 0.001
	10	1.23	0.05	-7.43	24	< 0.001
	20	1.19	0.05	-7.09	23	< 0.001
	30	0.95	0.05	-6.45	23	< 0.001
	40	0.98	0.07	-5.10	11	< 0.001
	50	0.91	0.08	-3.27	5	0.001

In both species, acute desiccation tolerance thresholds differed significantly among the starvation treatments, as determined by GLM analysis of the combined survivorship data from all starvation treatments of a given species. For *B. glandula*, desiccation tolerance differed significantly among each starvation treatment, with the 0 d control group having the highest tolerance threshold (pairwise comparisons among starvation treatments: z values < -3.36, df = 92, p < 0.001) and tolerance decreasing gradually for each sequential starvation treatment period (Figure 3.4A). For *N. ostrina*, however, the trend towards lower desiccation tolerance was not gradual. There was no significant difference in desiccation tolerance among 0, 10, and 20 d starvation treatments (pairwise comparisons: z values > 0.001, df = 90, p > 0.71). Desiccation tolerance was, however, significantly lower for longer starvation treatments (30, 40, and 50 d) when compared to the shorter starvation treatments (pairwise comparisons: z values < -1.76, df = 90, p < 0.005) (Figure 3.4B).

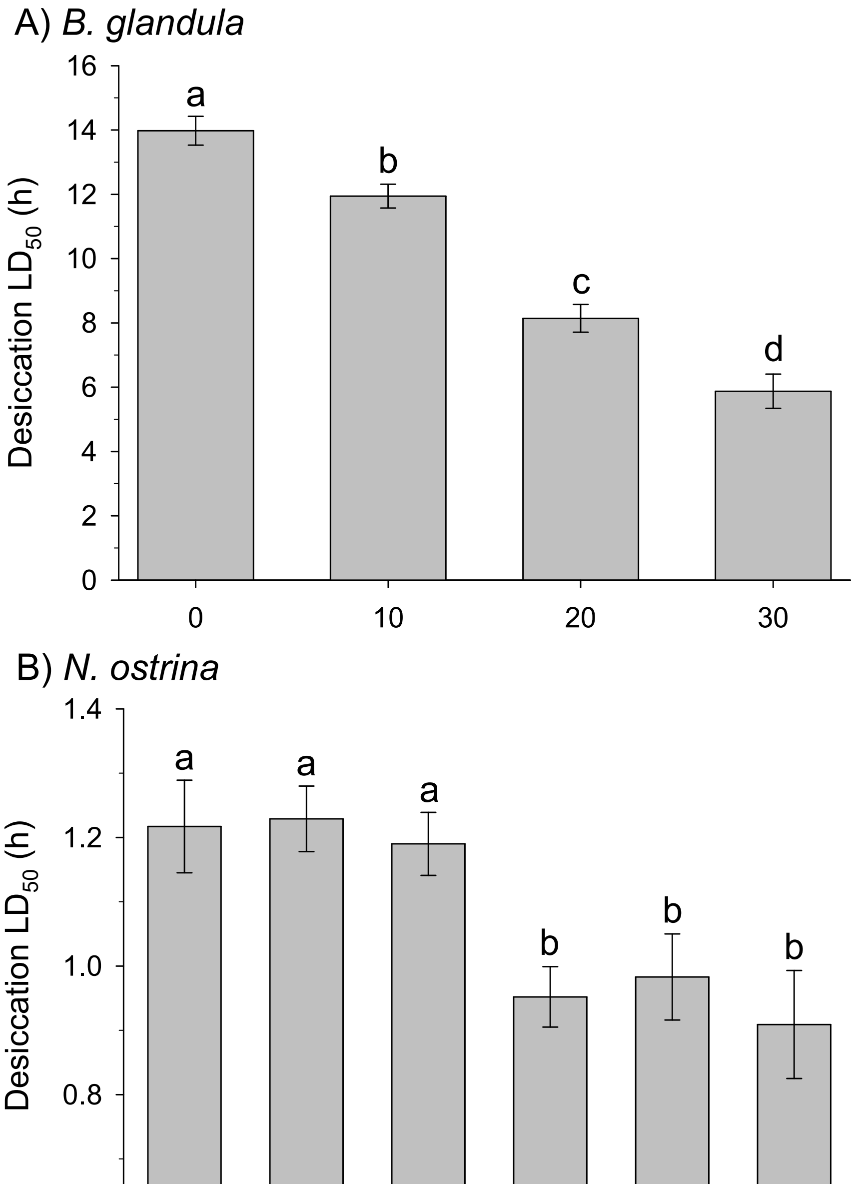

Figure 3.4. Acute desiccation tolerance thresholds, measured as LD_{50}, as a function of duration of starvation in EBP (A) *B. glandula* and (B) *N. ostrina*. Error bars represent SE. Bars identified with different letters are significantly different based on GLM analysis.

Effect of body mass on desiccation tolerance

Within each *N. ostrina* size class, survivorship decreased significantly as a function of the duration of the desiccation treatment (Figure 3.5), as determined by GLM analysis (Small size class: z value = -5.46, df = 17, $p < 0.001$; Large size class: z value = -6.26, df = 17, $p < 0.001$). In addition, when comparing size classes, the desiccation LD_{50} was significantly shorter for the small size class than for the large size class, as determined by GLM analysis of combined survivorship data from both size classes (z value = -5.46, df = 23, $p < 0.001$). Small individuals had a desiccation LD_{50} of 0.71 ± 0.05 h, whereas the desiccation LD_{50} of the large size class was 132% longer, at 1.65 ± 0.10 h.

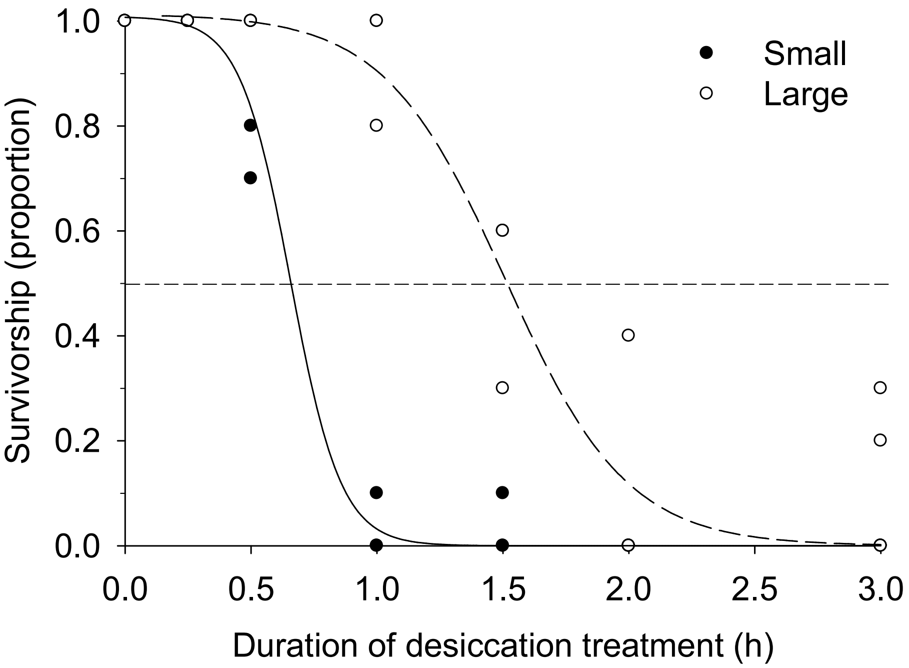

Figure 3.5. Effect of exposure to desiccation conditions on the survival of newly hatched *N. ostrina* of two size classes (Small = 0.074 – 0.192 mg; Large = 0.402 – 0.833 mg). Each point represents the proportion of individuals from a group of 10 that survived desiccation treatment for a given duration (h). The points where the curves intersect the dashed line represent the desiccation LD_{50} for each size class.

DISCUSSION

Effect of estimated energy reserves on tolerance to elevated emersion temperature

In early benthic phase (EBP) invertebrates, organic matter, a proxy for energy content, can decrease by half after only 10 d of starvation and approach a minimum after 40 – 50 d of starvation (Chapter 2), at which point nearly all metabolizable organic compounds have likely been utilized. Yet, the ability of EBP individuals to tolerate elevated emersion temperature was not impacted by the duration of the starvation period they had experienced. In both *Balanus glandula* and *Nucella ostrina*, EBP individuals starved for 50 or 60 d had comparable acute temperature tolerance thresholds to individuals that had not been starved. This suggests that the mechanisms employed by EBP individuals to tolerate acute elevated emersion temperatures are not energetically intensive. The production of heat-shock proteins (HSPs) is one common strategy among invertebrate species to cope with the physiological challenges of increased environmental temperatures (Sokolova et al. 2012). Since the production and activation of these specialized proteins is energetically expensive (Krebs and Loeschcke 1994; Somero 2002), individuals with more depleted energy reserves were expected to produce fewer HSPs and thus be more vulnerable to elevated temperatures. However, our results indicate that individuals with highly depleted energy reserves, as estimated by duration of starvation, were just as tolerant of acute exposure to high temperatures as individuals with a full complement of energy reserves. This suggests that temperature tolerance may be determined by other physiological and biochemical mechanisms that require minimal energy expenditure, such as metabolic rate depression (Storey 1998) and reduced ATP turnover rates (Sokolova & Pörtner 2001). Alternatively, benthic invertebrates may already possess protective mechanisms, such as a store of HSPs, upon entering the EBP, protecting them from the physiological stress of elevated temperature during emersion without requiring the use of stored energy. The quantity of stored HSPs, as well as the capacity of EBP invertebrates to utilize other suggested tolerance methods, should be the focus of future research.

Emersion temperature tolerance in *N. ostrina* hatchlings was significantly impacted by their initial body mass. For *N. ostrina* hatchlings, a greater initial body mass is indicative of having consumed more nurse eggs during embryonic development (Rivest 1983; Gallardo et al. 2004; Lloyd and Gosselin 2011), such that larger individuals have a larger reserve of stored energy (particularly lipids) upon hatching than their smaller counterparts (Moran and Emlet 2001). However, given that no relationship was detected in the previous experiment between temperature tolerance and duration of starvation, the effect of body mass on temperature tolerance was likely due to body size itself rather than to a difference in initial energy reserve. Body size can be an important determinant of tolerance thresholds, as found in this and other studies (Lowell 1984; Gosselin 1997, Hamilton and Gosselin 2020), largely owing to the lower surface area:volume (SA:V) ratio and increased water reservoir in the tissues of larger individuals. These traits would enhance temperature tolerance in large hatchlings by decreasing the rate of heat exchange with the external environment, and perhaps also the ability to carry out evaporative cooling, thus decreasing the rate of warming of the body. It is also possible that the capacity for other physiological or biochemical mechanisms, such as those discussed in the previous paragraph, may differ between snails of different body size. This remains speculative, however, and may be explored in future studies.

An interesting finding of the body size experiment was that tolerance thresholds also differed significantly among replicate groups of hatchlings of the same body size. These replicate groups, however, were collected from different field sites or on different dates, indicating some natural variation in temperature tolerance within and among cohorts of the same species. The mechanisms controlling this variation remain unclear but may involve geographical (i.e. interpopulation) and seasonal differences in tolerance thresholds.

Effect of estimated energy reserves on tolerance to desiccation

Unlike emersion temperature, vulnerability to desiccation was impacted by estimated initial energy reserves; in both species, EBP individuals starved for a longer duration of time had lower desiccation tolerance thresholds than those that had not been starved. This suggests the mechanisms or strategies employed by EBP intertidal invertebrates to withstand the challenge of desiccation are more dependent on energy-mediated processes than those used for acute temperature tolerance. Such physiological mechanisms of desiccation tolerance may include production of protective proteins, such as certain heat-shock proteins (i.e. HSP70) (Hayward et al. 2004), LEA proteins, and Trehalose (Hibshman et al. 2020), as well as the synthesis of new proteins to replace those that become damaged. Many of these responses have a significant energetic cost, such that individuals with reduced energy reserves may be unable to properly utilize these mechanisms and thus be less tolerant of desiccation stress (Sokolova 2013). The specific energy-dependent mechanisms of desiccation tolerance utilized by intertidal invertebrates require further research.

The effect of duration of starvation on desiccation tolerance thresholds appears to be different in the two species. In *B. glandula*, desiccation tolerance decreased gradually with increasing duration of starvation such that individuals in each starvation treatment had a significantly lower tolerance of desiccation than individuals starved for shorter durations. *B. glandula* transition through a non-feeding cyprid stage prior to completing an energetically expensive metamorphosis shortly after settlement (Anderson 1994, Lucas et al. 1979; Thiyagarajan et al. 2003), such that some individuals likely begin the EBP with very limited energy reserves. Any further depletion of energy, such as a delay in feeding, could also further restrict the physiological mechanisms of desiccation tolerance described above. For *N. ostrina*, desiccation tolerance thresholds were impacted differently; rather than decreasing gradually, desiccation tolerance was unchanged for groups starved for up to 20 d but was significantly reduced in groups starved for longer durations. Since *N. ostrina* hatchlings begin the EBP with relatively large energy reserves obtained through maternal provisioning (Spight 1976; Marko et al. 2014), it is likely they have enough stored energy to maintain physiological mechanisms of desiccation tolerance for a more extended period. Prior research has revealed that metabolizable energy reserves in EBP *N. ostrina*, as estimated by organic matter content, decline rapidly throughout the first 20 – 30 d when starved, decreasing by 58 – 69% during this period (Chapter 2). After that point, metabolizable energy reserves quickly approach a minimum, and are thus likely sufficiently reduced to begin impacting any energy dependent mechanisms of desiccation tolerance.

As was the case for emersion temperature tolerance, desiccation tolerance thresholds were also affected by initial body mass; *N. ostrina* hatchlings in the large size class had significantly higher tolerance thresholds than those in the small size class. The higher organic content, and thus larger reserves of energy, present in larger individuals likely contributed to the increased desiccation tolerance in the larger size class, in addition to the physical characteristics of larger individuals, such as having a smaller SA:V ratio and a larger reservoir of water in their tissues (Lowell 1984; Gosselin 1997). Together, these factors have a large impact on the ability of EBP individuals of different sizes to tolerate desiccation stress and likely determine tolerance thresholds.

Does the relationship between energy reserves and stress tolerance contribute to EBP mortality?

The present study investigated the indirect effects of initial energy reserves on EBP mortality, and more specifically on the vulnerability of EBP individuals to two of the most significant abiotic stressors in the intertidal zone, desiccation and elevated emersion temperature (Gosselin and Chia 1995; Miller et al. 2009). One approach used to obtain groups of individuals with distinct levels of initial energy reserve was to sort them according to initial body size, and vulnerability to both environmental stressors did vary as a function of body size, with larger individuals being more tolerant of desiccation and elevated temperatures than smaller individuals. This outcome, however, was likely primarily due to the physical characteristics of a larger body size, such as a smaller SA:V ratio, rather than difference in energy stores between the size classes, especially in the case of temperature tolerance.

The most interesting finding of this study is that depleted energy reserves, as estimated by duration of starvation, can indirectly lead to EBP mortality by reducing tolerance to desiccation. Desiccation stress is likely a more important selective pressure on EBP individuals than emersion temperature, regardless of energy reserves. Acute temperature tolerance thresholds of EBP individuals of these two species are higher than most temperatures experienced in the field during the summer. All temperature treatments in this study were within the range of low-tide summer temperatures recorded in the intertidal zone in Barkley Sound (Jenewein and Gosselin 2013). However, the temperatures necessary to cause significant mortality, even in the most vulnerable experimental groups, were at the high end of the natural temperature range (> 30°C) in the intertidal zone, and those high temperatures usually persist only for short periods of time (< 2.5 h) before the surfaces are immersed by the incoming tide (Jenewein and Gosselin 2013b). Desiccation tolerance thresholds, however, were lower than durations of aerial exposure occurring in most mid- and upper-intertidal habitats. The duration of desiccation required to cause significant mortality among individuals in all starvation treatments of both species was shorter than durations regularly experienced in mid- and upper-intertidal habitats in the study area, such that EBP individuals would be vulnerable to desiccation stress during many daytime summer low tides. This indicates EBP individuals are substantially more vulnerable to desiccation conditions in their natural habitats than to the temperature conditions in those same habitats. In addition, individuals with low energy reserves would be most vulnerable to desiccation stress, and thus less likely to survive the EBP, whereas those entering the EBP with greater stores of energy are more likely to survive desiccation during the critical first few days of the EBP. In this way, desiccation may be acting as a selective pressure favoring the evolution of greater energy reserves at the onset of the EBP, especially in species that inhabit areas of the intertidal with the greatest level of desiccation stress, such as the upper-intertidal zone.

The findings of this study suggest energy reserves at the start of the EBP may be an important indirect cause of EBP mortality by reducing desiccation tolerance thresholds. An additional consequence of this indirect effect is that variation in initial energy reserves would likely be a contributing factor in the extensive variation in intraspecific EBP mortality rates. In all the experiments of this study, however, tolerance thresholds to both stressors also varied among individuals and replicate groups within the same starvation treatment (i.e. with the same energy reserves). Replicate batches of individuals were obtained from different locations or at different times of the spring and summer, suggesting differences in tolerance thresholds may be linked to the location or time when each batch of animals was collected. This could have resulted from differences among populations, or temporal differences in environmental conditions experienced by individuals from different batches (Jarrett and Pechenik 1997; Jarrett 2003). Additionally, in the experiment examining the relationship between duration of starvation and emersion temperature tolerance, significant differences in tolerance thresholds also occurred among starvation treatments, but those differences were random with regards to the duration of starvation each group had experienced (i.e. the amount of remaining energy reserve). Together, these findings suggest tolerance thresholds are also influenced by factors other than initial energy reserves. Such factors could include latent effects resulting from the environmental conditions experienced during the embryonic and larval stages, altering their ability to tolerate similar stressors during the subsequent EBP (Pechenik 2006; Nasrolahi et al. 2016), or transgenerational effects resulting from the conditions experienced by the parents (Agrawal et al. 1999; Marshall 2008; Donelan and

Trussell 2015). Additionally, parasites or bacterial infections might negatively impact tolerance thresholds. A better understanding of the mechanisms controlling EBP mortality requires further research on how these additional factors influence acute tolerance thresholds in BP intertidal invertebrates.

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CHAPTER 4: General Conclusion

SUMMARY OF RESULTS

To further our understanding of the role of initial energy reserves on the high rates of early benthic phase (EBP) mortality among intertidal invertebrates, I (1) determined the proportion of individuals that begin the EBP with energy reserves that are close to or below a minimum threshold necessary for survival; (2) determined the effect of delayed feeding from the onset of the EBP on the performance of individuals; and (3) examined to what extent initial energy reserves affect acute tolerance thresholds to two major abiotic stressors encountered throughout the EBP: (a) desiccation and (b) high emersion temperature. The most important finding of this research is that, contrary to previous hypotheses, depleted energy reserves have minimal direct impact on EBP mortality, as all six species of invertebrates examined were highly tolerant of starvation throughout the critical first few days.

The first study of this thesis examined the direct impacts of estimated initial energy content on performance during the EBP. Past research has indicated that the period of highest mortality for many species of benthic invertebrates is the first 24 to 48 hours after the onset of the EBP, after which time mortality levels off (Gosselin and Qian 1997; Hunt and Scheibling 1997), and it has been hypothesized that depleted or insufficient energy reserves at the onset of EBP may be partially responsible for this early mortality. In the current study, all species were highly tolerant of starvation throughout the first 10 d after the onset of the EBP, with three species experiencing < 2% mortality, and the remaining three experiencing only 6 – 12% mortality during the first 10 d period. Furthermore, five of the six species that were examined only reached 50% mortality after >50 d of starvation with the exception of *N. lamellosa* which experienced 50% mortality at 27.9 d. This suggests EBP individuals can survive with only the energy stores acquired during their larval stages or provided by maternal inputs for an extended period, and leads us to conclude that the vast majority of individuals do not begin the EBP with energy reserves below or only slightly above the minimum threshold to survive.

This study also examined the effect of different durations of delay in feeding on the recovery and performance of EBP invertebrates of three species, and found that delayed feeding from the onset of the EBP negatively impacted performance (i.e. survivorship and growth) for all three species examined. For two of the species (*C. dalli* and *N. ostrina*), the duration of delay in feeding that resulted in only 50% of individuals being able to recover (i.e. survive and grow) was considerably shorter than the starvation LD_{50} from the previous experiment. This suggests that even if individuals can survive on their initial energy reserves for extended periods of time, they may nevertheless fail to recover if they are unable to feed and replenish their energy stores much sooner. For the final species, *B. glandula*, recovery was possible for all individuals, regardless of the delay in feeding. However, the rate of growth throughout the subsequent feeding period was greatly impacted, with non-starved individuals growing five times more than individuals who had experienced a 50 d delay in feeding. Such a drastic reduction in growth rate could impact survivorship by making individuals more vulnerable to other mortality factors for a longer period of time.

The second study in this thesis examined potential indirect effects of initial energy reserves on EBP mortality by determining the role of energy reserves in tolerance to two major environmental stressors, desiccation and high emersion temperature. For both species that were examined, the acute tolerance threshold to desiccation, but not to emersion temperature, was significantly reduced among individuals with lower energy reserves (i.e. starved for a longer duration from the onset of EBP). This suggests that having low energy reserves makes individuals more vulnerable to some environmental conditions experienced during the EBP, and thus may lead to increased EBP mortality. Additionally, initial body size had a significant effect on tolerance to both emersion temperature and desiccation, as larger individuals had higher tolerance thresholds to both stressors, indicating the body size at the onset of the EBP may play a larger role in determining vulnerability to environmental stressors than the amount of stored energy an individual possesses.

RELEVANCE OF FINDINGS FOR POLICY AND MANAGEMENT

The findings of this research have substantive implications for the following areas of policy and management: (1) climate change impacts on communities of coastal marine organisms, (2) marine invertebrate aquaculture operations, in particular hatcheries, and (3) marine invertebrate fisheries management.

Impacts of climate change on intertidal communities

The findings of this study highlight the vulnerability of EBP invertebrates to stressors such as desiccation and elevated emersion temperature, both of which are associated with the ongoing impacts of climate change. Average air temperature in coastal British Columbia is increasing at a faster rate than the global average (1.4 °C for coastal BC versus 0.8 °C global average over the past century), and extreme weather events, including periods of extreme heat, are becoming more common (Lemmen 2016). The warmest summer sea surface temperature has also increased over the past century at a rate of 0.81-1.13 °C (Iwabuchi and Gosselin 2019). With these long-term climatic changes, important abiotic stressors such as desiccation and emersion temperature are increasing in magnitude and are increasingly likely to reach or exceed lethal levels for many intertidal invertebrate species. An example of the acute effects of climate change on intertidal life was recently observed in British Columbia, encompassing the study area of this research. An extreme heat wave during the summer of 2021 lead to rock surface temperatures as high as 50 °C being recorded along the rocky intertidal zone of British Columbia, causing mass mortality of intertidal animals near Vancouver B.C. (Migdal 2021). This assessment, however, only examined adult individuals, that are significantly more tolerant to abiotic stressors than individuals in the EBP. It is thus likely that temperatures of this magnitude will also cause drastic mortality events for EBP individuals of many species. If these types of extreme weather events coincide with peak spawning or hatching periods, this could severely impact recruitment of those species affected, which could in turn lead to substantial impacts on population dynamics and community structure. As such, it is important that the protection of rocky intertidal ecosystems and the species that inhabit them are taken into consideration when developing regulations for climate change management and adaptation and future CO_2 emissions. Furthermore, since EBP individuals are substantially more vulnerable to these abiotic stressors (Lowell 1984; Gosselin 1997), it is key that tolerance thresholds of the most vulnerable life stage be considered when making decisions about climate change mitigation strategies.

Marine invertebrate aquaculture and hatchery operations

Marine invertebrate aquaculture is a thriving industry in coastal BC, with 25 different invertebrate species being farmed on the Pacific coast (DFO 2021), generating an estimated

$41 million annually (Pinfold 2013). For most invertebrate aquaculture industries, the most unpredictable and complex period is the hatchery and nursery phase, i.e. the rearing of larvae and early juveniles into healthy and adequately sized "seed" that can then be put into the "growout" phase of the farming operation. Survivorship is significantly lower through these early phases, and growth rates can be difficult to standardize (Roberts et al. 2001). While adequate feeding throughout the larval stage is undoubtedly critical for development and survivorship, the results of this study indicate that low food availability during the first 5-10 days following metamorphosis into the EBP would not directly cause significant mortality. However, this study also demonstrates that even a brief delay in feeding after the onset of the EBP can significantly impact growth for some species of marine invertebrates. Thus, to optimize growth and success it is critical that invertebrate hatcheries ensure adequate food is available to their stock immediately following the onset of the EBP. The variability among collection batches in the present study also demonstrates some evidence of the latent effects of conditions experienced during the larval or embryonic phase impacting tolerance to certain stressors, such as desiccation stress, later in life. Thus, if hatchery stock is exposed to inadequate feeding or stressful conditions (i.e. temperature, salinity, acidity, etc.) it could have impacts on survivorship during the growout phase of the farming operation. These types of latent impacts are too often obscured in the aquaculture industry, as the hatchery/nursery facilities are often separate from the farming facilities making it difficult to trace mortality events in the farm back to conditions experienced in the hatchery. Nevertheless, it is important to consider these effects as they have potential to greatly reduce the amount of product produced by farms.

Marine invertebrate fisheries management

Many species of marine invertebrate species such as crabs, shrimp, and prawns are actively harvested, either recreationally or as part of a commercial fishery. However, these invertebrate fisheries are often not as meticulously managed as finfish fisheries, and overharvesting, or unregulated harvesting, can lead to reduced populations of these species over time (Anderson et al. 2011). Many factors, such as sex, size, and location, are considered when fishery managers are establishing allowable catches. However, the results of this study highlight the importance of also considering rates of EBP survivorship on a local scale when making these management decisions. Invertebrates are considerably more vulnerable to abiotic changes in the environment during this early life stage (Hamilton and Gosselin 2020), and local disturbances could result in variable recruitment rates of EBP individuals to the adult population, which could greatly impact population size and distribution in future years. Monitoring these changes in recruitment could thus allow fisheries managers to set harvest quotas at an appropriate level to maintain a sustainable fishery. Failure to account for these small-scale differences in population recruitment could potentially lead to drastic changes in population abundance over time, which could in turn lead to shifting baseline syndrome, or even extirpation, for these important invertebrate species as has been observed in many finfish fisheries (Pauly 1995).

DIRECTIONS FOR FUTURE STUDY

The results of this study indicate that initial energy reserves at the onset of the EBP have minimal direct impact on EBP mortality rates but do influence mortality through indirect effects, such as reducing early growth and increasing susceptibility to desiccation stress. Yet there are many factors that influence survivorship throughout the critical first few days to weeks of the EBP that were not addressed in the current study. To further our understanding of the overall effects of initial energy reserves on EBP success, future research should examine the role of energy reserves on these other mortality factors. Focus should be placed on other abiotic stressors that are likely to increase in magnitude with future effects of climate change such as elevated sea surface temperature, reduced salinity, reduced dissolved oxygen, and increased ocean acidity (Talloni-Álvarez et al. 2019). Of further interest are the effects of low initial energy reserves on biotic mortality factors such as predation, competition, and food acquisition. Furthering our understanding of the relationships between initial energy reserves and these additional factors will allow us to better understand and predict changes in populations and how they will be impacted by future changes to their environment.

The current study demonstrates that tolerance to some stressors, such as desiccation, are likely mediated by energy dependent mechanisms. However, the physiological mechanisms of desiccation tolerance utilized by intertidal invertebrates are not well understood. Future research in this field should focus on determining the specific physiological and biochemical mechanisms responsible for the desiccation tolerance of these species. One method of desiccation tolerance suggested in this thesis is that benthic invertebrates may enter the EBP already possessing a large store of protective proteins, such as heat-shock proteins. This would allow them to tolerate adverse conditions experienced in the intertidal zone without depleting their energy reserves. The presence of these stores of protective proteins are, however, unknown. Future research should aim to determine the tolerance potential of pre-EBP (i.e. larval) individuals of intertidal species.

The findings of this study also demonstrate that tolerance thresholds are influenced by factors other than initial energy reserves, as replicate groups of the same starvation treatment (i.e. same amount of remaining energy reserves) but obtained at different collection sites or times exhibited some significant differences in acute tolerance thresholds. Previous studies have shown that for many species of intertidal invertebrates, environmental conditions experienced in the larval or embryonic stages can have significant latent effects on some aspects of later juvenile fitness (Emlet and Sadro 2006; Pechenik 2006; Pechenik and Tyrell 2015). However, little is known about how such latent effects could impact vulnerability during the EBP to the various environmental stressors to which they are exposed. Future research should aim to understand how environmental conditions experienced during the larval or embryonic stage might alter vulnerability to similar stressors following the onset of the EBP. Such latent effects could considerably impact population dynamics and fluctuations in population over time and space in the intertidal zone.

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APPENDIX A.

Table A1. Number of batches and number of individuals in each batch for all six benthic invertebrate species in the experiment assessing the survivorship of EBP individuals unable to replenish energy reserves.

Species	Batch #	# individuals	Total # of individuals
B. glandula	1	16	249
	2	41	
	3	34	
	4	9	
	5	21	
	6	88	
	7	25	
	8	15	
C. dalli	1	20	269
	2	34	
	3	17	
	4	27	
	5	171	
N. ostrina	1	29	449
	2	90	
	3	37	
	4	131	
	5	21	
	6	68	
	7	47	
N. lamellosa	1	196	930
	2	198	
	3	170	
	4	200	
	5	166	
M. trossulus	1	34	73
	2	39	
Petrolisthes spp.	1	72	112
	2	40	

Table A2. GLM equations for the relationship between % survivorship and duration of starvation, starting at the onset of the EBP, for all six species.

Species	Equation
B. glandula	y=1/(1+exp(-5.2253+0.0666x))
C. dalli	y=1/(1+exp(-4.7558+0.0686x))
N. ostrina	y=1/(1+exp(-3.3032+0.0591x))
N. lamellosa	y=1/(1+exp(-3.1759+0.1139x))
M. trossulus	y=1/(1+exp(-2.4995+0.0419x))
Petrolisthes spp.	y=1/(1+exp(-8.7306+0.1720x))

Table A3. Starvation LD20 and percent survivorship at 5 d starvation, and their associated standard errors (SE), calculated from generalized linear models (GLM) of survival as a function of duration of starvation for each size class of each batch of *Nucella ostrina* hatchlings. n represents sample size for each size class of each batch.

Batch	Size class	n	Starvation LD20 (d)	SE	Survivorship (%) at 5 d starvation	SE
1	Small	13	20.4	5.8	90.5	3.9
	Medium	29	44.7	8.7	93.9	2.6
2	Small	106	21.6	1.4	94.8	1.0
	Medium	90	44.3	1.6	98.9	0.4
	Large	217	69.0	3.4	97.6	0.5
3	Medium	37	20.7	3.4	94.0	2.6
	Large	56	38.3	3.0	94.6	1.5
4	Large	59	31.9	2.8	93.9	1.6
5	Small	11	26.3	3.9	97.6	2.1
	Medium	25	22.7	6.7	87.8	3.9
	Large	15	32.3	7.9	91.5	4.3
6	Small	57	12.9	2.2	88.9	2.5
	Medium	152	26.1	1.6	93.1	1.2
	Large	76	32.1	2.5	94.1	1.5
7	Small	34	10.8	2.5	87.9	3.4
	Medium	115	44.8	3.5	95.6	1.1
	Large	85	49.1	2.8	99.3	0.4
8	Small	68	30.2	2.1	96.5	1.3
	Large	34	39.1	3.5	99.3	0.7

Table A4. Recovery LD50 and associated standard error (SE) from linear model analysis of the proportion of individuals able to recover from different durations of starvation. The z statistic represents the strength of the relationship between the % survivorship and the duration of starvation; df = degrees of freedom.

Species	Recovery LD_{50} (d)	SE	F value	n	R^2	p
C. dalli	28.6	4.2	17.56	6	0.81	0.014
N. ostrina	20.9	6.6	7.08	6	0.64	0.056

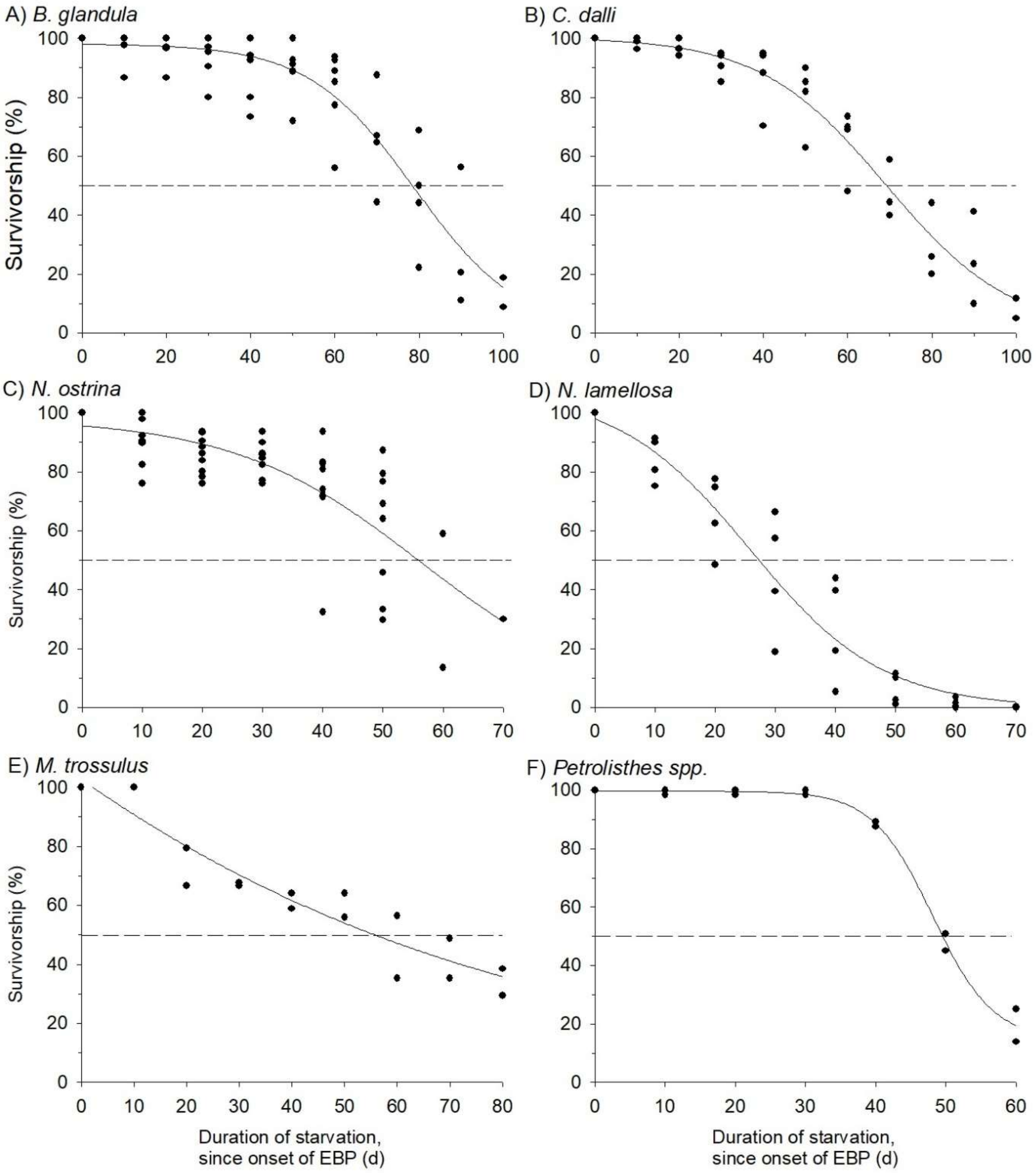

Figure A1. Relationship between % survivorship and the duration of starvation, starting at the onset of the EBP, for five species of benthic invertebrates. Results from the generalized linear models (GLMs) are shown in Table 2.1 in Chapter 2.

APPENDIX B.

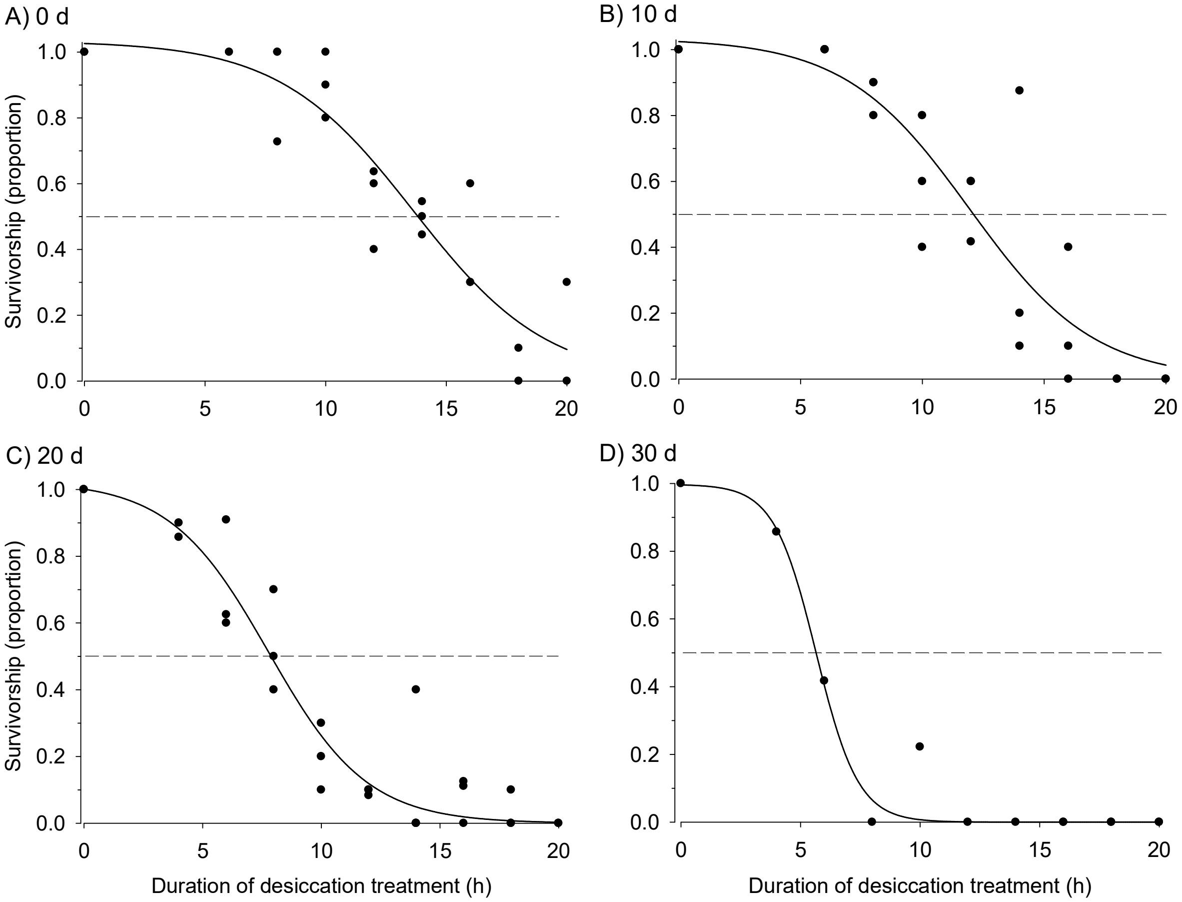

Figure B1. Relationship between the proportion of individuals surviving and the duration of the desiccation treatment to which they were exposed for groups of *B. glandula* starved for (A) 0 d, (B) 10 d, (C) 20 d, and (D) 30 d. Results from the generalized linear models (GLMs) are shown in Table 3.3 in Chapter 3.

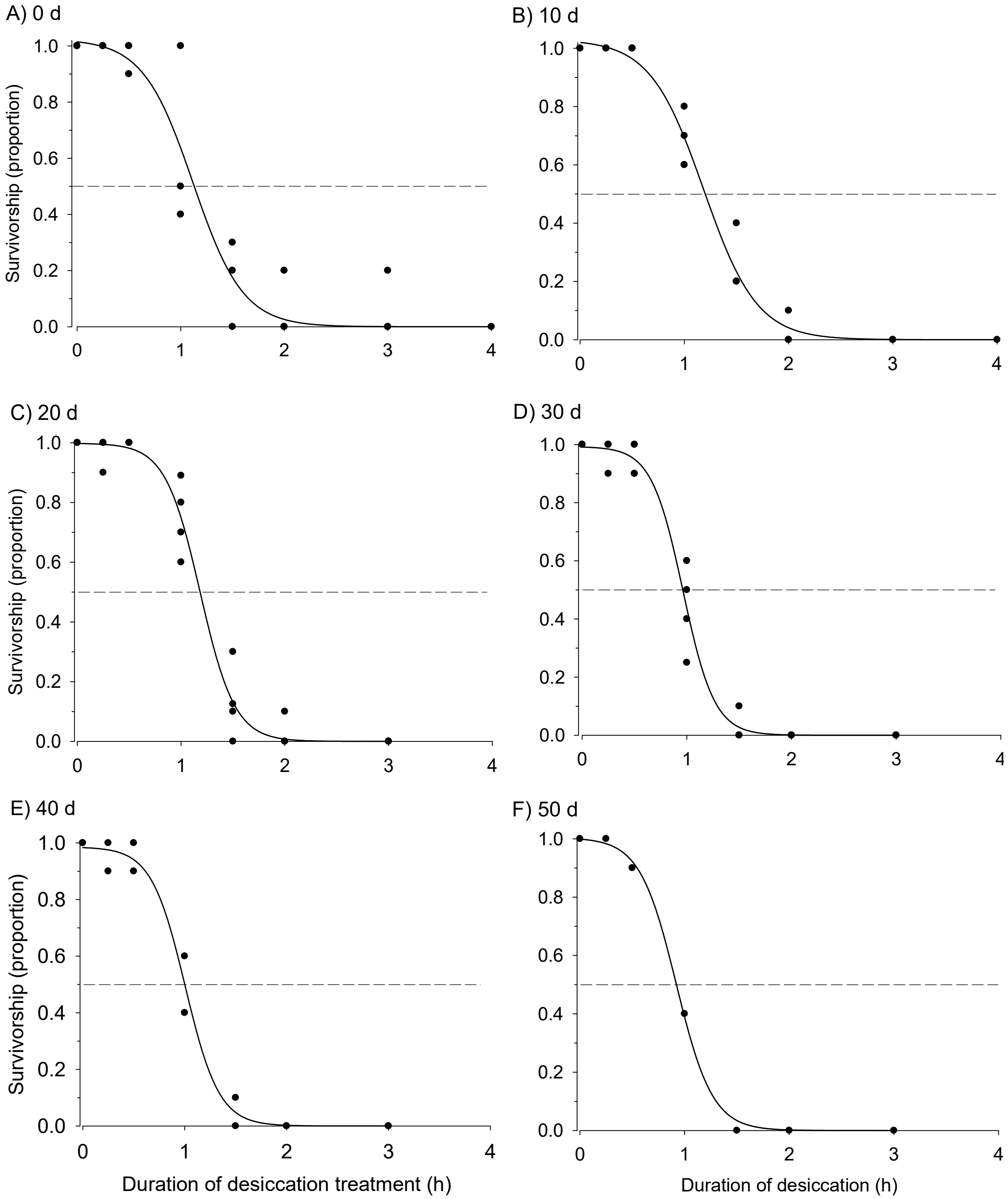

Figure B2. Relationship between the proportion of individuals surviving and the duration of the desiccation treatment to which they were exposed for groups of *N. ostrina* starved for (A) 0 d, (B) 10 d, (C) 20 d, (D) 30 d, (E) 40 d, and (F) 50 d. Results from the generalized linear models (GLMs) are shown in Table 3.3 in Chapter 3.