DETERMINING THE EXTENT TO WHICH WEATHER-RELATED ABIOTIC FACTORS INFLUENCE DAILY VARIATION IN EARLY BENTHIC PHASE MORTALITY OF INTERTIDAL MARINE INVERTEBRATES

by

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ABSTRACT

Populations of marine intertidal invertebrates vary substantially in abundance from year to year. These differences might be partly due to cohorts experiencing 60 – 99% mortality during the first few days after the transition from pelagic to benthic environments. However, the causes of this mortality, including the role of weather conditions experienced during low tide, are not well understood. This study aimed to improve our understanding of the influence of low tide environmental stress on survival through the early benthic phase by (1) determining the influence of temperature and desiccation stress on mortality of newly settled mussels and the ontogeny of sensitivity to these stresses through the early benthic phase; (2) examining the mortality of barnacle cyprids from settlement to metamorphosis and mortality of early juveniles up to the age of 10 days after metamorphosis; and (3) documenting the frequency of occurrence of lethal conditions occurring in the field at low tide during the recruitment season.

Laboratory experiments revealed that newly settled *Mytilus trossulus* of 1-2 mm shell length experienced a temperature tolerance threshold at 34°C and a desiccation tolerance threshold at a vapour pressure deficit level of 1.01 kPa. Mussels became highly tolerant to desiccation stress when they reached a size of 3 mm shell length, suggesting a size threshold of desiccation tolerance between 2-3 mm shell length. This size closely corresponds to the size at which some studies have reported juvenile *M. trossulus* relocate from protective filamentous algal habitat to adult habitat, suggesting ontogenetic shifts in habitat use by juvenile *M. trossulus* may be a response to changing vulnerability to desiccation stress.

A field survey of *Balanus glandula* recruitment revealed that cyprid and juvenile mortality varied greatly among daily cohorts and was significantly higher under *Fucus* spp. cover than on bare surfaces. Contrary to expectations, cyprid mortality was not significantly influenced by weather-related abiotic conditions. This was likely due to the study being conducted during a La Niña cycle that may have had lower temperature and desiccation stress than is typical for Barkley Sound.

Intertidal temperatures above the threshold tolerance levels for newly settled mussels were uncommon during the recruitment season, suggesting that temperature stress is not likely an important factor influencing early benthic phase mortality of mussels. The desiccation stress threshold level for newly settled mussels was frequently exceeded for several hours during the recruitment season, however, suggesting that desiccation stress may be an important factor influencing early benthic phase mortality in mussels that settle on open surfaces. In contrast, cyprid mortality on bare surfaces was not significantly influenced by desiccation stress or any other weather-related abiotic factors. I concluded that the survival of newly settled mussels likely depends upon the presence of protective microhabitats created by filamentous and fucoid algae, whereas barnacles experience reduced survival through the early benthic phase in the presence of fucoid algae. Changes in survival of these algae due to climate change could therefore have extensive influence on mortality through the early benthic phase and may subsequently affect population and community structure.

Keywords: early post-settlement mortality; mortality factors; ontogenetic shift; physiological stress; marine invertebrates; desiccation; temperature; climate change

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CHAPTER 1: General Introduction

Populations of marine intertidal invertebrates, such as mussels, barnacles, seastars, and crabs, vary in abundance over time (year to year) and space (from one location to another). In many species these variations can be substantial, differing by several orders of magnitude (Berger et al., 2006; Bao et al., 2007; Broitman et al., 2008; Pedersen et al., 2008). Several potential causes of these variations have been investigated, and for many benthic invertebrates the number of individuals colonizing intertidal habitats is affected by larval supply (Grosberg, 1982; Gaines et al., 1985; Minchinton & Scheibling, 1991) and settlement cues (Raimondi, 1988; Pawlik, 1992; Holmes et al., 2005; Jenkins, 2005). However, there is debate over whether these are the predominant influences of population abundance, or if factors affecting post-settlement survival are the most important indicators of abundance (Lively et al., 1993; Gosselin & Chia, 1995; Hunt & Scheibling 1997; Jarrett, 2000; Petraitis et al., 2003; Gosselin & Jones, 2010).

Settlement and metamorphosis occur in many invertebrate species with pelagic larvae, and these processes constitute a dramatic ecological transition into new habitat to which settlers must quickly adapt to survive (Werner & Gilliam, 1984); in most cases, individuals will experience air exposure within a few hours of settlement. Most cohorts experience 60 – 99% mortality in the first few days and weeks of life after settlement, (Gosselin & Qian, 1997; Pedersen et al., 2008), therefore it has been suggested that variations in survival through the first few days of life in this new habitat may be the reason for observed differences in population abundance (Osman et al., 1992; Gosselin & Chia, 1995). Variation in post-settlement survival may be influenced by both biological and environmental factors. Biological factors, including predation (Hurlbut, 1991; Lively et al., 1993), dislodgement (Dayton, 1971; Chan & Williams, 2003), and competition (Young & Chia, 1984; Dungan, 1985), are often documented as the cause of early post-settlement mortality. However, it has been suggested that environmental factors that fluctuate to extremes over a short period might be more important causes of mortality (Gosselin & Qian, 1997), which may include temperature stress (Gosselin & Chia, 1995; Chan & Williams, 2003), desiccation stress (Denley & Underwood, 1979; Shanks, 2009), reduced salinity (Chen & Chen, 1993; Berger et al., 2006; Thiyagarajan et al., 2002, 2007), and both ultraviolet radiation and visible light (Rawlings, 1996; Bingham & Reitzel, 2000). Temperature and desiccation stress appear to be the physical factors most likely to cause mortality during aerial exposure (Gosselin & Chia, 1995; Somero, 2002), although there has been relatively little research examining this hypothesis in newly settled invertebrates. Recent research suggests that if newly settled invertebrates are sensitive to these abiotic factors, then there may be a direct link between early benthic phase mortality and the weather parameters that influence the abiotic factors, including temperature, relative humidity, wind speed, ultraviolet radiation, and solar radiation (Gosselin & Jones, 2010).

For many intertidal species, little is known of the influence of abiotic factors on newly settled individuals, except that they tend to be more sensitive to temperature and desiccation stress than adults (Gosselin & Chia, 1995) because their high surface-to-volume ratio causes rapid water loss (Foster, 1971). Denley and Underwood (1979) found that survival of newly settled barnacles (*Tetraclitella purpurascens*) was significantly reduced in sunny areas, suggesting that early post-settlement mortality may be directly linked to desiccation and/or temperature stress. More recently, Gosselin and Chia (1995) found that newly hatched snails (*Nucella ostrina*) in the mid-intertidal were highly vulnerable to ambient levels of desiccation stress, but not temperature stress, suggesting that desiccation has a stronger influence on early post-settlement mortality than temperature stress. To date there has been relatively little research examining the extent of thermal and desiccation stress that newly settled invertebrates experience in their natural habitat, but recent research has begun to explore the role of various weather parameters that influence these stressors on early post-settlement mortality. In a study of newly settled *Balanus glandula*, Gosselin and Jones (2010) found that 65% of the variation in mortality rate from settlement to metamorphosis could be explained by daily fluctuations in ultraviolet radiation (UVR) levels. However, UVR itself was not responsible for all observed mortality, which lead the authors to suggest that mortality was caused by other weather conditions that covary with UVR intensity. High levels of

UVR typically occur on days with clear skies and low relative humidity, which increases both the temperature and evaporation rate at intertidal surfaces. Therefore, by monitoring weather parameters that influence desiccation and temperature stress, early benthic mortality rates of intertidal invertebrates might be predictable with a high degree of accuracy.

The ultimate goal of this thesis is to improve our understanding of the influence of low tide environmental stress on survival through the early benthic phase. More specifically, the objectives of this project are to: (1) determine the sensitivity of newly settled mussels to temperature and desiccation stress; (2) determine how sensitivity of mussels to temperature and desiccation stress changes with increasing size; and (3) determine the role of temperature, relative humidity, wind speed, ultraviolet radiation, and solar radiation in regulating temporal variation in early post-settlement mortality of barnacles. Defining these relationships is a crucial step toward understanding, and possibly predicting, early benthic mortality rates. In addition, this information will provide insight into the potential impacts of future climate change on early survival and recruitment of intertidal organisms and subsequent effects on community structure.

Chapter 2 focuses on the sensitivity of newly settled mussels (*Mytilus trossulus*) to temperature and desiccation stress, and explores the potential of an ontogenetic shift in sensitivity. This was accomplished with controlled laboratory experiments that exposed different sizes of mussels, ranging from 1-8 mm in shell length (SL), to the full range of temperature and humidity conditions experienced in their natural habitat. The lethal temperature and desiccation levels that caused 50% mortality of mussels after 6 h exposure was determined for newly settled (1-2 mm SL) individuals.

Chapter 3 focuses on a recruitment survey of barnacles (*Balanus glandula*). Individuals were monitored from the day of settlement up to 10 days after metamorphosis using the transparency mapping technique. Fucoid algae (*Fucus* spp.) were removed from within and around half of the survey quadrats to assess the impact of the algae on settlement and survivorship through the early benthic phase. During the survey, weather parameters were monitored near the survey site to determine the relationship between these parameters and cyprid and early juvenile mortality.

Chapter 4 provides a summary of the major findings of this thesis, including suggestions for future research directions.

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CHAPTER 2: Ontogenetic shift in stress tolerance thresholds of the mussel *Mytilus trossulus*: Do low tide temperature and desiccation stress influence early benthic phase mortality?

INTRODUCTION

The onset of intertidal life for many benthic marine invertebrates is characterized by a Type III survivorship curve, where most cohorts experience 60 – 99% mortality in the first few days and weeks of life after settlement (Gosselin & Qian, 1997; Hunt & Scheibling, 1997; Pedersen et al., 2008). The mechanisms causing variation in survival through this early benthic phase are poorly understood, but for intertidal organisms it is thought that abiotic environmental factors exert a significant influence (Gosselin & Chia, 1995; Gosselin & Jones, 2010). Sensitivity to abiotic factors could constitute a direct link between early benthic phase mortality and weather-related parameters such as temperature, humidity, wind speed, and solar radiation. It is therefore important to fully understand the role of weather conditions in controlling post-settlement mortality. In addition, if mortality is sensitive to weather conditions, then climate change may alter patterns of survivorship through the critical early benthic phase and further affect population abundance and community structure.

In benthic marine invertebrates with pelagic larvae, the processes of settlement and metamorphosis to the juvenile stage constitute a dramatic ecological transition (Werner & Gilliam, 1984); newly-settled individuals must rapidly adapt to the new habitat where they are exposed to aerial conditions for several hours each day. During aerial exposure, thermal and desiccation stress appear to be the physical factors most likely to cause mortality (Gosselin & Chia, 1995; Somero, 2002), although to date this has only been studied in a small number of species. Also, of particular importance, it is not clear how long sensitivity to these stressors persists during juvenile life, or the frequency of occurrence of conditions that are lethal for new settlers during the settlement season, and thus the likelihood that small juveniles might experience such conditions.

Mussels are often dominant members of intertidal communities and major components of intertidal food webs. Little is known of the sensitivity of newly metamorphosed mussels to abiotic stressors, but the responses of larger juvenile and adult mussels to thermal and desiccation stress have been studied extensively. Laboratory experiments have revealed that the physiological performance of *Mytilus* adults declines as body temperature rises above certain temperatures (22°C, *M. californianus*: Bayne et al., 1976; 24°C, *M. galloprovincialis*: Anestis et al., 2010; 25°C, *M. trossulus*: Buckley et al., 2001; Schneider et al., 2010), and mass mortalities of adult mussel populations have often been attributed to high temperature events (Suchanek, 1978; Tsuchiya, 1983; Petes et al., 2007; Harley, 2008). Studies of aerial exposure have confirmed that body temperatures occasionally reach or exceed these adult threshold temperatures during spring and summer (Hofmann & Somero, 1995; Roberts et al., 1997; Helmuth, 1999). Temperature variation might therefore substantially influence population dynamics of mussels.

The main mechanism adult mussels use to counteract thermal stress is evaporative cooling, which is achieved by opening shell valves during air exposure (Bayne et al., 1976; Helmuth, 1998). However, the drawback to this response is an increased risk of desiccation. Larger mussels have greater amounts of water available in their tissues than smaller mussels (Helmuth, 1998), which provides greater protection from desiccation and therefore enables them to use evaporative cooling for longer periods (Sukhotin et al., 2003; LeBlanc et al., 2005). Larger mussels are thus capable of enduring longer periods of thermal and desiccation stress than smaller mussels. This suggests sensitivity to temperature and desiccation might scale with body size, in which case the early benthic phase should be the most sensitive to stressful conditions. Although recent studies have begun to examine the causes of mortality through the early benthic phase, none have focused on the effects of temperature or desiccation on mussels <6 mm SL or on the ontogeny of their physiological tolerance.

The species examined in this study is the bay mussel, *Mytilus trossulus* Gould 1850, which colonizes the low- to mid-intertidal zone along the west coast of North

America from Alaska to central California (McDonald et al., 1991). On the west coast of British Columbia, *M. trossulus* colonizes the mid-intertidal zone between approximately 1.7 m and 2.9 m above mean lower low water (MLLW) and is typically exposed to air for 5-8 h per tide cycle (Suchanek, 1978; pers. obs.). *M. trossulus* larvae may settle and metamorphose year-round, although peak settlement on the west coast of British Columbia occurs in June and July (Strathmann, 1987; Johnson & Geller, 2006). Larvae tend to settle first on filamentous algae, such as *Cladophora columbiana* and *Endocladia muricata*, and on the byssal threads of adult mussels (Suchanek, 1978; Martel et al., 1999). Upon reaching 2.0 – 2.5 mm SL they relocate to open surfaces, sometimes at higher intertidal levels, via byssus drifting (Sigurdsson et al., 1976; de Blok & Tan-Maas, 1977). On open surfaces, these small mussels are typically only found within crevices and depressions (Suchanek, 1978).

The overall goal of this study was to determine the influence of temperature and desiccation stress on mortality of newly settled *M. trossulus*, and ontogeny of sensitivity to these stresses through the early benthic phase. This was accomplished by (1) determining the sensitivity of newly settled mussels to the full range of (a) temperatures and (b) desiccation stress levels that can be experienced in the field during the settlement season, (2) characterizing the changes in sensitivity to desiccation stress that occur as mussels increase in size, (3) determining the frequency of lethal desiccation conditions in the field during the period of peak recruitment, and (4) comparing the pattern of ontogenetic shift in sensitivity in juvenile *M. trossulus* to the size at which juveniles of this species leave filamentous microhabitats to relocate to open surfaces.

METHODS

Study site

This study was conducted from May to August during the summers of 2010 and 2011. Mussels were collected from Prasiola Point (N 48° 49’ 55”, W 125° 07’ 05”) in Barkley Sound on the west coast of Vancouver Island, a site with consistently high *Mytilus trossulus* settlement (A. Martel, pers. com.). Mussels were collected no more than 3 d prior to being used in experiments and were held in flowing seawater at 14°C. Laboratory experiments were conducted near the collection site at the Bamfield Marine Sciences Centre in Bamfield, British Columbia.

Abiotic conditions in the intertidal zone

To document the weather conditions occurring throughout the summer, when peak mussel recruitment normally occurs, I monitored six weather parameters from 26 June – 27 August 2010 and 4 May – 20 August 2011 using a weather station (Davis Instruments Vantage Pro2 Plus Integrated Sensor Suite 6327). The weather station was installed at Wizard Islet (N 48° 51’ 27’’, W 125° 09’ 38’’), a small rocky islet devoid of trees; Wizard Islet was used as a common weather monitoring site for all the experiments in this study and also for a separate study of barnacle mortality (Chapter 3). The weather station, mounted approximately 45 m inland from the intertidal zone, recorded air temperature, relative humidity (RH), wind speed, ultraviolet radiation, solar radiation, and rainfall. In addition, temperature was monitored on intertidal rock surfaces using 3 Thermocron® iButton (DS1921G) data loggers, and intertidal RH was monitored using 2 Lascar Electronics (EL-USB-2) data loggers. Loggers were placed at 2.75 m above MLLW, which is just below the upper limit of the vertical distribution of *M. trossulus*. The iButton loggers remained in the intertidal zone at all times during the same dates as the weather station. The RH loggers, however, would be damaged by water and therefore were only placed in the intertidal zone during low tide on six consecutive days in June 2011. All devices recorded data at 15 minute intervals and data from the weather station and iButton loggers were downloaded bi-weekly.

To determine the temperature and RH conditions occurring over the vertical range of mussel distribution in the intertidal zone, 6 RH data loggers were used to record temperature and RH at three intertidal heights (2.0 m, 2.25 m, 2.75 m), with 2 loggers per intertidal height. These heights correspond approximately to the lower, middle, and upper range of the mussels, respectively. At each intertidal height, one logger was placed on bare rock surface, while the other logger was placed under fucoid algae (*Fucus* spp.).

Data was logged at 5 minute intervals for 3-4 h during low tide on sunny days at two different field sites: Grappler Inlet (N 48° 40’ 55”, W 125° 07’ 05”) on 5 September 2012 and Wizard Islet on 6 September 2012.

Relative humidity (RH) is occasionally used as a measure of desiccation stress (Kennedy, 1976; Ricciardi et al., 1995; Montalto & Ezcurra de Drago, 2003; Miller et al., 2009), but vapour pressure deficit (VPD) is considered a better estimate of evaporation potential than RH. RH is a measure of the percent saturation in water vapour; as a result, for a constant RH, the actual amount of water that can be added to the air, and thus evaporation potential, changes with air temperature. VPD, on the other hand, is a measure of the actual amount of water vapour that can be added to the air (Anderson, 1936) and thus more accurately reflects desiccation stress. VPD has been used in several studies of the effects of desiccation stress on animals (squirrels, Baudinette, 1972; amphipods, Koch, 1989; mites, van Houten et al., 1995) and is used extensively in studies of plant physiology (Addington et al., 2004; Katul et al., 2009; Siqueira et al., 2012). VPD is defined as the difference between saturated and actual vapour pressure and is calculated as follows (WMO, 2008):

$$\mathrm{VPD} = e_s - e_a \qquad \text{(Equation 2.1)}$$

$$e_s = 0.6112 * e^x \text{ (kPa)}$$

$$x = \left(\frac{17.62 * T}{243.12 + T}\right)$$

$$e_a = (\mathrm{RH}/100) * e_s$$

where e_s = saturation vapour pressure
e_a = actual vapour pressure
T = air temperature (°C)

Sensitivity to temperature stress

The sensitivity of newly settled mussels to the full range of temperatures that occur in mid-intertidal rocky shore habitats of Barkley Sound was examined in two experiments carried out in July 2010 and July 2011. In the first experiment, 80 groups of 20 mussels measuring 1-2 mm in shell length (SL), i.e., ~ 1 month after settlement (A. Martel, pers. comm.), were separately placed in 80 sealed plastic Ziploc® containers measuring ~ 11 cm x 11 cm x 5 cm. Each group of mussels was subjected for 6 h to one of 8 temperature treatments in a controlled temperature chamber. The experimental design was as follows: 8 temperature treatments with 10 replicate containers per treatment and 20 mussels per container, for a total of 1600 mussels. The 6 h duration is representative of the average length of time that the mussels are emersed during low tide, thus it is an appropriate treatment duration to mimic field conditions and to obtain conservative measures of sensitivity. The 8 temperature treatments (10 - 45°C at 5° intervals) were representative of the range of rock surface temperatures occurring in the mid-intertidal zone at low tide, as determined by iButton measurements made in July 2010. VPD levels were kept to a minimum by placing a 5 x 5 cm piece of shammy cloth saturated with seawater in each sealed container. Mussels were positioned on this small square of shammy cloth ~ 1 mm apart to ensure the mussels were exposed to the temperature treatment equally. Temperature and RH, which are used to calculate VPD, were monitored by placing RH data loggers in 2 of the containers during each temperature treatment. Temperatures inside the containers equilibrated with the temperature inside the chamber within 30 min after the start of each experiment and remained relatively constant thereafter, fluctuating ± 0.5°C about the target temperature. After the treatment, mussels were placed in flowing seawater for 12 h and were then examined for mortality. Mussels were recorded as dead if gaping, not moving, and not responding to light tapping on their shells with a needle probe. Given that this first experiment revealed a tolerance threshold between 30°C and 35°C, a second experiment was carried out in July and August 2011 to resolve sensitivity at 1°C intervals from 30°C to 35°C. This second trial also included treatments at 28°C and 37°C to ensure mortality patterns were consistent with the first trial. The procedures and experimental design of this second experiment were the same as for the first experiment except for the treatment temperatures.

Sensitivity to desiccation stress

The sensitivity of recently settled juvenile mussels to desiccation stress was examined in July 2010. Forty groups of 20 mussels measuring 1-2 mm in SL were placed in separate sealed 4L glass jars and subjected for 6 h to one of four VPD treatments: 0.12 kPa (least stressful), 0.58 kPa, 1.17 kPa, and 1.75 kPa (most stressful). Treatment levels were determined by setting the temperature (20°C) and four levels of RH (95%, 75%, 50%, and 25% RH), and then calculating the corresponding VPD for each treatment. RH levels used in the treatments represented the full range of RH levels observed by the weather station in July 2010 (30 – 95% RH). Mussels were positioned on a 5 x 5 cm piece of dry shammy cloth ~ 1 mm apart to ensure the mussels were exposed to the treatment equally. All treatments were carried out in a controlled temperature chamber at 20°C, a temperature that occurs frequently in the field during the summer, and shown in the previous set of experiments to be benign for 1-2 mm SL *M. trossulus*. The experimental design was as follows: 4 VPD treatments, with 10 cages per treatment and 20 mussels per cage, for a total of 800 mussels. Different amounts of Drierite® desiccation crystals, which are composed of anhydrous calcium sulfate known to be chemically inert except toward water (W.A. Hammond Drierite Co. Ltd., 2012), were added to the bottom of the jars to obtain the two highest VPD levels. The two lowest VPD levels were obtained by adding a piece of shammy cloth wetted with different amounts of seawater to the jars. VPD levels equilibrated within 1 h from the start of the treatment, after which time VPD remained relatively constant, changing less than 0.1 kPa over 5 h. After the treatment, mussels were placed in flowing seawater for 12 h and were then examined for mortality.

Given that juvenile mussels prefer to settle within filamentous algae and then relocate to open surfaces later in life (Sigurdsson et al., 1976; de Blok & Tan-Maas 1977; Suchanek, 1978), we also examined whether filamentous algae maintain a low desiccation stress environment during low tide. To determine the amount of water retained by the algae throughout a low tide, 10 tufts of the filamentous green algae, *Cladophora columbiana*, were collected from Grappler Inlet during the last half hour of the daytime low tide on 4 September 2012 after being exposed for 8 h. The tufts were placed in sealed bags and returned to the laboratory where they were individually weighed, then blotted dry and reweighed.

Ontogeny of desiccation tolerance

The above experiments revealed that newly settled *M. trossulus* were more likely to be killed by ambient desiccation conditions than by temperature stress. The final experiment therefore examined how desiccation tolerance of juvenile mussels changes with increasing body size. The design of this experiment was similar to the desiccation experiment described above, except this experiment involved 3 VPD treatment levels and examined the responses of 4 size classes of juvenile *M. trossulus*: 1-2 mm, 3-4 mm, 5-6 mm, and 7-8 mm SL. The experimental design was as follows: 4 mussel size classes, each exposed to 3 VPD treatments (0.58 kPa, 1.17 kPa, 1.75 kPa), with 5 cages per treatment, and 20 mussels per cage, for a total of 1200 juvenile mussels. This experiment did not include a 0.12 kPa treatment because results from the previous desiccation experiment, described above, revealed no significant difference in mortality between the 0.12 kPa and 0.58 kPa treatments.

Data analysis

The proportion of dead mussels (percent mortality) was used as the dependent variable in all experiments. The assumptions of analysis of variance (ANOVA) were tested on the dependent variable using the Kolmogorov-Smirnov test for normality and Levene's test for homogeneity of variance. By nature of the data, proportion data follows a binomial distribution and violates the normality assumption of ANOVA. A modified Freeman and Tukey (1950) arcsine transformation was therefore applied to percent mortality data prior to statistical analysis. This transformation allowed the data to meet the assumptions of ANOVA, thus this test was used to assess the effects of temperature and VPD on percent mortality by comparing the mean proportion of dead mussels among treatment groups. Tukey post-hoc analysis was then used to determine patterns among treatment means.

The temperature at which 50% of the mussels died (LT_{50}) was estimated by fitting a sigmoidal curve to the mortality data, which is a special case of the logistic function often used in analysing dose-response relationships. The fraction of individuals, *S*, that are killed by a maximum temperature, *T* (°C), was therefore determined by the equation, modified from Denny et al. (2006):

$$S = \frac{1}{1+e^{-x}} \quad \text{where } x = (T\text{-}36.73)/0.3863$$

The VPD at which 50% of the mussels died (LD_{50}) was also determined as above. The formula for x was modified separately for temperature and VPD, and was determined using logistic regression.

RESULTS

Abiotic conditions in the intertidal zone

Maximum mid-intertidal rock surface temperature varied greatly throughout each summer. Although intertidal temperature data were lost from 28 June – 5 July 2010 and 18-28 July 2010 due to damage incurred to the iButton data loggers, reliable temperature data was obtained for 33 days in 2010, including some of the hottest days of the summer. Intertidal temperature recorded on those days reveal that the maximum rock surface temperature reached 45°C in July and also in August 2010. In 2011, the maximum daily rock surface temperatures peaked in late June at 40.5°C and again in late July at 42.5°C (Figure 2.1A). These peak temperatures occurred on days with mid-day low tides. The slightly lower intertidal temperatures recorded in August 2011, relative to June and July 2011, were likely due to the lower average amount of daily solar radiation, which was 10-33% lower than the rest of the summer and caused by the increased amount of fog and cloud cover that is typical of Barkley Sound in August.

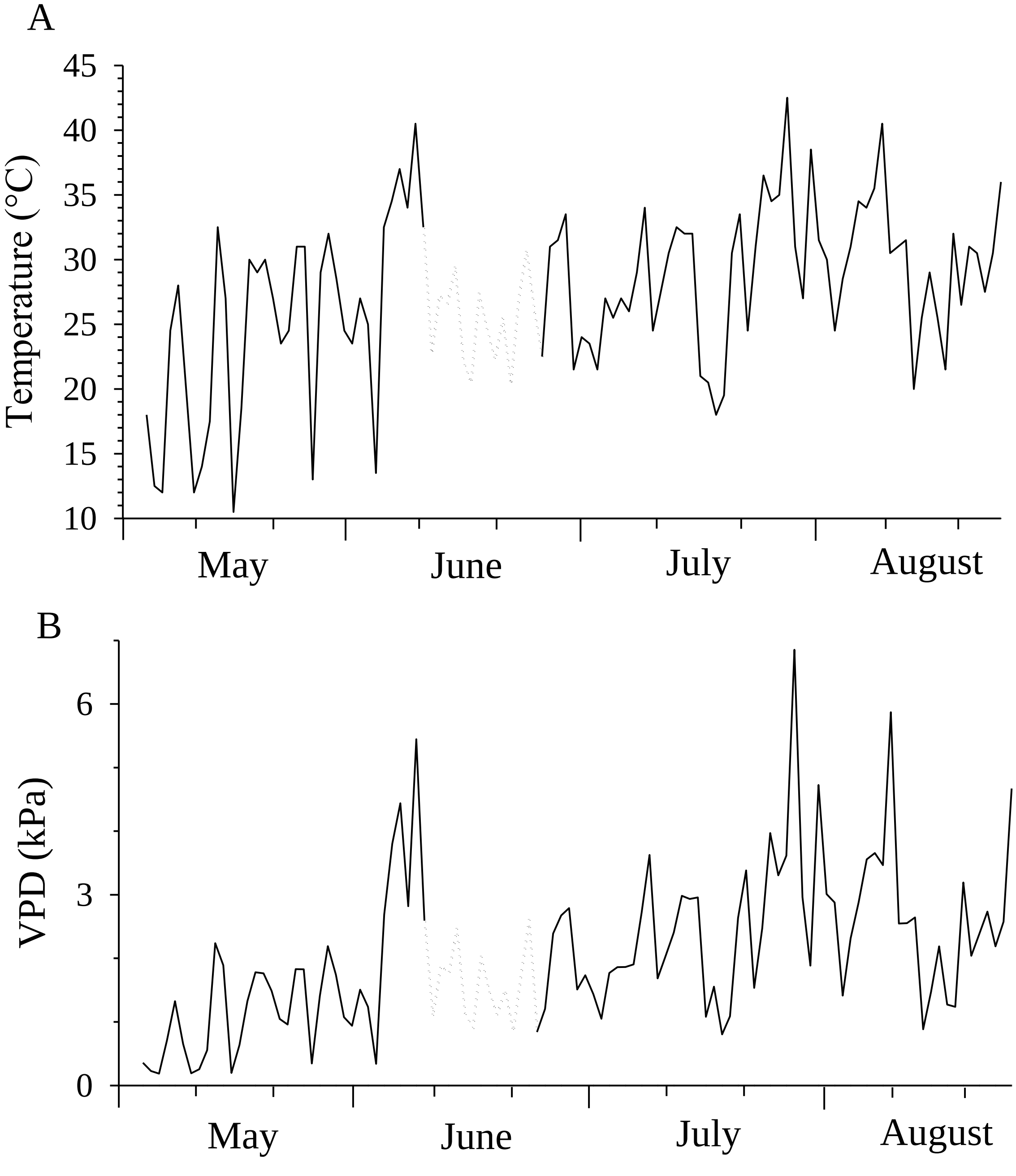


Figure 2.1. (A) Actual maximum daily mid-intertidal temperatures (°C) and (B) predicted maximum daily mid-intertidal VPD at the rock surface for May to August, 2011. Data for June 9-21 are based on predicted temperature values (dotted line), as measured data were lost due to damage incurred to data loggers.

Relative humidity measurements on intertidal rock surfaces were used to determine the frequency of stressful desiccation conditions occurring during the settlement season. Although RH data was not collected in 2010, intertidal RH data was recorded over six low tides in June 2011, and multiple regression analysis between intertidal RH measurements and weather station data was used to extrapolate intertidal RH data for the rest of the summer of 2011. Eight weather parameters recorded by the weather station were used as explanatory variables in the regression with intertidal RH as the response variable. The best-fit model (Table 2.1) was developed into a predictive equation for intertidal RH in 2011, and of the eight parameters examined, only three significantly influenced intertidal RH: air temperature, wind speed, and solar radiation. These three parameters could account for 89.7% of the variance. A small amount of autocorrelation was detected in the residuals, which is thoroughly examined in Appendix A, and suggests that the predictive equation may be slightly improved by accounting for autocorrelation. However, modifying the equation to account for such a small amount of autocorrelation is unlikely to make much difference in the resulting long-term predictions, therefore I opted to accept the simpler model. The equation developed from this model predicted intertidal RH values very close to actual measured values, though tended to underestimate RH below 48%. After calculation, the predicted RH values were converted to VPD. The predicted daily maximum mid-intertidal rock surface VPD levels ranged from 0.16 kPa to 6.52 kPa over the summer of 2011, with daily maximum VPD

Table 2.1. Multiple regression best-fit model predicting intertidal RH from weather station parameters in 2011. Temp= air temperature (°C), WindSpd= wind speed (km/h), SolRad = solar radiation (kW/m^2), β= partial regression coefficient

Coefficients	β	Estimate	Std. Error	t	p
Intercept	0.923	1.814	0.065	27.745	<0.001
Temp	0.729	- 0.062	0.005	- 12.289	<0.001
WindSpd	0.364	0.003	5.753×10^{-04}	4.504	<0.001
SolRad	0.803	$- 2.395 \times 10^{-04}$	1.541×10^{-05}	- 15.537	<0.001

$F_{3,133}=393.6$, $R^2 = 0.897$, $p < 0.001$

levels gradually increasing from May to August (Figure 2.1B). This suggests that the mussels encountered increased desiccation stress as the recruitment season progressed.

Data loggers that were placed at 3 different intertidal heights (2.0 m, 2.25 m, 2.75 m) during low tide on Wizard Islet revealed that intertidal rock surface temperatures and intertidal VPD levels reached and exceeded the juvenile mussel tolerance thresholds, and were thus potentially lethal, at all 3 intertidal heights (Figure 2.2A&B). However, high

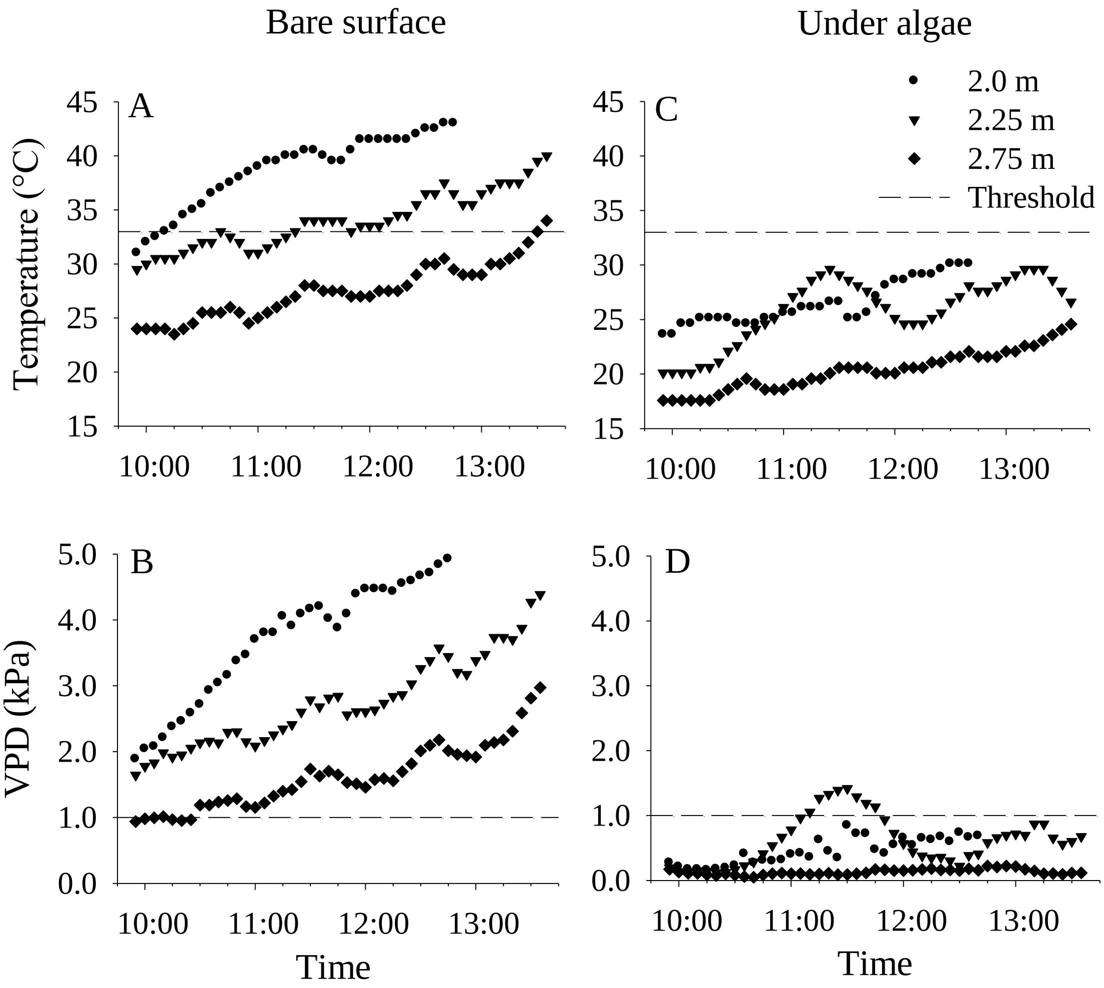


Figure 2.2. Temperature (°C) and vapour pressure deficit (kPa) data collected at 3 intertidal heights on Wizard Islet on 6 September 2012. The left column (A&B) represents conditions on bare rock surface, and the right column (C&D) represents conditions under fucoid algae. Horizontal dashed lines represent the juvenile mussel tolerance threshold, as determined by laboratory experiments.

temperatures did not persist for more than 2.5 h before the surfaces at 2.0 m and 2.25 m were re-immersed by the incoming tide. Although data were not collected after the tide rose to 2.25 m, there was potential for further temperature and VPD increases at 2.75 m over another 2 h before the tide re-immersed that surface. Newly settled mussels are therefore exposed to potentially lethal temperatures and desiccation levels over their full vertical range, but this exposure likely occurs for the longest time near the upper limit of distribution, which is occasionally emersed for up to 13 h when the water level at the lower high tide of the day is ≤ 2.7 m above MLLW. Data loggers placed under fucoid algae showed that rock surface temperatures and VPD levels did not reach potentially lethal levels at any intertidal height (Figure 2.2C&D); the exception was a short period of time when VPD levels at 2.5 m briefly peaked above the threshold, which may have been due to the angle of the sun temporarily allowing sunlight to penetrate through a small gap in the algal fronds (pers. obs.), thus heating the surface. Data from Grappler Inlet, examined in Appendix B, indicate patterns among intertidal heights were very similar to those revealed on Wizard Islet, though temperatures did not ever exceed threshold values.

Unexpectedly, temperatures at 2.0 m were consistently higher than those at 2.25 m, and both were higher than temperatures at 2.75 m. Observations of the field site indicated that the rock surfaces in the low- and mid-intertidal tend to be coated with brown/black biofilm and encrusting algae, whereas the higher intertidal level was mostly light grey-colored bare rock (Figure 2.3). The lower intertidal surfaces therefore likely absorbed more sunlight and thus heated faster than the lighter gray surfaces of the high-intertidal, accounting for the difference in temperatures observed.

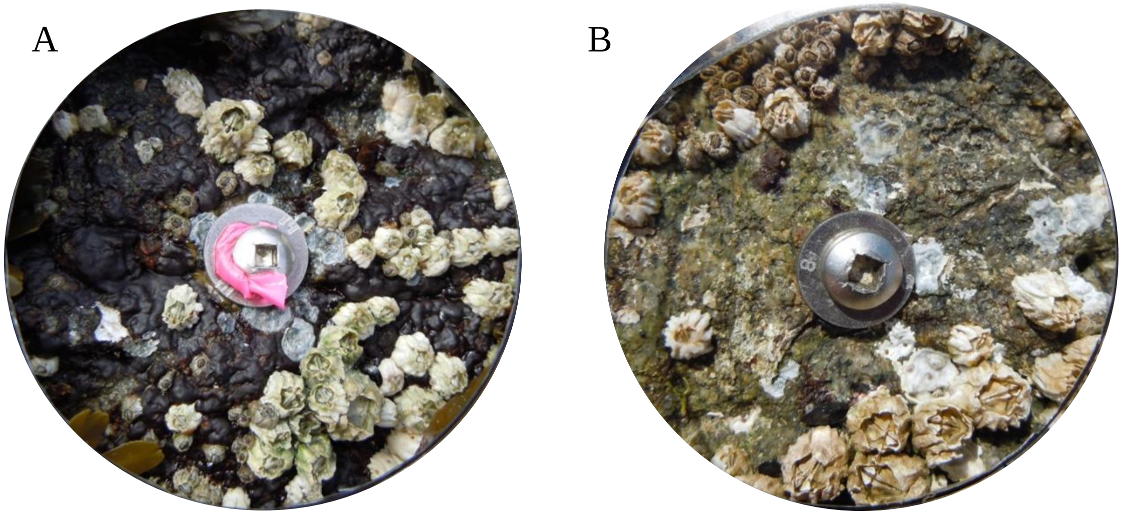
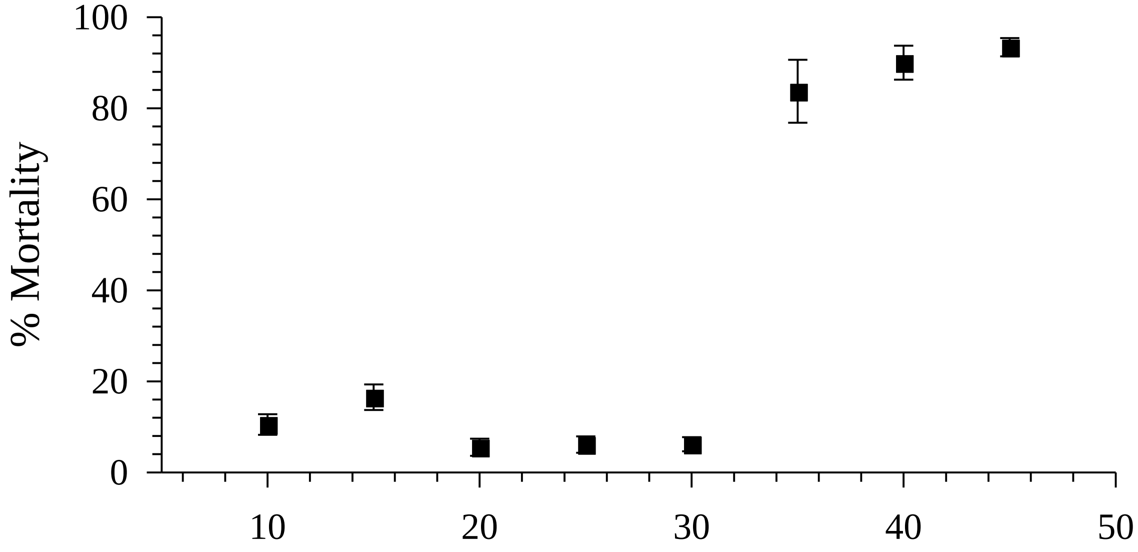


Figure 2.3. Differences in rock surface colour at A) 2.5 m and B) 2.75 m above MLLW. The darker surface in (A) is due to a thick layer of brown/black biofilm and encrusting algae coating the surface, which is nearly absent in (B). Photos were taken as part of a separate study of barnacle mortality (Chapter 3).

Sensitivity to temperature stress

Juvenile mussels were tolerant of a wide range of temperatures, but did experience increased mortality at high experimental temperatures. In the first temperature tolerance trial, there was a significant difference among temperature treatments in the average proportion of dead mussels (ANOVA: $F_{7,72}$=104.9, p<0.001). Tukey post-hoc comparisons revealed that mortality was significantly higher at 35°C than at 30°C, with juvenile mussels experiencing 77% more mortality at 35°C than at 30°C (Figure 2.4A). There was no significant difference in mortality among treatments ranging from 10°C to 30°C, nor among treatments ranging from 35°C to 45°C. This suggested the threshold of temperature tolerance was between 30°C and 35°C. The second trial, focusing on the 30°C to 35°C range of temperatures, also revealed a significant difference among temperature treatments in the average proportion of dead mussels (Figure 2.4B; ANOVA: $F_{7,72}$=96.8, p<0.001). Tukey post-hoc comparisons revealed that mortality differed significantly between the 33°C and 34°C treatments (a 58% difference) and between the 35°C and 37°C treatments (a 34% difference), indicating a temperature tolerance

A) Temperature trial 1, July 2010



B) Temperature trial 2, July 2011

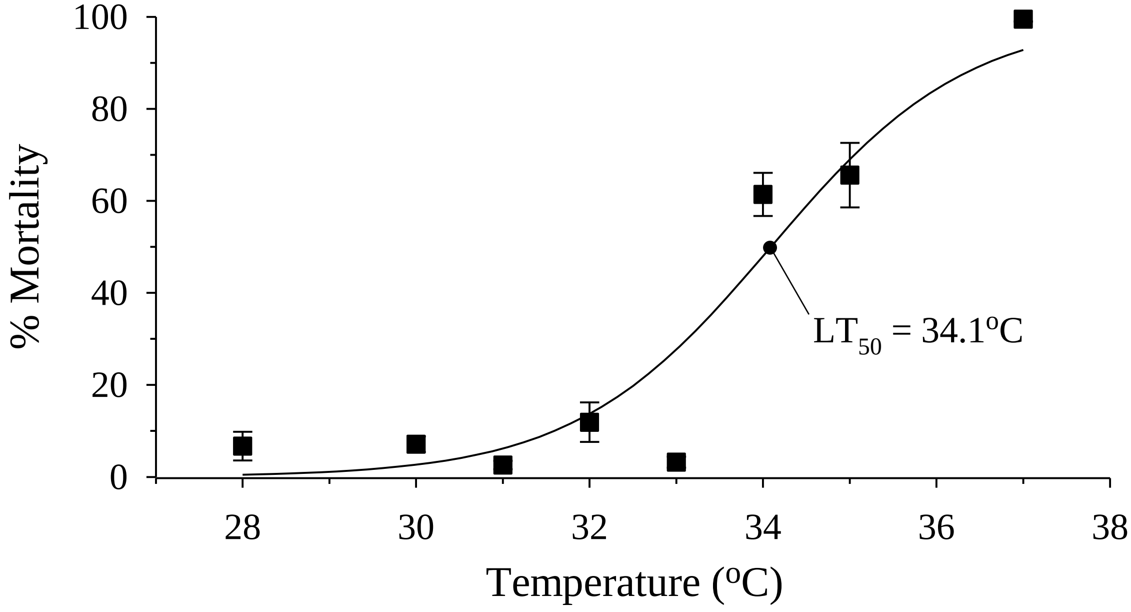


Figure 2.4. Effect of prolonged exposure to various temperature treatments on mortality of newly settled (1-2 mm SL) mussels. (A) First temperature trial exposing mussels to temperatures ranging from 10-45°C in 5°C intervals. (B) Second temperature trial exposing mussels to the narrower temperature range of 28-37°C in 1°C intervals to determine the threshold of temperature tolerance. Each point represents the average % mortality for a treatment ± SE. Solid line in (B) is the sigmoidal curve used to determine the LT_{50}.

threshold of 33°C. There was no significant difference in mortality among treatments ranging from 28°C to 33°C, nor between the 34°C and 35°C treatments. Fitting a sigmoidal curve to the data revealed an LT_{50} of 34.1°C for juvenile *M. trossulus*.

The temperature loggers recorded a total of 12 days in 2010 and 13 days in 2011 that exhibited rock surface temperatures exceeding 33°C (i.e., above the temperature tolerance threshold for mussels <2 mm SL) and persisting for at least 1 h (Figure 2.5). The majority of these days had temperatures that persisted above 33°C for only 1-2 h, but twice in July 2010 and once in July 2011 the temperatures persisted above 33°C for 8 h. August 2011 had slightly fewer days exceeding 33°C than August 2010. In addition, June 2011 had cooler temperatures than July and August 2011, with only 3 days that exceeded 33°C and never for more than 3 h. Temperatures in May 2011 never exceeded 33°C. Newly settled mussels are therefore exposed to potentially lethal temperatures in the field, but this occurs relatively infrequently throughout the recruitment season, and temperatures above the threshold do not often persist for periods longer than 2 h.

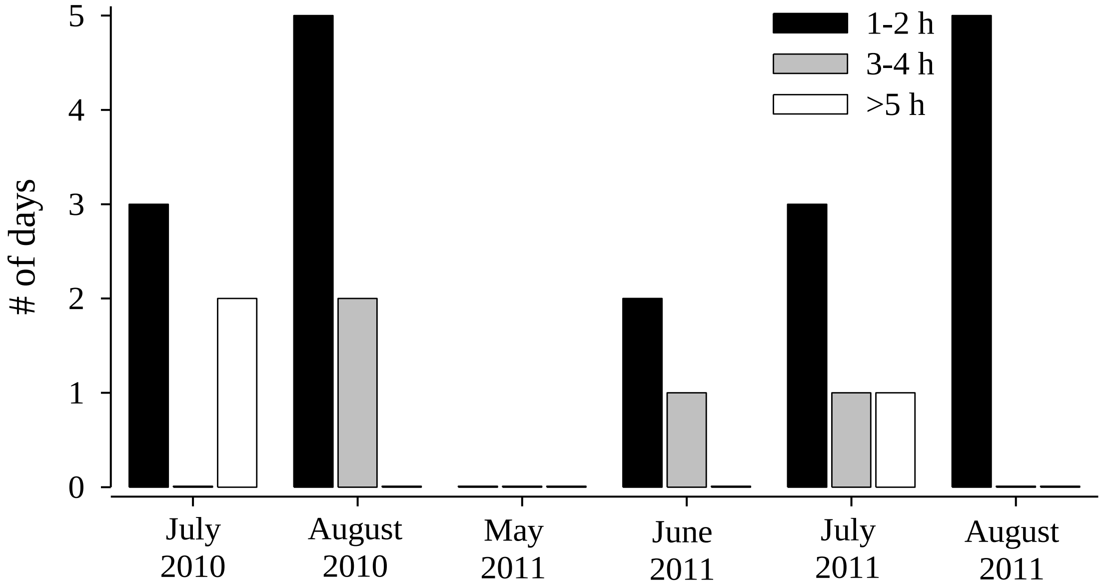


Figure 2.5. Number of days during the 2010 and 2011 settlement seasons when the intertidal rock surface temperature near the upper limit of adult mussel distribution exceeded 33°C for different durations at low tide.

Sensitivity to desiccation stress

Juvenile mussels had low tolerance to high VPD levels (Figure 2.6). There was a significant difference in average mortality among VPD treatments (ANOVA: $F_{3,36}$=68.42, $p<0.001$), and Tukey post-hoc comparisons indicated that mortality differed significantly between the 0.58 kPa and 1.17 kPa treatments, mortality being 72% higher in the latter treatment. There was no significant difference between the 0.12 kPa and 0.58 kPa treatments, nor between the 1.17 kPa and 1.75 kPa treatments. This suggests the threshold VPD lies between 0.58 kPa and 1.17 kPa. Fitting a sigmoidal curve to the data suggests an LD_{50} of 1.01 kPa for 1-2 mm SL *M. trossulus*.

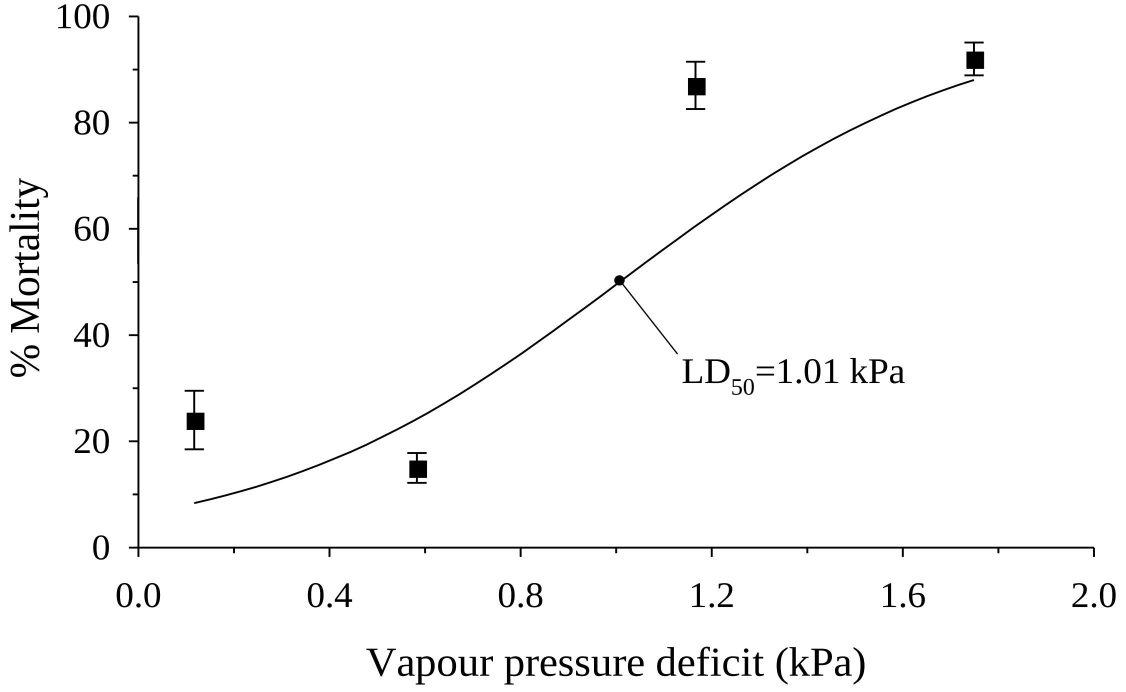


Figure 2.6. Effect of prolonged exposure to various vapour pressure deficits (kPa) on mortality of juvenile (1-2 mm SL) mussels (*Mytilus trossulus*). Each point represents the average % mortality ± SE. Solid line is the sigmoidal curve used to determine the LD_{50}.

The majority of days during summer 2011 had estimated low tide VPD levels that met or exceeded the LD_{50} for juvenile mussels and were often sustained for many consecutive hours (Figure 2.7). May had few days with potentially harmful VPD levels, with 67% of the month having estimated VPD levels below the threshold level. In contrast, every day in July had at least 1 hour of estimated VPD levels over the threshold level, and on most days harmful VPD levels were estimated to be sustained for at least 5 hours. Harmful VPD levels that were estimated to be sustained for at least 9 hours, spanning almost the full duration of low tide, occurred in June, July, and August.

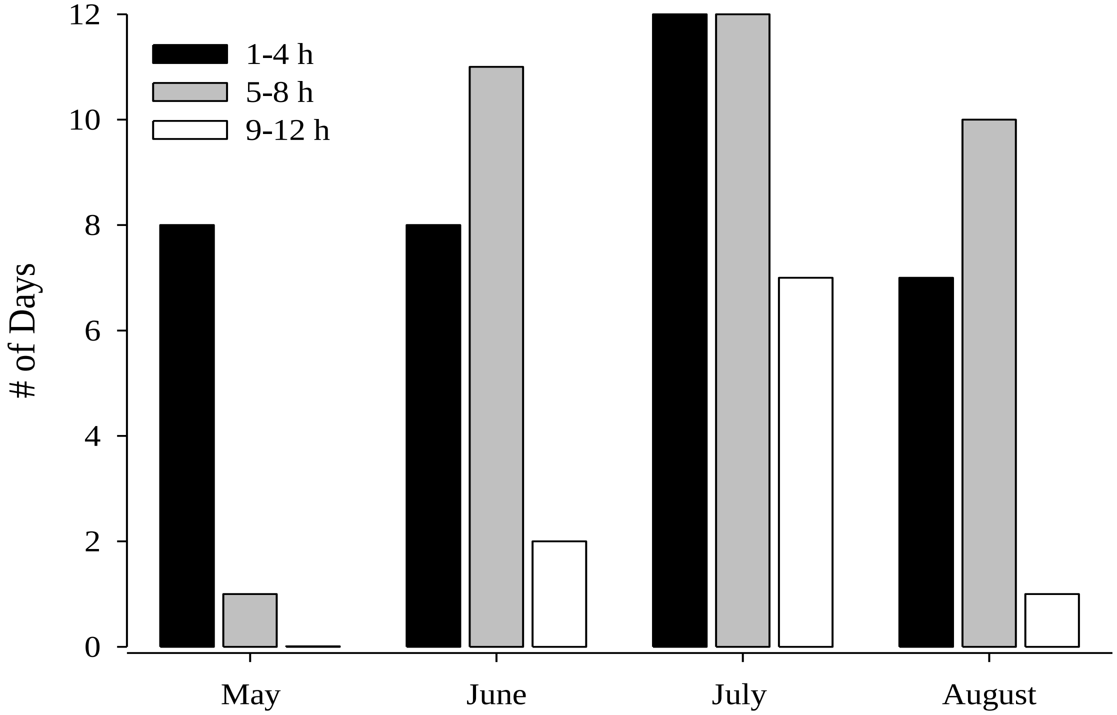


Figure 2.7. Number of days during the 2011 settlement season that vapour pressure deficit was ≥ 1.01 kPa (mussel threshold) for different durations during low tide.

Cladophora columbiana maintained a moist environment among its filaments even after prolonged exposure to desiccating conditions. After 8 h aerial exposure, the last 5 h of which were in direct sunlight, the tufts of algae still contained an amount of water equal to an average of 26% of their blotted dry weight. The amount of water that was contained within tufts of algae after low tide aerial exposure was proportional to the blotted dry weight of the algae (Figure 2.8, Linear regression: $F_{1,8} = 26.5$, $R^2 = 0.77$, $p < 0.001$).

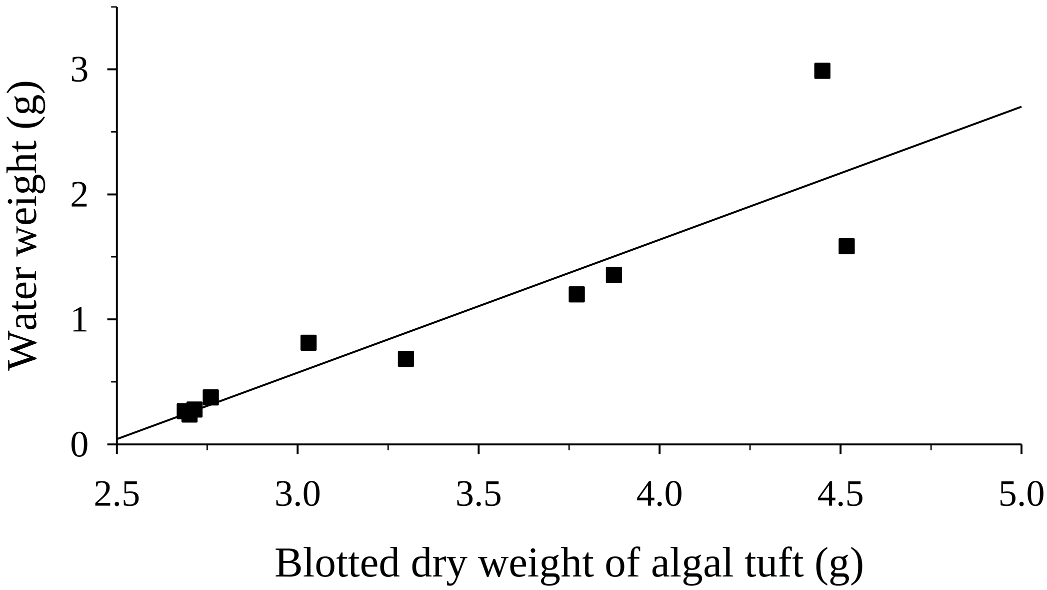


Figure 2.8. Weight of water (g) contained within tufts of *Cladophora columbiana* after 8 h aerial exposure as a function of the blotted dry weight (g) of the algae.

Ontogeny of desiccation tolerance

Mussel mortality differed among VPD treatments and among size classes. A significant interaction between size class and VPD (Table 2.2) revealed that the trend in average mortality among VPD levels differed among size classes. Figure 2.9 reveals that higher treatment VPD levels led to increased mortality in mussels 1-2 mm SL, but not in mussels ≥ 3 mm SL.

In this experiment, the 1-2 mm SL mussels experienced lower mortality at 1.17 kPa and 1.75 kPa than would be predicted based on the previous desiccation threshold experiment (65% and 50%, respectively); nevertheless, the overall trend of increasing desiccation stress causing higher mortality for 1-2 mm SL mussels is consistent between both experiments.

Table 2.2. Two-factor ANOVA of the average mortality of mussels in each of 4 size classes exposed to various VPD levels for 6 h.

Source	*df*	MS	F	p
Size Class	3	0.363	43.532	<0.001
VPD	2	0.082	9.840	<0.001
Size Class x VPD	6	0.026	3.088	0.012
Residual	48	0.008		

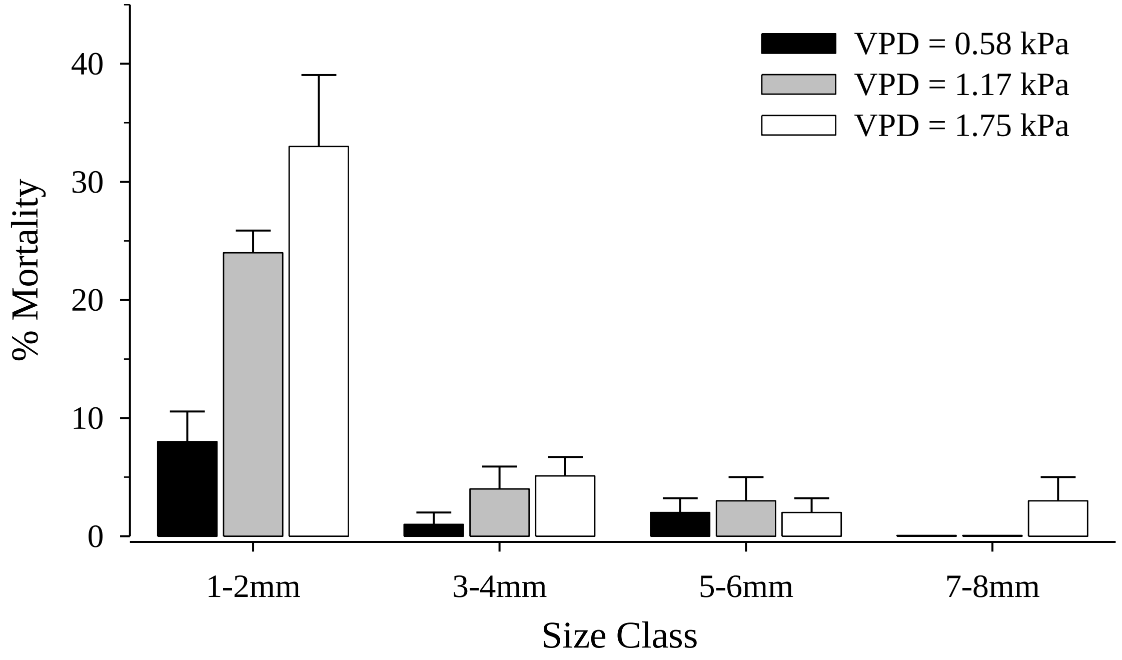


Figure 2.9. Effect of relative humidity on mortality of mussels within different size classes. Bars represent average % mortality ± SE.

DISCUSSION

Sensitivity to temperature stress

Extreme temperatures may not be as important a mortality factor as previously presumed for recently settled *M. trossulus* on Vancouver Island, British Columbia. Mortality of 1-2 mm SL juvenile *Mytilus trossulus* did increase at high temperatures, but only at temperatures exceeding 33°C, indicating that these recent settlers are able to tolerate the majority of temperature conditions experienced in their natural environment when humidities are high. To my knowledge, this is the first study to experimentally determine a lethal temperature for juvenile mussels during air exposure. Intertidal rock temperatures frequently reached or exceeded lethal levels (i.e., > 33°C), but they were most often sustained for only 1-2 h. Although not tested directly in this study, 1-2 h is not likely long enough to kill many newly settled mussels since 6 h exposure to 34°C was required to obtain 50% mortality of juvenile mussels in laboratory experiments. In July 2010 and 2011, there were a similar number of days when temperatures reached or exceeded lethal levels for at least 1 h. These high temperature events also occurred with similar frequency in August of 2010 and 2011, suggesting that the frequency of such temperature events is high. However, these high temperature events occurred on less than 20% of the total number of days monitored during the settlement season, and less than 1% had a temperature event that persisted above the threshold level for at least 5 h. Prolonged exposure to lethal temperatures was therefore very infrequent in the field during the period of larval settlement and early juvenile growth. Adding to this, the actual body temperature of mussels is typically lower than the temperature of the rock surface because other factors, such as solar radiation, air temperature, wind speed, and evaporative cooling, contribute to determining the actual body temperature of the mussels (Helmuth, 1998). This further reduces the likelihood of temperature being an important mortality factor for newly settled *M. trossulus*. My results are consistent with other studies that reported field temperatures within the tolerance limits of early juvenile limpets (Wolcott, 1973) and snails (Gosselin & Chia, 1995).

Prolonged temperature events at or above the threshold level are infrequent in Barkley Sound, but temperature events repeatedly occurring near the threshold for 1-2 mm SL mussels might nevertheless reduce their ability to survive. In the present study there were several days during which temperatures at or above the threshold level were sustained for 1-2 h, and several days during which temperatures were sustained 1-3°C below the threshold level for 3-4 h. These conditions are not likely to directly kill newly settled mussels. However, a recent study by Jones et al. (2009) indicated that repeated exposure to sublethal temperatures for 6 h each day lowered the thermal tolerance threshold of adult *M. edulis* by up to 5 °C. This may occur because sublethal stress can cause irreparable protein damage in mussels (Hofmann & Somero, 1995; Tomanek & Zuzow, 2010). In addition, desiccation could potentially interact with temperature to lower the tolerance threshold; for example, Miller et al. (2009) determined that a change in RH from 100% to 50-60% over 7 h caused the LT_{50} of *Lottia gigantea* to decrease by nearly 5°C. An interaction such as this, coupled with repeated exposure to sublethal temperatures, might therefore reduce the survivorship of 1-2 mm SL mussels during the first few days and weeks of life in the intertidal zone.

Sensitivity to desiccation stress

Unlike extreme temperature, exposure to high desiccation stress appears to be an important factor influencing the mortality of 1-2 mm SL *M. trossulus* on the west coast of Vancouver Island. These recently settled juvenile *M. trossulus* were highly sensitive to VPD levels above 1.01 kPa, a level that was exceeded in the field nearly every day and for prolonged periods. Peak settlement for *M. trossulus* on the northwest coast of North America occurs in June and July (Strathmann, 1987; Johnson & Geller, 2006), which coincided with the highest levels and longest durations of desiccation stress in the field. This likely occurs as a consequence of high food availability at this time of year, which supports larval growth in the water column (Mackas et al., 2007). Therefore, the benefits provided to mussels settling at this time of year likely would have outweighed the costs incurred by exposure to high desiccation stress. It is not clear whether climate change will alter this balance in the future. Substantial mortality was observed after exposing mussels to 6 h of high desiccation stress in laboratory experiments. In addition, lethal levels of desiccation stress occurred over the full range of the intertidal distribution of *M. trossulus*. Therefore, the frequent occurrence of days where the threshold VPD was reached or exceeded for at least this long suggests that juvenile mussels would have a low probability of survival if they were to settle on open rock surfaces.

Early juvenile mortality of other intertidal invertebrates, including barnacles (Shanks, 2009; Gosselin & Jones, 2010), limpets (Wolcott, 1973), and snails (Gosselin & Chia, 1995), has also been attributed to desiccation stress. Although not examined in this study, the duration of exposure to desiccation stress may also be an important factor influencing mortality of recently settled mussels. For example, LeBlanc et al. (2005) reported that mortality of 8 mm SL *M. edulis* after 11 h exposure to a VPD level of 1.98 kPa (calculated from temperature and RH values provided by the author) was >50%, whereas 6 h of exposure to the similarly high desiccation stress level of 1.75 kPa caused less than 5% mortality of 8 mm SL *M. trossulus* in the present study.

To ensure survival, it is critical for settlers to reduce their exposure to lethal desiccation conditions. *M. trossulus* settle primarily in filamentous algae (Suchanek, 1978; Martel et al., 1999), a structurally complex microhabitat. I determined that tufts of filamentous algae are able to retain large amounts of water when exposed at low tide, thus eliminating desiccation stress within the tufts. I also determined that the amount of water retained is proportional to the size of the algal tuft; as these algae are most often found in large, dense mats rather than small tufts in Barkley Sound (pers. obs.), the algae are unlikely to ever completely dry up even on the warmest days of mid-summer. Additionally, temperature and humidity measurements under fucoid algae during mid-summer revealed substantially lower temperature and substantially higher humidity under the algae than on bare surfaces during mid-day low tides. The function of primary settlement of *M. trossulus* among the microhabitats created by filamentous algae may therefore be to obtain protection from frequent exposure to desiccation stress. This would be consistent with Gosselin and Chia (1995), who demonstrated that similar microhabitats provide protection from desiccation stress to newly hatched *Nucella emarginata*.

Ontogeny of desiccation tolerance

Tolerance to desiccation stress changed rapidly with increasing body size in juvenile *M. trossulus*. Larger mussels were much more tolerant to the range of experimental desiccation conditions than newly settled mussels. More specifically, *M. trossulus* juveniles 1-2 mm SL were highly sensitive to moderate desiccation stress, yet mussels in the next size class, 3-4 mm SL, were significantly more tolerant. The tolerance threshold to desiccation stress by juvenile *M. trossulus* therefore increases substantially when the individual reaches ~2 mm SL. My results revealed that bare rock surfaces within the vertical range of *M. trossulus* in the intertidal zone (≈2.0 m – 2.75 m) are frequently exposed to desiccation stress levels at or above the tolerance threshold for newly settled mussels. In addition, these high levels of desiccation stress were often sustained for the full duration of low tide, which typically lasted 5-8 h but on some days spanned 13 h. To my knowledge, intertidal growth rates of newly settled *M. trossulus* have not been published; however, the average growth of newly settled individuals of a closely related species, *M. edulis*, was reported as 0.025 mm d^{-1} (Bayne, 1964), indicating growth from settlement (~ 0.5 mm) to 2 mm could take at least 40 d. This suggests 1-2 mm SL *M. trossulus* may be repeatedly exposed to potentially lethal levels of desiccation stress for several weeks before growing to desiccation-resistant size.

The difference in desiccation tolerance observed between mussels in the two desiccation experiments only slightly changes the likelihood of exposure to potentially harmful VPD levels during the settlement and early growth season. The two desiccation tolerance experiments were conducted on different years, thus inconsistency in mussel mortality between the experiments may reflect natural variability in tolerance among yearly cohorts of settlers. I fitted a second sigmoidal curve to mortality data for 1-2 mm SL mussels from the ontogeny of desiccation tolerance experiment, which revealed an LD_{50} of 2.09 kPa. Using this as the threshold value, there would still be 42 d (38.9%) of the settlement and early growth season when VPD levels reached or exceeded the higher estimated desiccation tolerance level of 2.09 kPa for at least 1 h, and on most days harmful VPD levels were sustained for over 4 h. Although the tolerance of 1-2 mm SL mussels may differ slightly among yearly cohorts, the broad range of observed VPD levels and frequent and prolonged occurrence of levels above 2.09 kPa in the field nonetheless suggests desiccation stress is likely to be a potentially important mortality factor for 1-2 mm SL *M. trossulus*.

The size threshold at which mussels become resistant to desiccation stress also closely corresponds to the size at which mussels experience a shift in microhabitat use. After primary settlement in filamentous algae, many *Mytilus* species relocate via byssus drifting (Bayne, 1964; Sigurdsson et al., 1976; Lane et al., 1985) to adult mussel beds or open surfaces during secondary settlement (*M. edulis*: Bayne, 1964; Newell et al., 1991; *M. galloprovincialis*: Porri et al., 2007; *M. trossulus*: L. Gosselin, pers. obs.). This constitutes a shift in microhabitat from being protected within the extensive branching of filamentous algae to being more directly exposed to ambient conditions of the mid-intertidal zone. Many *Mytilus* species are known to be able to repeatedly shift habitats until they reach 2.0 – 2.5 mm SL (Bayne, 1964; Sigurdsson et al., 1976; de Blok & Tan Maas, 1977). In addition, Hunt and Scheibling (1998) found that up to 82% of *M. trossulus* and *M. edulis* that colonized natural substrata were >2 mm SL, suggesting that dispersal is common for mussels <2 mm SL. These findings suggest the size of juvenile mussels at final settlement in adult habitat (2.0 – 2.5 mm SL) may be the minimum size where mussels are able to tolerate most desiccation conditions experienced in the field. This close correspondence between the size at which juvenile *M. trossulus* become considerably more tolerant of desiccation conditions and the size at which juvenile mussels relocate from filamentous algal habitat to adult habitat suggests ontogenetic shifts in habitat use by juvenile *M. trossulus* may be a response to changing vulnerability to desiccation stress, similar to findings by Gosselin (1997) for hatchling snails.

Finally, climate forecasts for the south coast of British Columbia predict air temperatures to be warmer by 1.7°C and air to be dryer, with a 13% decrease in precipitation, by 2050 (Rodenhuis et al., 2007). These changes could increase the frequency of days with conditions exceeding threshold desiccation tolerance levels for juvenile *M. trossulus*. Survival of newly settled mussels, and thus possibly the persistence of mussel populations, will therefore likely depend even more upon the persistence of protective microhabitats created by filamentous and fucoid algae as climate changes. A recent report examining intertidal community changes over 10 y following ocean warming on the west coast of California revealed that the abundance of algae dominating the intertidal zone, such as filamentous *Endocladia muricata*, has declined by over 50% (Schiel et al., 2004). This raises the question of whether the algae that serve as protective microhabitats for early benthic phase *M. trossulus* will be able to withstand the predicted climate changes.

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CHAPTER 3: Is daily variation in early benthic phase mortality of the barnacle *Balanus glandula* influenced by low tide weather conditions or *Fucus* spp. cover?

INTRODUCTION

Our understanding of the factors that influence variability in the abundance of marine intertidal invertebrate populations has increased considerably over the last 30 years. For many species, however, there is still debate over whether this variability is primarily influenced by larval supply and settlement (Gaines & Roughgarden, 1985; Minchinton & Scheibling, 1991; Hunt & Scheibling, 1998) or postsettlement survival (Lively et al., 1993; Petraitis et al., 2003; Jennings & Hunt, 2010, 2011). Many benthic marine invertebrates have pelagic larvae that settle into benthic habitats and undergo metamorphosis, which constitutes a dramatic ecological transition from a consistently wet habitat to one exposed to aerial conditions for several hours per day (Werner & Gilliam, 1984). During this transition period, most cohorts typically experience 60-99% mortality (Gosselin & Qian, 1997; Pedersen et al., 2008). It has therefore been suggested that variations in survival during the transition from pelagic to intertidal habitat may be the cause of observed differences in population abundance (Osman et al., 1992; Gosselin & Chia, 1995).

In the first few hours and days after settlement, mortality can be severe, but is also often highly variable among cohorts. For example, Gosselin and Qian (1996) reported that first day mortality of 3 daily cohorts of *Balanus glandula* cyprids ranged from ~30-40%, and second day mortality of the same cohorts ranged from ~5-20%. Similarly, mortality of *Semibalanus balanoides* cyprids during the 2 d transition from settlement to metamorphosis ranged from 15-43% among 5 daily cohorts (Jarrett, 2000). Few studies have followed daily mortality of individual recruits during the short transition period from settlement to metamorphosis; Minchinton and Scheibling (1993) demonstrated that sampling individuals during this period at 1.3 d intervals can result in determining significantly higher, and likely more accurate, mortality rates than longer sampling intervals. Because recruitment studies have widely varied in sampling intervals, the processes specifically influencing mortality during the transition into benthic habitat have been difficult to identify.

Several factors may cause mortality during the first few hours and days after settlement, including dislodgement, predation, poor physiological condition, and abiotic stress (Gosselin & Qian, 1997; Hunt & Scheibling, 1997; Jarrett, 2000). In particular, newly settled juveniles of some invertebrate species are known to be sensitive to such abiotic factors as temperature stress (oysters, Roegner & Mann, 1995; barnacles, Chan & Williams, 2003), desiccation stress (whelks, Gosselin & Chia, 1995; barnacles, Shanks, 2009), reduced salinity (barnacles, Berger et al., 2006; Thiyagarajan et al., 2007), and both ultraviolet radiation and visible light (tunicates, Hurlbut 1993; Bingham & Reitzel, 2000; barnacles, Gosselin & Jones 2010). However, the direct influence of these abiotic factors on daily variation in mortality through the early benthic phase has yet to be determined. The sensitivity of new settlers to abiotic factors such as temperature and desiccation stress could constitute a direct link between early benthic phase mortality and the weather-related parameters that influence these factors, such as wind speed, temperature, relative humidity, solar radiation, ultraviolet radiation, and precipitation.

Barnacles in particular are directly exposed to abiotic stress; once settled, they cannot move to a more suitable microclimate if conditions become unfavourable. Several experiments have determined that newly settled barnacles are unable to survive prolonged periods of high temperature stress (Crisp & Ritz, 1967; Foster, 1969; Thiyagarajan et al., 2000; Shanks, 2009) and desiccation stress (Foster, 1971). Other studies have reported a negative influence of weather conditions on survivorship, such as gales (Connell, 1961) and sun exposure (Denley & Underwood, 1979), but specific sensitivity to the abiotic factors that influence these weather conditions were not determined. It is therefore not clear if daily changes in the weather parameters that influence temperature and desiccation stress in the intertidal zone are directly linked to daily variation in mortality through the early benthic phase. Understanding the role of weather-related abiotic conditions in controlling post-settlement mortality may provide insight into the effects of climate change on patterns of survivorship through the critical early benthic phase, including impacts to overall population abundance and community structure.

This study aims to improve our understanding of the factors that influence mortality during the first hours and days after the transition from pelagic to benthic environments. This was accomplished by examining the mortality of barnacle cyprids from settlement to metamorphosis, as well as mortality of early juveniles up to the age of 10 d after metamorphosis. The specific goals of the study were therefore to (1) determine the range of weather-related abiotic conditions occurring daily in the upper intertidal zone, a habitat heavily colonized by barnacles, (2) determine daily settlement and mortality rates of barnacle cyprids and juveniles in the upper intertidal zone, (3) characterize the role of temperature, relative humidity, wind speed, solar radiation, ultraviolet radiation, and rainfall at low tide in controlling variation in mortality of daily cohorts, and (4) determine the effect of cover by the macroalgae *Fucus* spp. on settlement and mortality of barnacle cyprids and juveniles.

METHODS

Study site and organism

This field study was conducted from May to August 2011 in Barkley Sound along the west coast of Vancouver Island, British Columbia. Field work was carried out on Wizard Islet (N 48° 51’ 27’’, W 125° 09’ 38’’), a small islet with moderate exposure to waves (Gosselin & Rehak, 2007) and wind (pers. obs.). The intertidal zone at the field site consists of a long, gently sloping bench that is not shaded by trees or rocky cliffs. The mid-intertidal zone is colonized by fucoid algae (*Fucus* spp.), which can create an extensive network of branching fronds over the rock surface. This algal cover is typically greatest in the spring; it is often considerably reduced by the high heat of mid-summer, but may persist all season when summer temperatures are below average (Haring et al., 2002; L. Gosselin, pers. obs.).

This study examined the acorn barnacle, *Balanus glandula* Darwin 1854, a species well suited to address the goals of this study because new settlers are exposed to abiotic stress daily and for extended periods. *B. glandula* densely colonizes the upper intertidal zone from approximately 2.4 m – 3.0 m above mean lower low water (MLLW). The barnacles are occasionally exposed to air for up to 13 h when the water level at the lower high tide of the day is below the vertical range of distribution, though typical emersion time is 8-9 h per low tide (Gosselin & Jones, 2010; pers. obs.).

Weather-related abiotic conditions in the upper intertidal zone

To document the range of weather conditions occurring throughout the summer, a weather station (Davis Instruments Vantage Pro2 Plus Integrated Sensor Suite 6327) was mounted on Wizard Islet approximately 45 m inland from the field site. Wizard Islet was used as a common monitoring site for the present study and also for a separate study of mussel mortality (Chapter 2). From 4 May to 20 August 2011, the weather station recorded the following weather parameters: air temperature, relative humidity (RH), wind speed, ultraviolet radiation, solar radiation, and rainfall. Temperature was monitored on intertidal rock surfaces using 3 Thermochron® iButton (DS1921G) data loggers, and intertidal RH was monitored using 2 Lascar Electronics (EL-USB-2) data loggers. The loggers were placed at 2.75 m above MLLW, which is ~0.35 m above the lower limit of the vertical distribution of *B. glandula*. The iButton data loggers remained in the intertidal zone for the same time period as the weather station, but the RH data loggers were only placed in the intertidal zone during low tide on 6 consecutive days in June 2011 because they could be easily damaged by water. All devices were set to record data at 15 minute intervals, and data from the weather station and iButtons were downloaded bi-weekly. Each parameter was cumulated over 48 h to coincide with total cyprid cohort mortality.

Although RH values were measured, vapour pressure deficit (VPD) was used as an estimate of desiccation stress (see Chapter 2 for justification). Therefore, RH values were converted to VPD values using Equation 2.1.

Daily settlement, cyprid mortality, and juvenile mortality

To determine patterns of mortality through the early benthic phase, a field survey of *Balanus glandula* recruitment was conducted in June 2011 at Wizard Islet using the transparency mapping technique (Gosselin & Qian, 1996). This consisted of using a 20X magnifying lens to identify settlers within a quadrat and then marking their position on clear transparency sheets. Within a long horizontal bench of the intertidal zone, 30 sites were selected between 2.5 m and 2.75 m above MLLW. The sites were selected based on the following features (Gosselin & Jones, 2010): 1) being within the *B. glandula* zone; 2) having at least a 12 cm diameter circular area of flat rock surface; 3) the rock surface being horizontal or on a very modest slope; 4) not being located in a tidepool; and 5) being already colonized by several juvenile or adult *B. glandula*. Of the 30 sites, 20 were randomly chosen for this study. At each site, one 5 cm diameter quadrat was established. All barnacle cyprids and small juveniles were then removed from each quadrat using a needle probe; all other juveniles and adults were left intact. From 1-20 June 2011, new settlers and their subsequent fate were surveyed daily on each quadrat. A cohort of settlers was considered as all new barnacles (all quadrats pooled together) that had settled within the 24 h prior to the survey day; thus, there was one cohort per survey day. New settlers were recorded up to June 13, after which only the fate of existing cohorts was monitored. In total, 13 daily cohorts were monitored during the survey. The fate of individuals was categorized as (a) attached cyprid, (b) dead (or missing) cyprid, (c) metamorphosed live early juvenile, or (d) metamorphosed dead early juvenile. A cyprid was considered dead if the carapace was shrivelled and/or it had not completed metamorphosis by the third day after settlement (Gosselin & Jones, 2010). Dead cyprids and early juveniles were carefully removed using a needle probe. Prior to statistical analysis, a modified Freeman and Tukey (1950) arcsine transformation was applied to cohort mortality data.

Effect of weather-related abiotic conditions on cyprid mortality

Cyprid cohort mortality was defined as the total number of cyprids that died or disappeared from the rock surface by day 2 after settlement. To determine if variation in weather-related abiotic conditions influences variation in cyprid cohort mortality, the conditions experienced by each cohort were estimated as the sum or average of observations for each weather variable from 6 am to 8 pm over 2 consecutive days, starting on the day the cyprid cohort settled and only including observations made during low tide. The only exception to this was calculations of wave height (m), which were based entirely on observations made during high tide. Several of the weather variables were highly correlated, which violates most parametric linear model assumptions. To analyze the effect of weather-related abiotic conditions on cyprid cohort mortality, I therefore began by employing principal component analysis (PCA) to eliminate multicollinearity among weather variables. PCA eliminates multicollinearity by converting observations to a set of uncorrelated variables called principal components. A multiple linear regression was performed using the uncorrelated PCA factors as explanatory variables and cyprid cohort mortality as the response variable. Cyprids that settled on bare surfaces were analyzed separately from cyprids that settled under *Fucus*.

Effect of *Fucus* cover on settlement and mortality of cyprids and juveniles

To determine if cover by the macroalgae *Fucus* spp. affects cyprid settlement and mortality through the early benthic phase, all *Fucus* spp. were removed from within a 12 cm radius of half (10) of the quadrats. Randomized complete block analysis of variance (ANOVA) was used to compare mortality between bare surfaces and *Fucus*-covered surfaces using the day of settlement as the blocking factor (n=13). ANOVA and linear regression models were used to analyze potential causes of variation in mortality between surfaces.

RESULTS

Weather-related abiotic conditions in the upper intertidal zone

Temperature and RH data at the intertidal rock surface were used in combination with weather station data to estimate the temperature and desiccation conditions experienced by settlers at low tide on bare surfaces during the settlement season, from May to June 2011. Intertidal temperature and RH data was recorded over 6 low tides in June 2011, and multiple regression analysis was used to extrapolate intertidal temperature and RH data for the rest of the settlement season. Multiple regression models were developed using either intertidal temperature or intertidal RH as the response variable and the weather conditions recorded by the weather station as explanatory variables. The best-fit models (Tables 2.1 & 3.1) were developed into predictive equations for intertidal temperature and intertidal RH. Of the eight parameters examined, only four significantly influenced intertidal temperature: air temperature, RH of air, wind speed, and solar radiation. These four parameters could account for 91.3% of the variance in intertidal temperature. The equations developed from these models predicted intertidal values very close to actual measured values, though tended to underestimate temperature above 27°C and RH below 48%. After calculation, the predicted values for intertidal RH were converted to VPD.

Table 3.1. Multiple regression best-fit model that predicts intertidal temperature from weather station parameters. Temp= air temperature (°C), TRH= arcsine transformed relative humidity of air, WindSpd= wind speed (km/h), SolRad = solar radiation (kW/m^2), β= partial regression coefficient

Coefficients	β	Estimate	Std. Error	t	p
Intercept	0.395	-28.960	5.856	- 4.945	<0.001
Temp	0.624	2.661	0.290	9.183	<0.001
TRH	0.396	10.750	2.168	4.959	<0.001
WindSpd	0.627	- 0.200	0.022	- 9.235	<0.001
SolRad	0.818	0.010	6.334×10^{-04}	16.365	<0.001

$F_{4,132}$ =356.4, R^2 = 0.913, n=137, p<0.001

Maximum mid-intertidal temperature and VPD on bare surfaces varied greatly during the settlement season. Both intertidal temperature and VPD generally increased from May to June, with peak values occurring in early June. The maximum rock surface temperature that occurred during May and June was 28.8°C and 36.8°C, respectively (Figure 3.1A). VPD levels at the rock surface were also highest on the days when these maximum temperatures were observed; the maximum VPD level for May was 2.23 kPa and the maximum VPD level for June was 4.9 kPa (Figure 3.1B). These peak temperature and VPD levels occurred on clear, sunny days with mid-day low tides.

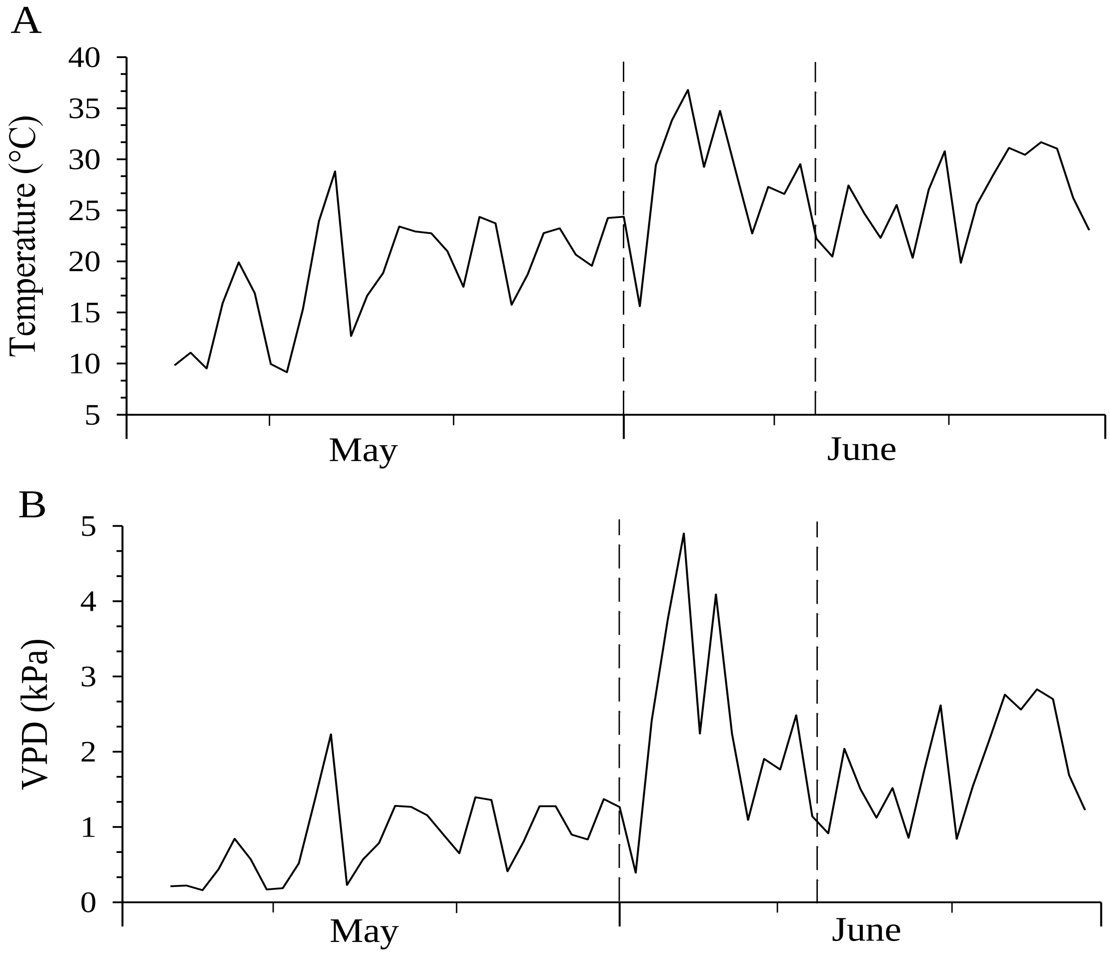

Figure 3.1. (A) Predicted maximum daily mid-intertidal temperatures (°C) and (B) predicted maximum daily mid-intertidal VPD at the rock surface for May and June 2011. Vertical dashed lines indicate the start and end of the settlement period monitored during the recruitment survey.

During the recruitment survey, abiotic conditions in the intertidal zone were highly variable from day to day. Settlement days 2 and 9 were overcast days with some rainfall (<3 mm). Days 3-5 were mainly sunny, whereas the remaining days were generally characterized by overcast mornings with sunny afternoons. The highest temperatures, VPD levels, solar radiation levels, and UV radiation levels occurred over days 4 and 5 (Figure 3.2, cohort 4). Average wind speed generally increased throughout the survey, ranging from 9.1 – 16.7 km/h. Settlers were exposed to aerial conditions for a total of at least 17.5 h over 48 h, up to a maximum total of 24 h in a 48 h period. The maximum duration of a single emersion event during daylight was 13 h. Given the high daily variability of abiotic conditions observed, each cohort of barnacle settlers experienced conditions at settlement that differed from those experienced by other cohorts.

Daily settlement, cyprid mortality, and juvenile mortality

Cyprid settlement and mortality varied greatly among daily cohorts. Daily monitoring for settlers before and after the survey period suggest the full settlement season for *Balanus glandula* in 2011 occurred for ~ 5 weeks from 23 May to 26 June. The 13 daily cohorts monitored during the settlement survey therefore represented ~43% of the total number of daily cohorts during the 2011 settlement season. A total of 2066 cyprids settled in the 20 quadrats during the survey. Two high-settlement events occurred on June 3 and 10 (Figure 3.3A, cohorts 3 and 10), on the days immediately following the only high tide rainfall events in the study period. Within the quadrats, there was an average of 101.2 ± 83.6 (SD) cyprid settlers per daily cohort, with daily settlement slightly increasing during the latter half of the survey. Cyprid mortality was high, averaging 52.7 ± 22.7% (SD), and differed greatly among the daily cohorts (Figure 3.3B).

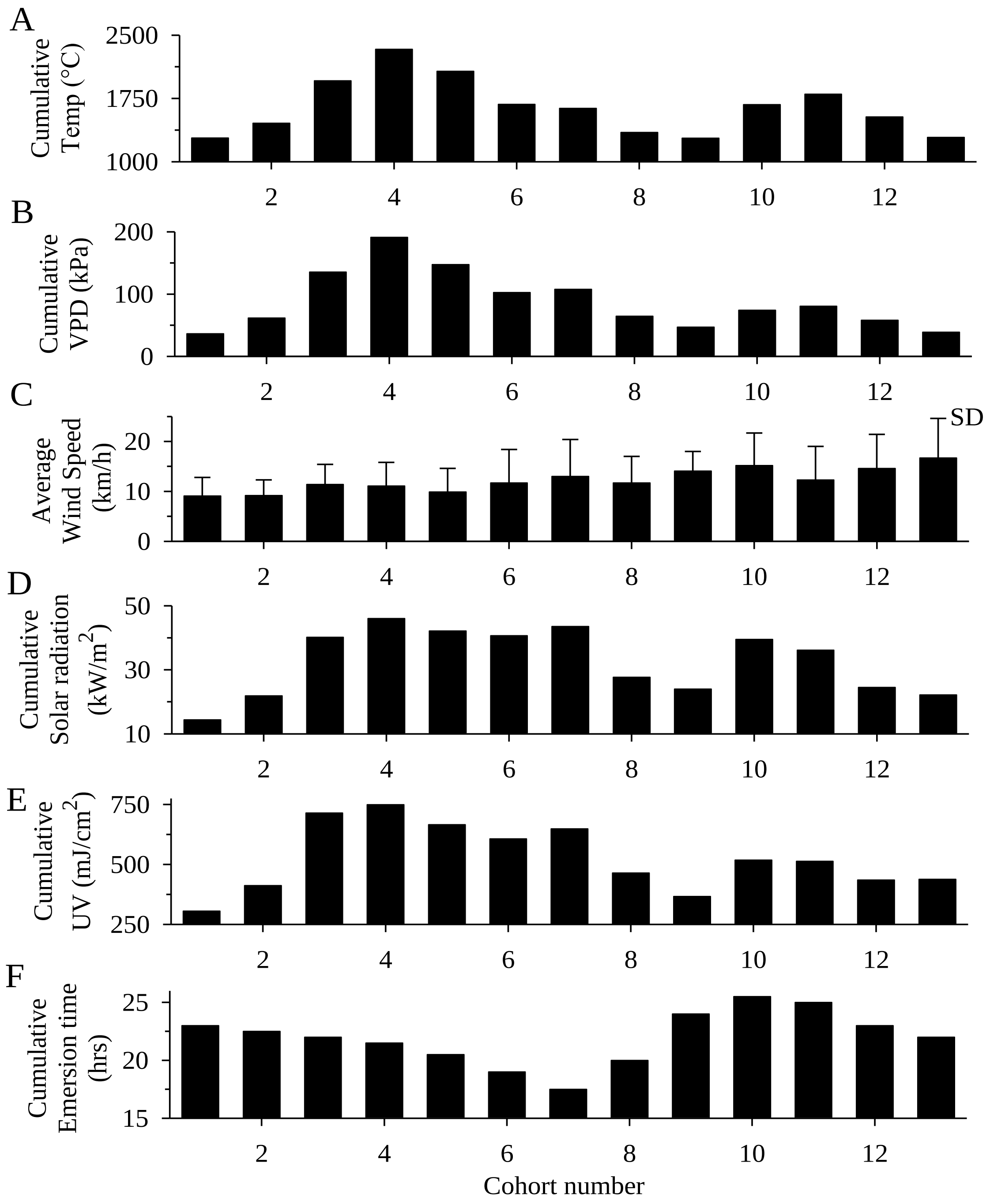

Figure 3.2. Weather conditions experienced by each cohort of *Balanus glandula* on bare surfaces during the first 2 d after settlement. (A) Cumulative predicted intertidal temperature (°C). (B) Cumulative predicted intertidal VPD (kPa). (C) Average wind speed (km/h) ± SD. (D) Cumulative solar radiation (kW/m^2). (E) Cumulative UV dose (mJ/cm^2). (F) Total emersion time (hrs). Cumulative emersion time was determined from tide tables retrieved from the Canadian Hydrographic Service (2011).

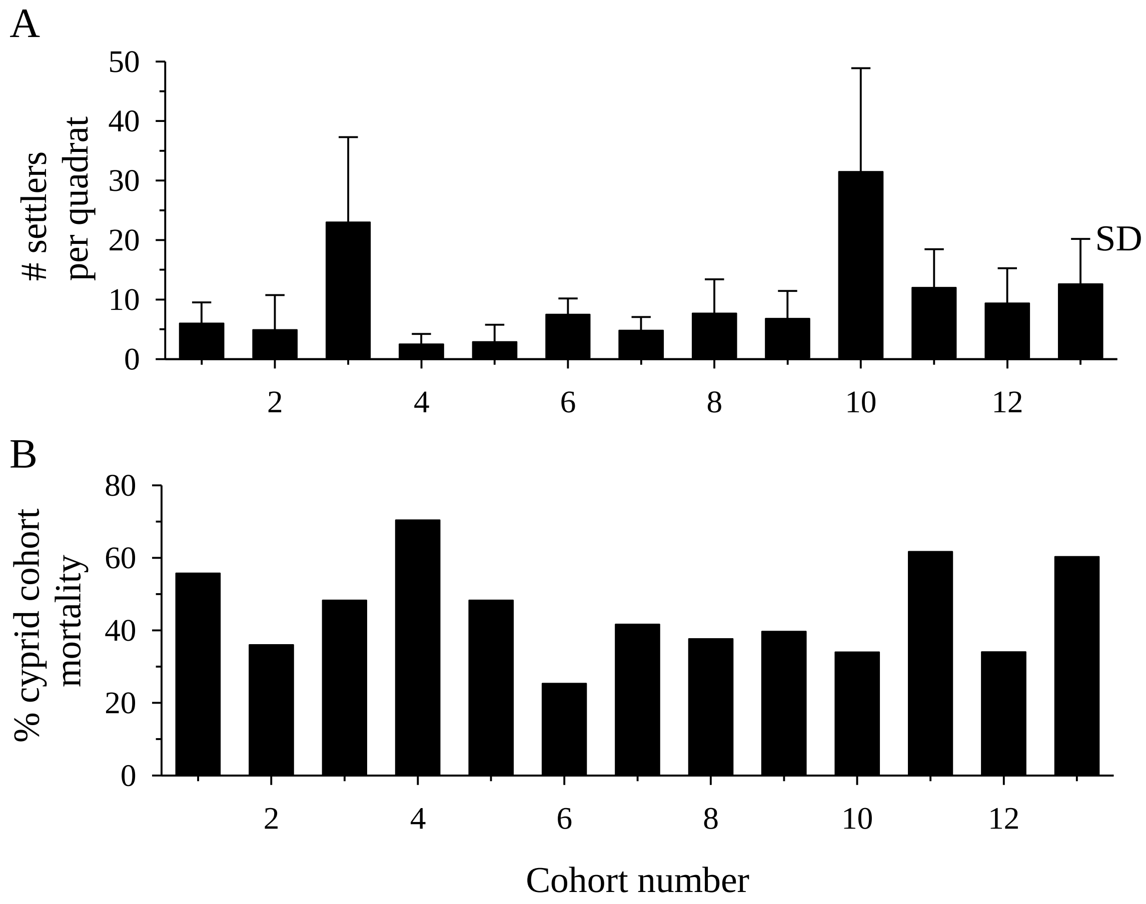

Figure 3.3. *Balanus glandula.* A) Number of daily cyprid settlers per quadrat (average ± SD). B) Total cyprid cohort mortality (%). Mortality data are pooled results of cyprids from all 20 quadrats.

Juvenile survivorship up to 10 d post-metamorphosis did not depend on how quickly cyprids completed metamorphosis. Of all settlers, 63% were first observed as a new cyprid settler and spent 24 – 48 h as a cyprid before completing metamorphosis. The remaining 27% were first observed as small juveniles, having settled and completed metamorphosis during the 24 h period since the last observation. A randomized complete block ANOVA using cohort number as a blocking factor (n=13) revealed that for cyprids settling on the same surface type (i.e., bare surfaces or under *Fucus* spp. cover), there was no significant difference in survivorship up to 10 d post-metamorphosis between cyprids that metamorphosed within 24 h of settlement and those that metamorphosed 24 – 48 h after settlement ($F_{1,36}$=0.096, n=40, p=0.758). Overall, an average of 70.6 ± 16.3% (SD) of all metamorphosed individuals were alive 10 d after metamorphosis (Figure 3.4).

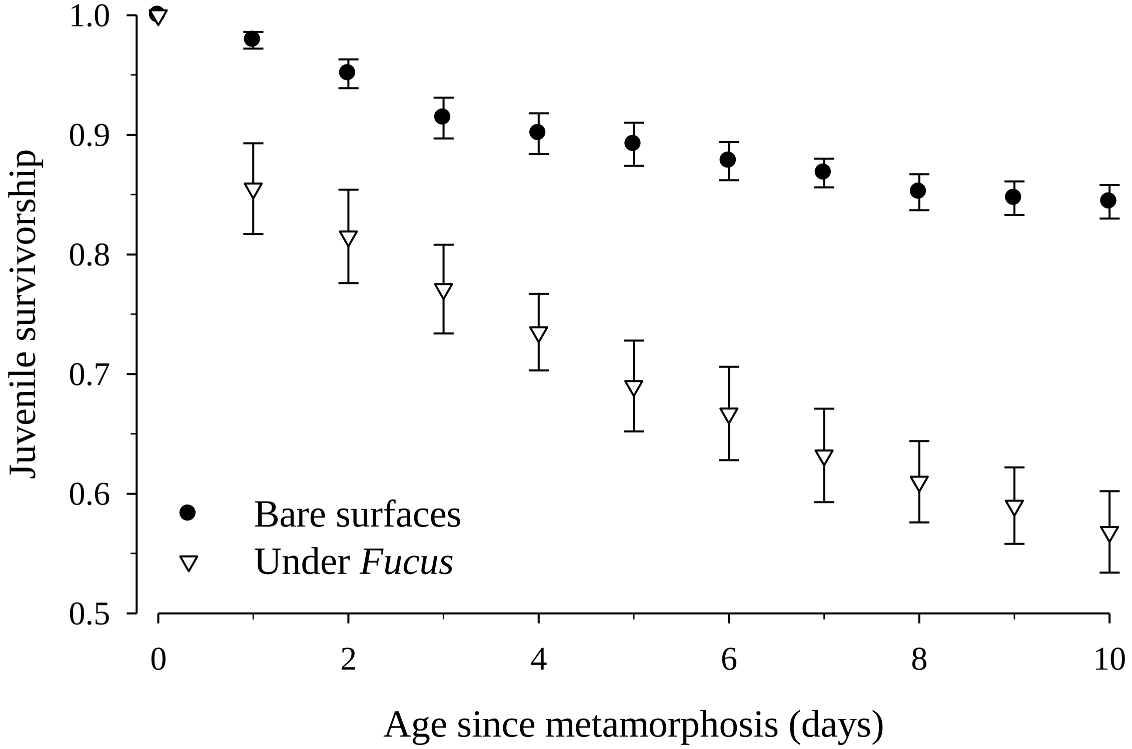

Figure 3.4. Survivorship of juvenile *Balanus glandula* up to 10 d post-metamorphosis on bare surfaces and under *Fucus* spp. cover. Data points represent the average proportion alive ± SE.

Effect of weather-related abiotic conditions on cyprid mortality

Weather-related abiotic conditions did not significantly influence cyprid mortality on bare surfaces. Principle component analysis (PCA) revealed 3 PCA factors that explained 91.4% of the variance in the weather-related abiotic conditions on bare surfaces (Table 3.2). Multiple regression revealed that mortality on bare surfaces was not significantly influenced by these PCA factors (Table 3.3). The cyprid cohort that experienced the highest mortality (70.4%), however, also experienced the most stressful weather-related conditions during aerial exposure; in the first 2 d after settlement, these cyprids were exposed to the highest temperature, VPD, solar radiation, and ultraviolet radiation levels observed during the recruitment survey. This suggests that cyprids are generally tolerant to the range of weather-related abiotic conditions experienced during the first 2 d after settlement, but these conditions might occasionally exceed cyprid tolerance limits and cause high mortality.

Table 3.2. Results of PCA of weather parameters on bare surfaces and under *Fucus* at Wizard Islet. Only factors that explained > 10% of the variance and components with loadings > 0.2 or < -0.2 are shown, and components are listed in order of descending loadings. Weather parameters: AT = cumulative air temperature (°C); AV = cumulative air VPD (kPa); IT = cumulative intertidal temperature (°C); IV = cumulative intertidal VPD (kPa); W = average wind speed (km/h); SR = cumulative solar radiation (kW/m^2); UV = cumulative UV radiation dose (mJ/cm^2); ET = cumulative emersion time (h); WH = average wave height (m)

Surface	Eigenvalue	% Variance Explained	Components
Bare surfaces			
Factor 1	3.508	58.5	IV, IT, SR, UV, -ET
2	1.057	17.6	W, ET, SR, IT
3	0.915	15.3	ET, -W, -UV, IT
Under *Fucus*			
Factor 1	3.077	44.0	ET, -WT, -UV, -SR, -AV, AT
2	2.257	32.3	-AT, -AV, -SR, WH, -ET, -UV
3	0.984	14.1	-W, -WH

Table 3.3. Multiple regression best-fit model analyzing the influence of PCA factors on cyprid cohort mortality. β= partial regression coefficient

Coefficient	β	Estimate	Std. Error	t	p
Intercept	0.986	0.706	0.039	17.992	<0.001
Factor 1	0.392	0.028	0.022	1.278	0.233
Factor 2	0.340	0.043	0.040	1.084	0.307
Factor 3	0.299	0.040	0.043	0.939	0.372

$F_{3,9}$=1.231, n=13, R^2=0.054, p=0.354

Weather-related abiotic conditions significantly influenced cyprid mortality under *Fucus* spp. cover. Principle component analysis (PCA) revealed 3 PCA factors that explained 90.3% of the variance in the weather-related abiotic conditions on *Fucus*-covered surfaces (Table 3.2). Multiple regression revealed that mortality under *Fucus* was significantly influenced by factor 3 (Figure 3.5; $F_{1,11}$= 7.69, R^2=0.411, p=0.018). Factor 3 had a strong negative association with average wind speed (km/h) and average wave height (m) (Table 3.2), suggesting that high cyprid mortality under *Fucus* is associated with low values of average wind speed and average wave height. Linear regression revealed that average wave height does not directly influence cyprid mortality ($F_{1,11}$=1.73, R^2=0.06, p=0.215), but average wind speed appears to have a negative relationship with cyprid mortality ($F_{1,11}$=4.65, R^2=0.23, p=0.054). The relationship between cyprid mortality and wind speed may be clarified by observing mortality of a greater number of daily cyprid cohorts.

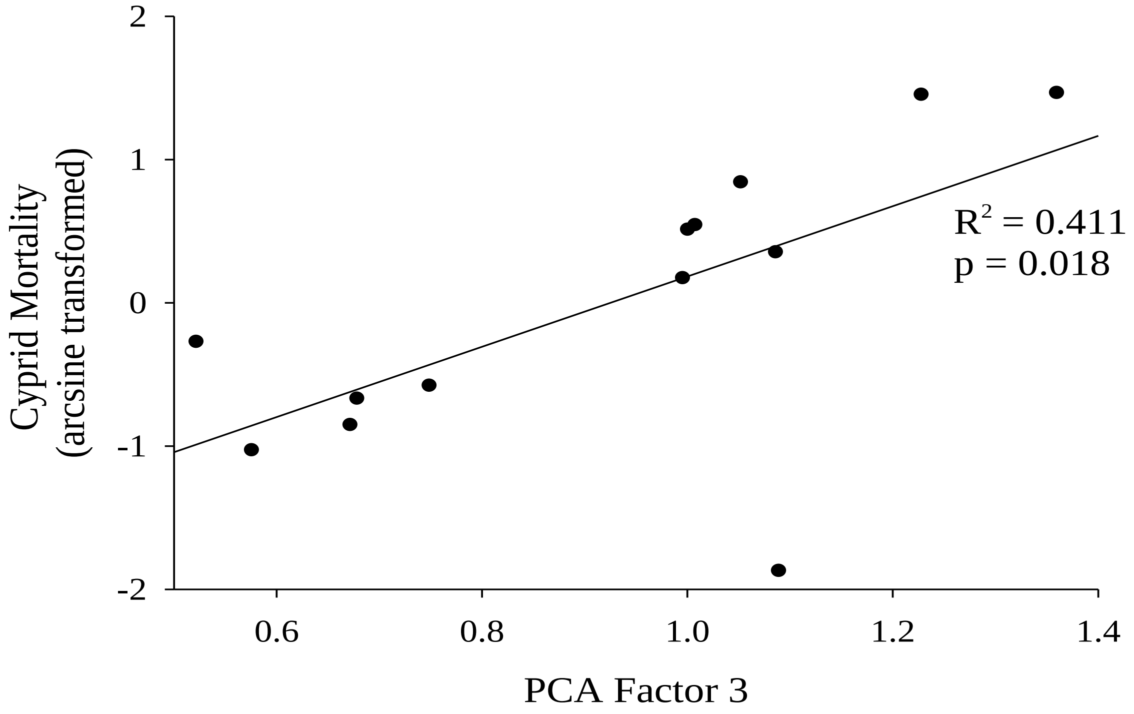

Figure 3.5. Cyprid cohort mortality (arcsine transformed data) as a function of principal component factor 3, which is negatively associated with wind speed and wave height. Solid line represents the linear regression.

Effect of *Fucus* cover on settlement and mortality of cyprids and juveniles

The presence of *Fucus* spp. cover had a strong effect on cyprid settlement and mortality. Settlement in each daily cohort was significantly higher on bare surfaces than under *Fucus* spp. cover (Table 3.4A), with settlement being an average of 108.6 ± 34.5% (SD) higher on bare surfaces than under *Fucus* spp. cover (Figure 3.6A). In addition, cyprid mortality was significantly higher under *Fucus* spp. cover than on bare surfaces (Table 3.4B), with mortality being an average of 21.0 ± 24.4% (SD) higher under *Fucus* spp. cover than on bare surfaces (Figure 3.6B).

Table 3.4. Random complete block ANOVA of (A) settlement and (B) mortality in each daily cohort on bare surfaces and under *Fucus* spp. cover, with the day of the survey as the blocking factor.

Source	*df*	MS	F	p
(A) Settlement				
Surface Type	1	17214	29.94	<0.001
Day of Survey	12	3500	6.09	<0.001
Residuals	12	575		
(B) Mortality				
Surface Type	1	0.31	9.34	0.01
Day of Survey	12	0.06	1.68	0.19
Residuals	12	0.03		

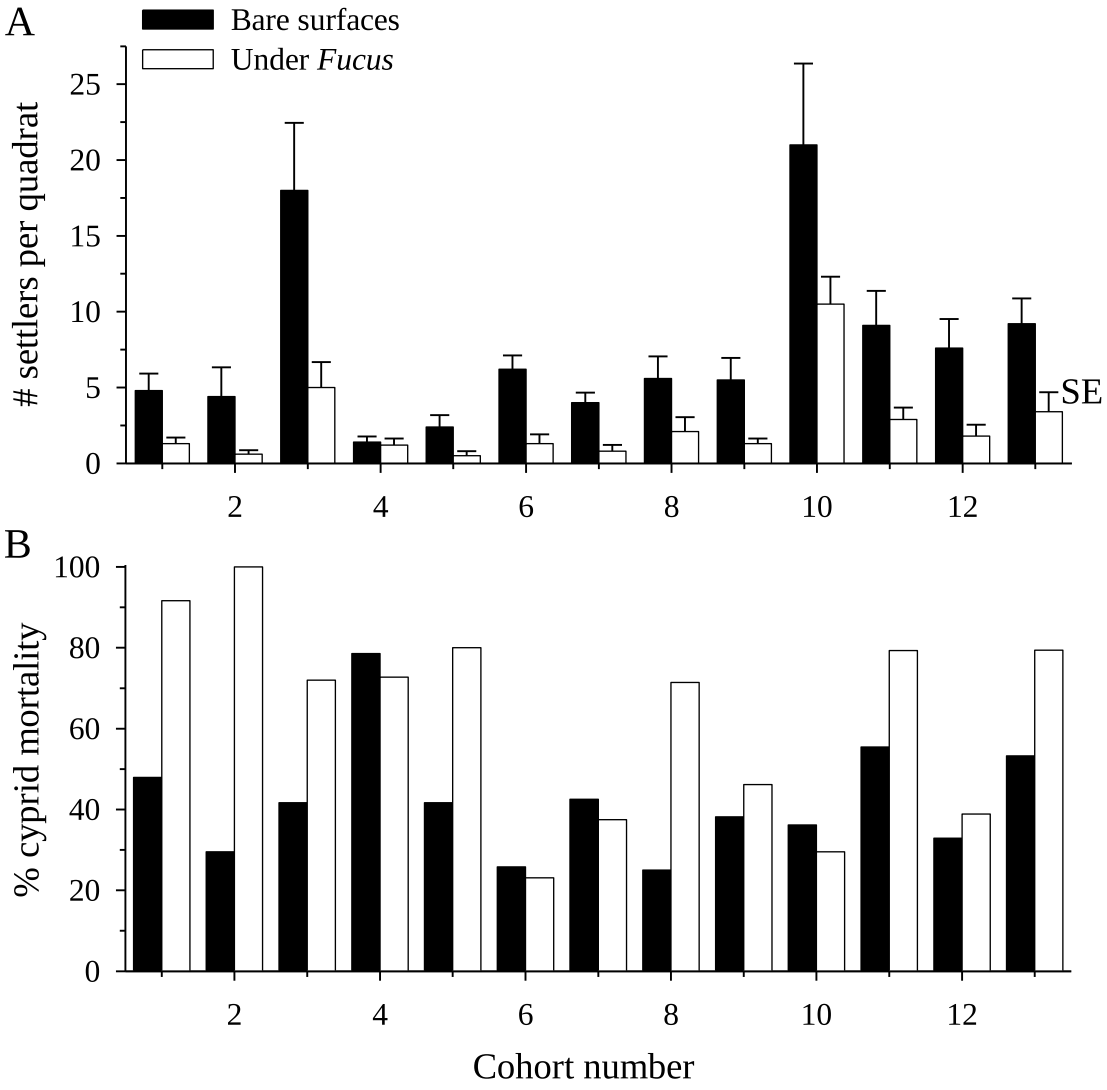

Figure 3.6. Comparison of surfaces with and without the cover of *Fucus* spp. A) Number of *Balanus glandula* cyprid settlers in each daily cohort (average ± SE). B) Total mortality of *B. glandula* cyprids in each daily cohort.

Of all the cyprids that died during the recruitment survey, 78.1% of cyprids died or the body disappeared from the substrate by day 2 after settlement; the other 21.9% of settlers that died as cyprids remained attached to the substrate up to day 3 after settlement. The number of cyprids that remained attached to the substrate up to day 3 after settlement differed between bare surfaces and *Fucus*-covered surfaces (χ^2(1, n=580) = 28.4, p<0.001), with 20.2% more cyprids remaining attached to bare surfaces up to day

3 after settlement than *Fucus*-covered surfaces (Figure 3.7). Lastly, *Fucus* spp. cover significantly influenced juvenile survivorship up to 10 d post-metamorphosis (ANOVA: $F_{1,10}$=79.1, n=22, p<0.001); survivorship to day 10 (Figure 3.4) was 27.6% greater on bare surfaces than under *Fucus* spp. cover.

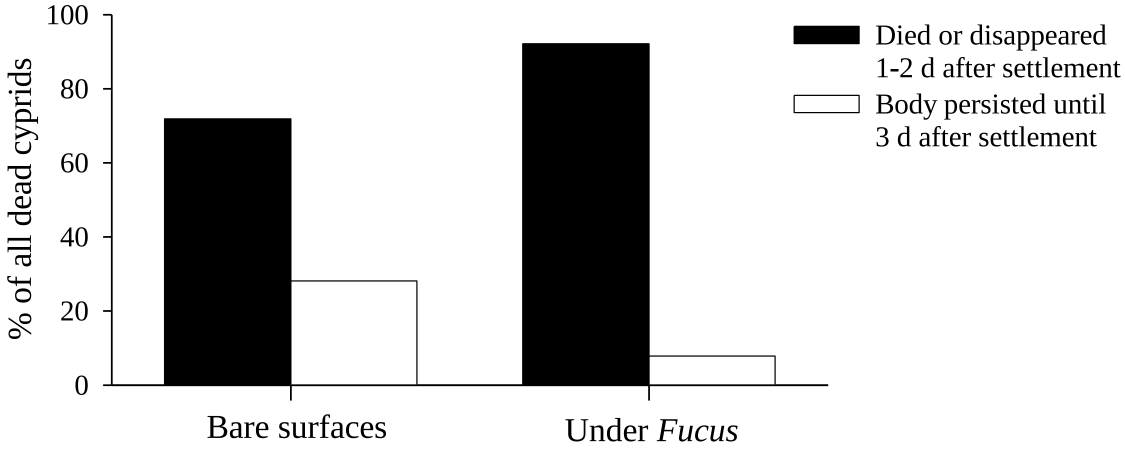

Figure 3.7. Proportion of dead *Balanus glandula* cyprids that were dislodged in the first 2 d after settlement and of those that remained attached to the substratum up to day 3 after settlement, in each of the 2 treatments.

Fucus spp. cover reduced average daily rock surface temperature and VPD levels, with differences between bare and *Fucus*-covered surfaces being the greatest on afternoon low tides under clear, sunny weather (Figure 3.8). Data loggers placed in the intertidal zone during low tide revealed that both temperature and VPD levels were significantly higher on bare surfaces than under *Fucus* spp. cover (ANOVA: temperature: $F_{1,5}$ = 23.5, n=12, p=0.005; VPD: $F_{1,5}$= 64.1, n=12, p<0.001). However, analysis of covariance using a model with common slopes and different intercepts revealed that differences in cyprid mortality between bare and *Fucus*-covered surfaces were not significantly influenced by temperature ($F_{1,9}$=0.54, n=12, p=0.48) or VPD ($F_{1,9}$=0.29, n=12, p=0.61). As reported above, cyprid mortality under *Fucus* spp. cover appears to only be influenced by average wind speed.

Table 3.5. Randomized complete block ANOVA of (A) temperature and (B) VPD on bare surfaces and under *Fucus* spp. cover, with the date as the blocking factor.

Source	*df*	MS	F	p
(A) Temperature				
Surface Type	1	69.77	23.48	0.005
Date	5	30.28	10.19	0.012
Residual	5	14.86		
(B) VPD				
Surface Type	1	3076.16	64.08	<0.001
Date	5	132.00	2.75	0.146
Residual	5	240.01		

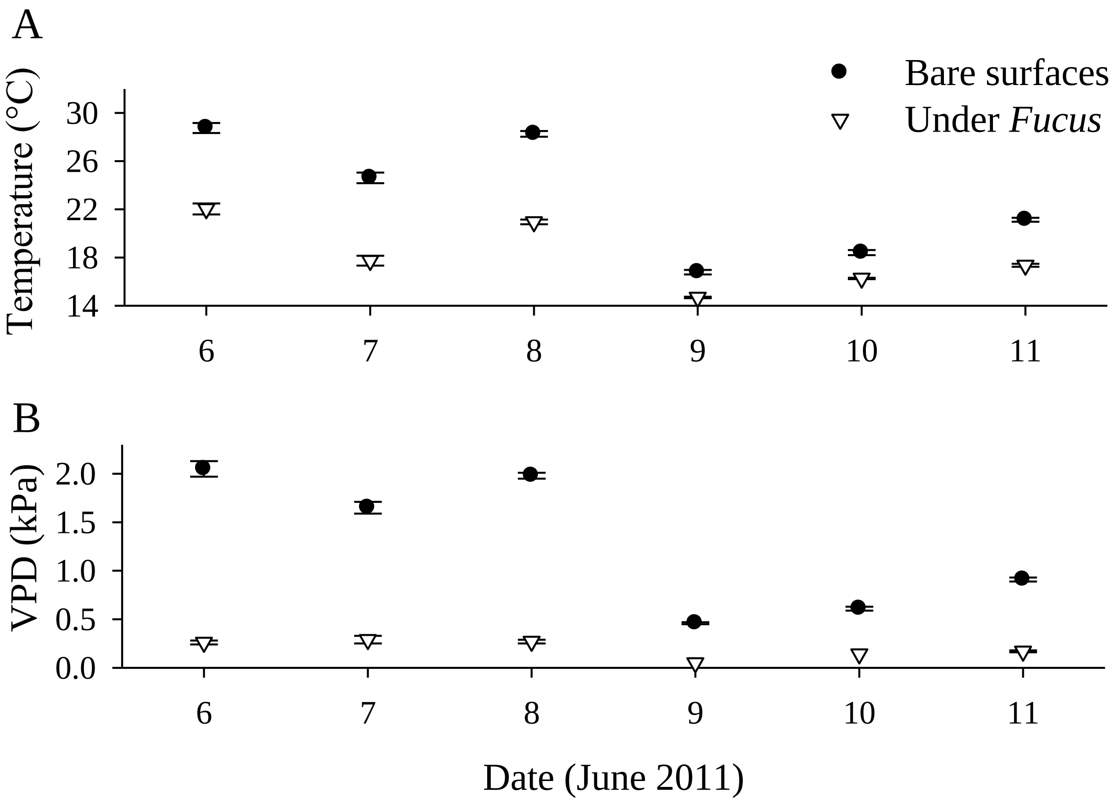

Figure 3.8. Comparison of (A) average temperature (°C) ± SE and (B) average VPD (kPa) ± SE between rock surfaces with and without the presence of *Fucus* spp. during the final hour of low tide on 6 of the settlement days. Low tides on June 6-8 had clear, sunny weather, and low tides on June 9-11 were mainly overcast.

DISCUSSION

Weather-related abiotic conditions in the upper intertidal zone

Weather-related abiotic conditions varied considerably from day to day during the settlement and early growth season, indicating that each daily cohort experienced distinct levels of stress. A wide range of conditions was experienced by the daily cohorts during the survey; the weather parameters that varied the most during the survey were intertidal temperature, intertidal VPD, solar radiation, and ultraviolet radiation. Predicted intertidal temperature conditions during the settlement and early growth season were mostly within the known tolerance limit of newly settled barnacles. Temperatures above 33°C have been demonstrated to cause high mortality of *Balanus glandula* cyprids in the laboratory (Shanks, 2009); however, maximum surface temperatures above 33°C occurred during only 3 out of 58 days (0.05%) of the settlement and early growth season. Desiccation conditions occasionally exceeded levels expected to cause high cyprid mortality. Foster (1971) experimentally determined desiccation stress at VPD levels above 1.58 kPa (calculated from temperature and RH values provided by the author) can cause 50% mortality of *Semibalanus balanoides* cyprids after an average of 6.6 h exposure; daily maximum surface VPD levels above this level occurred during 18 out of 58 days (31%) of the settlement season. Temperature stress is therefore unlikely to influence cyprid mortality at the study site, but the frequency of occurrence of potentially lethal desiccation levels suggest desiccation stress may be an important mortality factor for newly settled cyprids.

Daily settlement, cyprid mortality, and juvenile mortality

The number of cyprids that settled during the survey greatly varied from day to day. Settlement slightly increased during the latter half of the survey, with an overall average of 101 settlers per daily cohort. The two days of peak settlement occurred on the days immediately following high tide rainfall events, suggesting a possible effect of rainfall on settlement intensity. It is possible that these rainfall events caused a temporary decline in sea surface salinity and upon return to typical levels, barnacle settlement was induced. Reductions in sea surface salinity to as low as 15 psu and extending 2 m below the surface following summer storm events have been observed in Barkley Sound (Garza & Robles, 2010), and Thiyagarajan and colleagues (2007) discovered that temporary exposure to similarly low salinity (10 psu) caused *B. amphitrite* cyprids to metamorphose upon transfer back to full strength seawater (34 psu). The rainfall events in the present study, however, did not result in a large quantity of rain and only lasted up to 4 h; although rainfall seems a possible cause of increased settlement, it is unknown if the amount of rainfall that occurred in this study was enough to cause a rapid drop and recovery of salinity near the sea surface in the short duration of high tide.

A substantial proportion of cyprid settlers were unable to survive the transition from pelagic to intertidal habitat. Cyprid mortality was high, with an average of 53% mortality per daily cohort. This is consistent with the high cyprid mortality rates obtained in previous recruitment studies (Young, 1991; Minchinton & Scheibling, 1993; Gosselin & Qian, 1996; Jarrett, 2000; Gosselin &Jones, 2010). In addition, daily mortality after metamorphosis was much lower than cyprid mortality, with an average of 71% of all metamorphosed individuals still alive 10 d after metamorphosis. These findings therefore suggest that the transition from pelagic to intertidal habitat is a critical period for survival in barnacles, and may constitute a bottleneck period for recruitment (Gosselin & Qian, 1997).

Effect of weather-related abiotic conditions on cyprid mortality

Cyprid mortality on bare surfaces was not significantly influenced by the weather-related abiotic conditions experienced during the peak period of transition from pelagic to intertidal habitat. The recruitment survey occurred in the middle of a La Niña cycle that spanned from June 2010 – May 2012 when ocean temperatures were below average (CPC, 2012). This was likely to also result in lower than average temperature and desiccation stress in the intertidal zone. The factors expected to cause cyprid mortality, desiccation and possibly temperature, were not responsible for variation in mortality among daily cyprid cohorts. This was surprising given cyprids on bare surfaces were directly exposed to these factors, which are generally considered major stresses and causes of early benthic phase mortality (Denley & Underwood, 1979; Gosselin & Chia, 1995; Somero, 2002; Gosselin & Jones, 2010). This may have been because cyprids preferentially settle in crevices and depressions (Wethey, 1986; Raimondi, 1988; Savoya & Schwindt, 2010), and space for settlement in these areas was not likely limited during this study; these sites protect cyprids from temperature and desiccation stress because they are more shaded and damp than raised areas and smooth surfaces (Foster, 1971). The cyprid cohort that experienced the highest mortality (70%, cohort 4), however, also experienced higher intertidal temperature, VPD, solar radiation, and ultraviolet radiation within the first 2 d after settlement than any other cyprid cohort. Weather-related abiotic factors that influence temperature and desiccation stress may therefore exert more influence on cyprid survival during average or El Niño cycles when high temperature and desiccation levels are expected to occur more frequently during the settlement and early growth season. In addition, the relationship between cyprid mortality and weather-related abiotic conditions may be a threshold relationship rather than linear, in which case the conditions during the present study may not have exceeded threshold values to cause mortality; this may be further explored with controlled laboratory experiments similar to those conducted in Chapter 2.

For the cyprids that died during the recruitment survey, a greater proportion of cyprids under *Fucus* spp. cover were removed/dislodged before day 3 than on bare surfaces. This may be due to the algal fronds dislodging and sweeping cyprids off the rock surface during immersion (Grant, 1977; Jenkins et al., 1999; Leonard, 1999; Hancock & Petraitis, 2001). However, as mentioned above, my study suggests that wave action was not responsible for cyprid mortality, which would be expected to increase the sweeping action of the algal fronds. This indicates that other factors related to *Fucus* spp. cover may have been indirectly responsible for variation in cyprid cohort mortality. For example, intertidal organisms that prey upon cyprids or dislodge cyprids from the rock surface while grazing are more active under fucoid algae canopy than on open surfaces (Menge, 1978; Jernakoff, 1985; Leonard, 1999; Miller & Carefoot, 1989), therefore greater cyprid mortality under *Fucus* spp. cover may have been due to increased predation and bulldozing. Alternatively, the biofilm layer and the encrusting algae on the rock surfaces covered by *Fucus* spp. may have prevented cyprids from achieving firm adhesion to the rock surface.”.

Effect of *Fucus* cover on settlement and mortality of cyprids and juveniles

The presence of *Fucus* spp. cover had a negative impact on cyprid mortality. I had predicted that fucoid algae cover would decrease early benthic phase mortality by reducing environmental stress (Gosselin & Chia, 1995). My study confirmed that temperature and desiccation stress were much lower under *Fucus* spp. cover. However, mortality under *Fucus* spp. cover was higher, not lower, than on bare surfaces. Mortality under *Fucus* spp. cover was also not associated with wave action and therefore was probably not caused by dislodgement by algal whiplash, but mortality may be associated with wind speed. Although the direct cause of cyprid mortality under *Fucus* spp. cover could not be identified in this study, high mortality was likely due to an indirect effect of *Fucus* spp. cover, which may include factors such as predation, bulldozing, and biofilm production.

The time it takes for a cyprid to complete metamorphosis after attaching to the substratum is not likely to impact long-term survival on either bare surfaces or under *Fucus* spp. cover. Overall survivorship at 10 d post-metamorphosis did not differ significantly between cyprids that completed metamorphosis within 24 h of settlement and those that took 24 – 48 h. This is contrary to several studies that suggested cyprids delaying metamorphosis may have lower metamorphic success and post-metamorphic survival due to limited energy reserves (Jarrett & Pechenik, 1997; Qiu et al., 1997; Pechenik et al., 1993, 1998; Thiyagarajan et al., 2003, 2007; Pechenik, 2006). The cause of delayed metamorphosis after attachment to the substratum is still unknown (Pechenik, 2006), but my results suggest this delay is not likely to influence survival through the early benthic phase and is therefore not expected to influence the overall abundance of barnacle populations.

Conclusions

I found that weather-related abiotic conditions were highly variable during the recruitment survey, indicating that each daily cohort of barnacle settlers experienced conditions at settlement that differed from those experienced by another daily cohort. Contrary to expectations, cyprid mortality was not significantly influenced by weather-related abiotic conditions. Although the direct cause of high cyprid mortality could not be determined, *Fucus* spp. cover negatively influenced the survival of newly settled cyprids and juveniles.

Changes to weather-related abiotic conditions caused by climate shifts (Rodenhuis et al., 2007) could increase the levels of temperature and desiccation stress in the intertidal zone during low tide aerial exposure. Although modest increases in temperature and desiccation stress might not directly influence barnacle early benthic phase mortality, these changes could have indirect effects. Specifically, additional stress may lead to a decrease in the population of fucoid algae: a recent study reported some dominant populations of intertidal algae on the California coast, including those of the genus *Fucus,* have declined by over 50% in the 10 y following ocean warming (Schiel et al., 2004); this suggests future increases in temperature and desiccation stress may reduce the abundance and limit the distribution of fucoid algae populations (Haring et al., 2002; Martinez et al., 2012). The negative influence of *Fucus* spp. cover on the survival of cyprid and early juvenile barnacles revealed in the present study suggests that future declines in fucoid algae populations may result in an increase in barnacle survival through the early benthic phase and may subsequently affect barnacle population abundance. In addition, a reduction of cover by fucoid algae may result in weather-related factors becoming more important than other factors in causing mortality of newly settled barnacle cyprids.

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CHAPTER 4: General Conclusion

SUMMARY OF RESULTS

To improve our understanding of the influence of low tide environmental stress on survival through the early benthic phase, I (1) determined the sensitivity of newly settled mussels to temperature and desiccation stress; (2) determined how sensitivity of mussels to temperature and desiccation stress changes with increasing size; (3) determined the frequency of occurrence of lethal conditions in the field during the recruitment season; and (4) determined the role of temperature, relative humidity, wind speed, ultraviolet radiation, and solar radiation in regulating temporal variation in early post-settlement mortality of barnacles. The most important findings of these studies were: (1) newly settled mussels experienced a threshold of temperature tolerance at 34.1°C and a threshold of desiccation tolerance at a VPD level of 1.01 kPa; (2) *Fucus* spp. cover negatively influenced the survival of newly settled barnacle cyprids and juveniles; and (3) the frequency of occurrence of lethal temperature and desiccation conditions in the field suggests desiccation, but not temperature, may be an important mortality factor for mussels during the first hours and days after transition from pelagic to intertidal habitat.

The first study in this thesis examined the influence of temperature and desiccation stress on mortality of newly settled *Mytilus trossulus*, as well as the ontogeny of sensitivity to desiccation stress through the early benthic phase. The critical temperature at which 50% of newly settled mussels of 1-2 mm shell length (SL) died was 34.1°C, although this temperature was rarely reached on intertidal rock surfaces during the recruitment season. The critical vapour pressure deficit (VPD) value of 1.01 kPa, however, occurred very frequently and for prolonged periods during the recruitment season, and thus posed a high threat to newly settled mussels. I concluded that desiccation stress appears to exert the most influence on mortality during the early benthic phase, and a final laboratory experiment characterized the changes in sensitivity to desiccation stress that occur as mussels increase in size. The mussels became highly tolerant to all experimental VPD values once they reached the size of 3 mm SL, suggesting a size threshold of desiccation tolerance between 2-3 mm SL. This size also closely corresponded to the size at which mussels have been reported to shift microhabitat use, from filamentous algae that provide protection from temperature and desiccation stress to rock surfaces and adult mussel beds that have much more variable environmental conditions during aerial exposure. Ontogenetic shifts in habitat use by juvenile *M. trossulus* may therefore be a response to changing vulnerability to desiccation stress.

The second study in this thesis examined the influence of weather-related abiotic conditions and *Fucus* spp. cover on early survivorship of *Balanus glandula* during the first hours and days after the transition from pelagic to benthic environments. Weather-related abiotic conditions were highly variable during the recruitment survey, indicating that each daily cohort of barnacle settlers experienced conditions at settlement that differed from those experienced by another daily cohort. Cyprid mortality varied greatly among daily cohorts and was significantly higher under *Fucus* spp. cover. Juvenile survivorship up to 10 d post-metamorphosis was also significantly lower under *Fucus* spp. cover than on bare surfaces. Contrary to expectations, cyprid mortality on bare surfaces was not significantly influenced by weather-related abiotic conditions. On *Fucus*-covered surfaces, however, high cyprid mortality may have been due to an indirect effect of the presence of *Fucus* spp., which may include factors such as predation and bulldozing.

Newly settled mussels and barnacles responded similarly to intertidal temperature stress but differently to intertidal desiccation stress. Intertidal temperatures above the threshold tolerance levels for newly settled mussels and barnacles did not occur frequently during the settlement and early growth season, suggesting that temperature stress is not likely an important factor influencing early benthic phase mortality. The desiccation stress threshold level for newly settled mussels was frequently exceeded for several hours during the recruitment season, however, suggesting that desiccation stress may be an important factor influencing early benthic phase mortality in mussels that settle on open surfaces. Cyprid mortality on bare surfaces, however, was not significantly influenced by desiccation stress or any other weather-related abiotic factors.

The differing response of these two species to desiccation stress is likely due to differences in mechanisms for coping with stress: Mussels settle within filamentous algae that retain moisture during low tide likely to reduce exposure to potentially lethal levels of desiccation stress. Barnacle cyprids, however, experience reduced survival through the early benthic phase in structurally complex habitats, and therefore likely utilize alternate mechanisms to reduce the harmful effects of desiccation stress. This may include preferentially settling in crevices and depressions of rough surfaces (Wethey, 1986; Raimondi, 1988; Savoya & Schwindt, 2010), which are usually more shaded and damp than raised areas and smooth surfaces (Raimondi, 1990). Cyprids may also possess physiological mechanisms (Foster, 1971) that cause evaporative water loss to occur at a slower rate than in newly settled mussels; this might explain why *B. glandula* cyprids have higher tolerance to desiccation stress than newly settled *M. trossulus*, with threshold tolerance levels at 1.58 kPa and 1.01 kPa, respectively.

CONCLUSIONS AND FUTURE DIRECTIONS

Through these studies I have concluded that the survival of newly settled mussels likely depends upon the presence of protective microhabitats created by filamentous and fucoid algae, whereas barnacles experience reduced survival through the early benthic phase in the presence of fucoid algae. This information is critical in light of the expected shift to warmer and dryer summer conditions on the west coast of British Columbia by 2050 (Rodenhuis et al., 2007); summer mortality of filamentous and fucoid algae populations may increase, as the predicted increases in temperature and desiccation stress may severely inhibit physiological activity of these algae (Haring et al., 2002; Ji & Tanaka, 2002; Martinez et al., 2012). This might simultaneously result in decreased mussel survival and increased barnacle survival during the first hours and days after the transition from pelagic to intertidal habitat. Changes in survival through the early benthic phase could in turn affect the abundance of adult populations (Osman et al., 1992; Roegner & Mann, 1995; Hunt & Scheibling, 1997). This indicates the importance of considering species interactions when developing predictions for the population abundance of a species; many other species of the intertidal community rely on mussels and barnacles for resources, such as food, shelter from predators, and protection from environmental stress (Gosselin & Chia, 1995; Crain & Bertness, 2006; Koivisto & Westerbom, 2010). Changes in the abundance of adult populations of these species could therefore have severe impacts on the entire ecosystem, including loss of community biodiversity, increased vulnerability to invasive species, reduction in productivity, and extinction at local ‘hot spots’ that are sensitive to climate change (Helmuth et al., 2002; Harley et al., 2006; Koivisto & Westerbom, 2010). The ability to modify, maintain, and create habitats that facilitate the existence of other species classifies *M. trossulus* and *B. glandula* as ecosystem engineers (Jones et al., 1994; Crain & Bertness, 2006), and suggests conservation of these species should be a top priority.

In this thesis I have demonstrated that newly settled mussels and barnacles are sensitive to temperature and desiccation stress, and the frequent occurrence of potentially lethal desiccation levels suggests desiccation stress may be an important factor in early benthic phase mortality. Specifically, weather-related abiotic factors that influence desiccation stress may sometimes be important mortality factors through the early benthic phase, and future studies should continue to identify the specific conditions and types of intertidal populations where this is the case (Raimondi, 1990). Of particular importance are: (1) determining the indirect effects of weather-related abiotic factors on early benthic phase mortality, such as the influence of these factors on predator and grazer activity below algal canopy; and (2) determining the direct effects of weather-related abiotic factors at the extremes of climate pattern oscillations (i.e., El Niño/La Niña), which may be achieved by monitoring settlement and early benthic phase mortality through several consecutive recruitment seasons. This information would strengthen our ability to understand and predict population fluctuations, and to characterize the role of early benthic phase mortality as a mechanism influencing population abundance.

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APPENDIX A: Autocorrelation analysis of multiple regression models predicting intertidal relative humidity and intertidal temperature on bare surfaces.

Complete analysis of time-series data includes inspecting model residuals for an indication of autocorrelation. Autocorrelation violates the ordinary least squares (OLS) assumption that the error terms are uncorrelated. While it does not affect the OLS coefficient estimates, positive autocorrelation of the errors at low lags tends to cause underestimation of the standard errors of a model and overestimation of the t-scores. If autocorrelation is found in model residuals, it suggests the model may require modification to include information that is not yet accounted for, which is typically done by adding additional terms to the model.

In this thesis, two multiple regression models were developed to predict intertidal relative humidity and intertidal temperature (Tables 2.1 and 3.1, respectively). Plots of the model residuals as a function of time (Figure A.1-A&C) do not reveal any patterns, suggesting the model has captured the patterns in the data quite well; however, there is a small amount of positive autocorrelation left in the residuals, indicated by the significant correlations at lag(1) and lag(17) for the intertidal temperature model (Figure A.1-B) and at lag(1) for the intertidal relative humidity model (Figure A.1-D). Ideally, an additional term should be added to each model to account for this correlation; this would likely result in a slightly more accurate forecast of intertidal temperature and relative humidity over the short-term than the original model. However, as the predicted data were used to determine the frequency of intertidal temperature and relative humidity conditions above threshold values, a minor change in the individual predicted values (i.e., <1°C or <2% RH change) caused by adding an additional term to the model is unlikely to change the overall results of the study; therefore, I did not opt to fit a more complicated model.

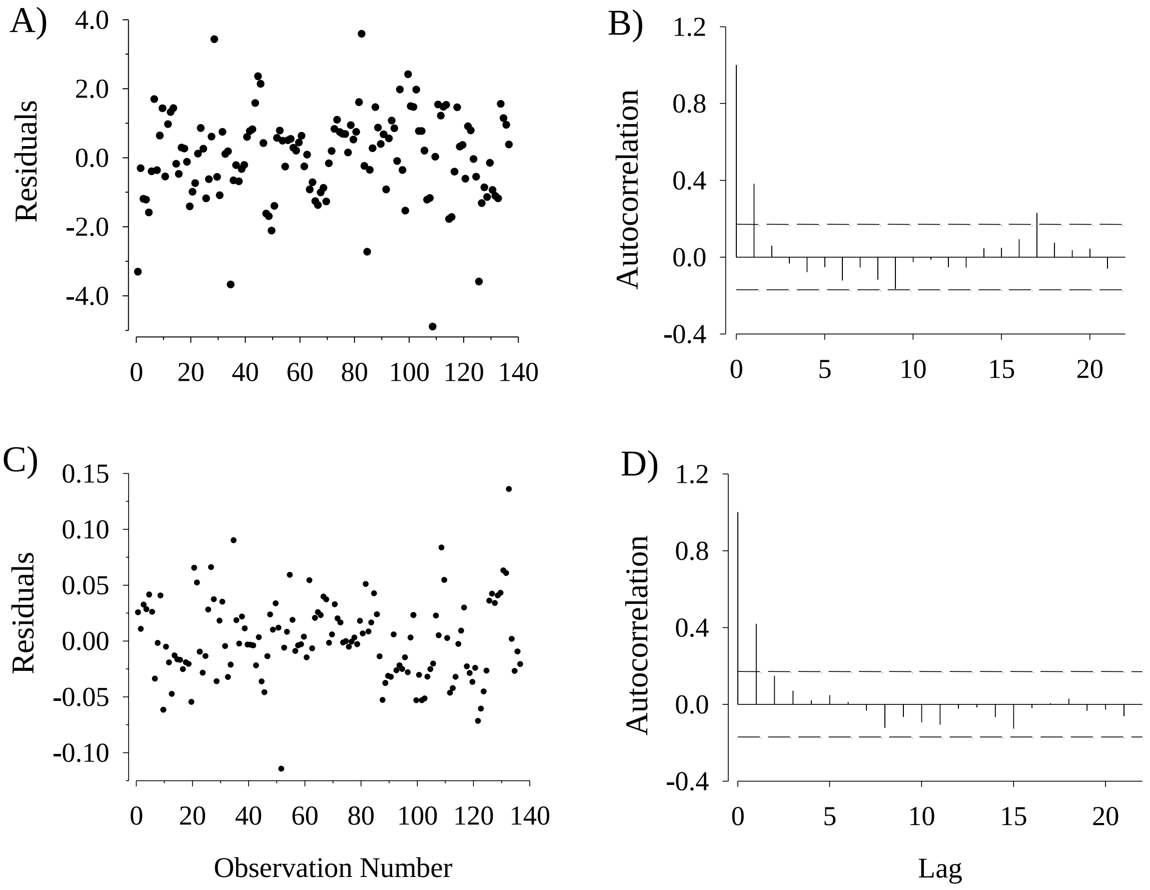

Figure A.1. Results of autocorrelation analysis of residuals from multiple regression models predicting intertidal temperature (A&B) and intertidal relative humidity (C&D). A&C represent scatterplots of the model residuals as a function of time in the form of consecutive observations. B&D represent bar plots of autocorrelation of residuals as a function of lag time. Bars outside the limits of the 95% CI (dashed lines in B&D) suggest significant autocorrelation at the associated lag value.

APPENDIX B: Intertidal temperature and relative humidity conditions during low tide at Grappler Inlet.

To determine the temperature and relative humidity (RH) conditions occurring over the vertical range of mussel distribution in the intertidal zone, RH data loggers were placed in the intertidal zone at Grappler Inlet during low tide on a sunny day (See Chapter 2).

Data loggers that were placed at three different intertidal heights (2.0 m, 2.5 m, 2.75 m) revealed the unexpected finding that intertidal rock surface temperature at 2.0 m and 2.5 m could reach the same or higher rock surface temperatures as those occurring at 2.75 m. On bare rock surfaces, temperatures did not reach the juvenile mussel tolerance threshold until the last half hour of low tide and were thus not likely to be lethal (Figure A.1-A). Although data was not collected after the tide rose to 2.5 m, there was potential for further temperature increases at 2.75 m over another 2 h before the tide re-immersed that surface. Data loggers placed under fucoid algae showed that rock surface temperatures did not reach potentially lethal levels at any intertidal height (Figure A.1-D).

VPD levels were above the juvenile mussel threshold for the majority of the low tide (Figure A.1-C). VPD levels at 2.0 m and 2.5 m could get as high as those recorded at 2.75 m; however, the rising tide caused potentially lethal VPD levels at 2.0 m to persist for less time than at 2.5 m. Although data was not collected after the tide rose to 2.5 m, there was potential for further VPD increases at 2.75 m over another 2 h before the tide re-immersed the surface. Levels of VPD that are lethal to recently settled *M. trossulus* therefore occur over the full range of their intertidal distribution, but this exposure likely occurs for the longest time near the upper limit of their distribution, where emersion can occasionally last up to 13 h. Data loggers placed under fucoid algae showed that VPD levels did not reach potentially lethal levels at any intertidal height (Figure A.1-F).

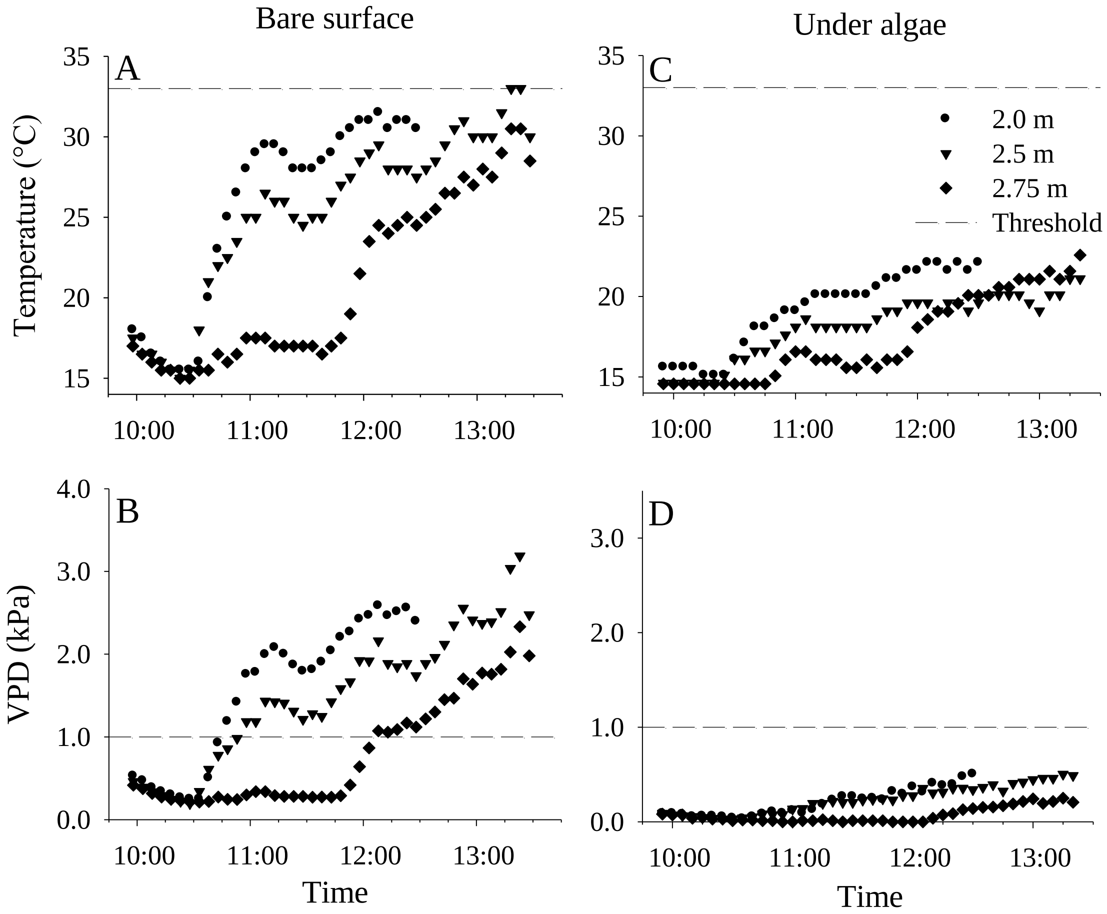

Figure B.1. Temperature (°C) and vapour pressure deficit (kPa) data collected at 3 intertidal heights at Grappler Inlet on 5 September 2012. The left column (A&B) represents conditions on bare rock surface, and the right column (C&D) represents conditions under fucoid algae. Horizontal dashed lines represent the juvenile mussel tolerance threshold, as determined by laboratory experiments (Chapter 2).