

Ontogenetic shift in stress tolerance thresholds of *Mytilus trossulus*: effects of desiccation and heat on juvenile mortality

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ABSTRACT: We examined the sensitivity of newly settled *Mytilus trossulus* to heat and desiccation, as well as the ontogeny of sensitivity through the early benthic phase. Laboratory experiments were conducted to determine the sensitivity of mussels to the full range of temperatures and desiccation levels experienced in the field. Mussels of 1 to 2 mm shell length (SL) experienced a threshold of heat tolerance at 34°C and a threshold of desiccation tolerance at vapour pressure deficit levels of 1.01 kPa. Extended periods of temperatures reaching or exceeding lethal levels for newly settled *M. trossulus* occurred relatively rarely in Barkley Sound, British Columbia, Canada, whereas lethal levels of desiccation occurred often during the recruitment season and were usually sustained for several hours. Desiccation, therefore, appears to be a substantially greater threat to recently settled *M. trossulus* than heat. A final laboratory experiment characterized the changes in sensitivity to desiccation that occur as mussels increase in size. Mussels became highly tolerant to desiccation when they reached a size of 2 to 3 mm SL. This size closely corresponds to the size at which juvenile *M. trossulus* relocate from protective filamentous algal habitat to adult habitat, suggesting ontogenetic shifts in habitat use by juvenile *M. trossulus* are a response to changing sensitivity to desiccation. If so, the future survival of newly settled mussels, and thus possibly the local persistence of mussel populations, may depend upon the persistence of protective algal microhabitats under changing climate conditions.

KEY WORDS: Early post-settlement mortality · Mortality factors · Physiological stress · Weather · Settlement · Recruitment · Population regulation · Climate change

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INTRODUCTION

The onset of intertidal life for many benthic marine invertebrates is characterized by a Type III survivorship curve, where most cohorts experience 60 to 99% mortality in the first few days or weeks of independent benthic life (Gosselin & Qian 1997, Hunt & Scheibling 1997, Pedersen et al. 2008). The mechanisms causing variation in survivorship 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 heat, humidity, wind speed, or 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.

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For intertidal 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 intertidal habitat where they are exposed to aerial conditions for several hours each day. During aerial exposure, heat and desiccation 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 heat and desiccation 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 heating is evaporative cooling, which is achieved by opening shell valves during air exposure (Bayne et al. 1976, Helmuth 1998). However, the drawback of this response is an increased risk of desiccation. Large mussels have greater amounts of water available in their tissues than small ones (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). Large mussels are, thus, capable of enduring longer periods of high heat and desiccation than small ones. This suggests sensitivity to heat and desiccation might scale with body size, in which case the early

benthic phase should be the most sensitive to stressful conditions. Recent studies have begun to examine the causes of mortality through the early benthic phase, but none have focused on the effects of heat or desiccation on mussels <6 mm shell length (SL) or on the ontogeny of their physiological tolerance.

We examined 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 ~1.7 m and 2.9 m above mean lower low water (MLLW) and is typically exposed to air for 5 to 8 h per tide cycle (Suchanek 1978, authors' 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* or *Endocladia muricata*, and on the byssal threads of adult mussels (Suchanek 1978, Martel et al. 1999). Upon reaching 2.0 to 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, Hunt & Scheibling 1998). On open surfaces, these small mussels are typically only found within crevices and depressions (Suchanek 1978).

Our overall goal was to determine the influence of heat and desiccation on mortality of newly settled *Mytilus trossulus*, and the 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 levels that can be experienced in the field during the settlement season, (2) characterizing the changes in sensitivity to desiccation that occur as mussels increase in size, and (3) determining the frequency of lethal desiccation conditions in the field during the period of peak recruitment. This information was then used to conclude as to whether ontogenetic shifts in habitat use by juvenile *M. trossulus* might be a response to changing vulnerability to desiccation.

MATERIALS AND METHODS

Study site

We conducted our study from May to August during the summers of 2010 and 2011. Mussels were collected from Prasiola Point (48°49'55" N,

125° 07' 05" W), in Barkley Sound on the west coast of Vancouver Island, a site with consistently high *Mytilus trossulus* settlement (A. Martel pers. comm.). Laboratory experiments were conducted nearby 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, we monitored 6 weather parameters from June 26 to August 27, 2010 and May 4 to August 20, 2011 using a weather station (Davis Instruments Vantage Pro2 Plus Integrated Sensor Suite 6327). The weather station was installed at Wizard Islet (48° 51' 27" N, 125° 09' 38" W), 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 (unpubl. data). The weather station, mounted ~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 nearby intertidal rock surfaces using 3 Thermocron® iButton (DS1921G) data loggers, and intertidal RH was monitored using 2 Lascar Electronics (EL-USB-2) data loggers. The intertidal zone at the monitoring site is a long, nearly horizontal bench facing SSW with rock surfaces fully exposed to the sun during low tide. Loggers were placed at 2.75 m above MLLW, which is just below the upper limit of the vertical distribution of *Mytilus trossulus*. The iButton loggers were attached to the rock by placing them in small grey mesh bags, resembling the colour of the rock surface, and bolting these to the rock. The mesh bags were stiff and kept the iButton loggers in good conductive contact with the rock during low tide. These 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 6 consecutive days in June 2011. All devices recorded data at 15 min intervals and data from the weather station and iButton loggers were downloaded bi-weekly.

To determine if the temperature and RH conditions occurring near the upper limit of mussel distribution in the intertidal zone are comparable to those occurring over the full range of mussel distribution, 6 Thermocron® iButton (DS1923) temperature and

humidity data loggers were used to record temperature and RH at 3 intertidal heights (2.0, 2.25, 2.75 m). The 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 one was placed under fucoid algae (*Fucus* spp.). Data were logged at 5 min intervals for 3 to 4 h during low tide on sunny days at 2 different field sites: Grappler Inlet (48° 40' 55" N, 125° 7' 05" W) on 5 September 2012 and Wizard Islet on 6 September 2012. Data loggers at 2.0 and 2.25 m were removed from the intertidal zone just before their location was covered by the rising tide; the loggers at 2.25 and 2.75 m were removed at the same time.

RH is occasionally used as a measure of desiccation potential (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 a given volume of air (Anderson 1936) and, thus, more accurately reflects desiccation potential. VPD has been used in several studies of the effects of desiccation 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 (World Meteorological Organization 2008):

$$\text{VPD} = e_s - e_a \quad (1)$$

$$e_s = 0.6112 \times e^x \text{ (kPa)} \quad (2)$$

$$x = \left(\frac{17.62 \cdot T}{243.12 + T} \right) \quad (3)$$

$$e_a = (\text{RH}/100) \times e_s \quad (4)$$

where e_s = saturation vapour pressure, e_a = actual vapour pressure, and T = air temperature at the rock surface (°C).

Sensitivity to heat

The sensitivity of newly settled mussels to the full range of temperatures that occur in mid-intertidal rocky shore habitats of Barkley Sound was exam-

ined in 2 experiments carried out in July 2010 and July 2011. In Expt 1, 80 groups of 20 mussels measuring 1 to 2 mm in SL (i.e. ~1 mo after settlement) were separately placed in 80 sealed plastic Ziploc® containers measuring ~11 × 11 × 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 to 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 × 5 cm piece of shammy cloth saturated with seawater in each sealed container. 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 around 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 blunt probe. Given that Expt 1 revealed a tolerance threshold between 30 and 35°C, Expt 2 was carried out in July and August 2011 to resolve sensitivity at 1°C intervals between 28 and 37°C. The procedures and experimental design of Expt 2 were the same as for Expt 1 except for the treatment temperatures.

Sensitivity to desiccation

The sensitivity of recently settled juvenile mussels to desiccation was examined in July 2010. Forty groups of 20 mussels measuring 1 to 2 mm in SL were placed in separate sealed 2 l glass jars and subjected for 6 h to one of 4 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 4 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 recorded by the weather station in July 2010 (30 to 95% RH). 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 to 2 mm SL *Mytilus trossulus*. Temperature and RH were monitored by placing RH data loggers in 2 of the jars during each experiment. The experimental design was as follows: 4 VPD treatments, with 10 jars per treatment and 20 mussels per jar, for a total of 800 mussels. Different amounts of Drierite® desiccation crystals¹, which are composed of anhydrous calcium sulfate known to be chemically inert except toward water, were added to the bottom of the jars to obtain the 2 highest VPD levels. The 2 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 <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 and 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 September 4, 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 *Mytilus trossulus* were more likely to be killed by ambient desiccation conditions than by heat. The final experiment, therefore, examined how desicca-

¹W.A. Hammond Drierite Co. Ltd. (2012) Drierite [Material Safety Data Sheet]. Date prepared: 2-21-2012. https://secure.drierite.com/catalog3/REGULAR_MSDS.pdf (accessed 26 Nov 2012)

tion 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, 3–4, 5–6, and 7–8 mm SL. The experimental design was as follows: 4 mussel size classes, each exposed to 3 VPD treatments (0.58, 1.17, and 1.75 kPa), with 5 containers per treatment, and 20 mussels per container, 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 and 0.58 kPa treatments.

Data analysis

Temperature and RH data from the intertidal rock surface were used in combination with weather station data to determine the frequency of stressful conditions occurring during the settlement season. Although intertidal RH measurement data were only recorded on 1 d in 2010, intertidal temperature and RH data were recorded over 6 daytime low tides in June 2011; multiple regression analysis of temperature or RH (dependent variables) with weather station data (independent variables) was used to extrapolate intertidal temperature and RH data for the rest of the 2011 settlement season.

The proportion of dead mussels (percent mortality) was used as the dependent variable in all experiments. A modified Freeman and Tukey (1950) arcsine transformation was, therefore, applied to percent mortality data prior to statistical analysis. Analysis of variance (ANOVA) 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):

$$S = \frac{1}{1 + e^{-x}} \text{ where } x = (T - 36.73)/0.3863 \quad (5)$$

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

The best-fit models obtained from multiple regression analysis were developed into predictive equations for intertidal temperature and intertidal RH. Of the 8 weather station parameters examined, only 4 were significantly related to intertidal temperature: air temperature, RH of air, wind speed, and solar radiation ($F_{4,132} = 356.4$, $R^2 = 0.913$, $n = 137$, $p < 0.001$). Three of these parameters were also significantly related to intertidal RH: air temperature, wind speed, and solar radiation ($F_{3,133} = 393.6$, $R^2 = 0.897$, $n = 137$, $p < 0.001$). Unexpectedly, RH at the weather station did not contribute to predicting intertidal RH; this is likely because the main source of humidity at the rock surface is the water evaporating from the intertidal rocks, algae, and animals rather than the humidity of the air mass. The equations developed from these models predicted intertidal values very close to actual measured values, explaining 89.7 to 91.3% of their variation, although they slightly underestimated temperatures $> 27^\circ\text{C}$ and RH values $< 48\%$. The predictive equation for temperature described above was used to estimate intertidal rock surface temperature during the 13 d in June 2011 when iButton loggers failed. After calculation, the predicted values for intertidal RH were converted to VPD.

Conditions on mid-intertidal rock surfaces varied greatly throughout the summers of 2010 and 2011. Although a few weeks of intertidal temperature data were lost in both years due to damage incurred to the iButton data loggers, reliable temperature data were obtained for 33 d in 2010 and 96 d in 2011, including some of the hottest days of the summer. Intertidal temperature measurements on those days reveal that rock surface temperature reached a maximum of 45°C in July and August 2010. The maximal daily rock surface temperatures in 2011 peaked in late June at 40.5°C and again in late July at 42.5°C (Fig. 1A). These peak temperatures occurred on days with mid-day low tides. The intertidal temperatures measured in August 2011 were slightly lower than in June and July 2011; this was likely due to the mean amount of daily solar radiation being 10 to 33% lower

in August than in the rest of the summer, caused by the increased amount of fog and cloud cover that is typical of Barkley Sound in August. The predicted daily maximal mid-intertidal rock surface VPD levels ranged from 0.16 to 6.85 kPa over the summer of 2011, with daily maximal VPD levels gradually increasing from May to the beginning of August (Fig. 1B). This suggests that mussels encountered increased desiccation levels as the recruitment season progressed, up to early August when the increased amount of fog and cloud cover resulted in lower levels of desiccation.

Data loggers that were placed at 3 different intertidal heights (2.0, 2.25, 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 (Fig. A1A,B in Appendix 1). However, high temperatures did not persist for >2.5 h before the surfaces at 2.0 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 furoid algae showed that rock surface temperatures and VPD levels did not reach potentially lethal levels at any intertidal height (Fig. A1C,D in Appendix 1); the exception was a short period of time when VPD levels at 2.5 m briefly peaked above the threshold, likely due to the angle of the sun temporarily allowing sunlight to penetrate through a small gap in the algal fronds, thus heating the surface. Data from Grappler Inlet showed very similar patterns to those recorded on Wizard Islet.

Sensitivity to heat

Juvenile mussels were tolerant of a wide range of temperatures, but did experience increased mortality after 6 h exposure to high experimental temperatures. In the first temperature tolerance trial, there was a significant difference among temperature treatments in the mean proportion of dead mussels (ANOVA: $F = 104.9$, $df = 7$, $n = 10$, $p < 0.001$). 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 (Fig. 2A). There was no significant difference in mortality among treatments ranging from 10 to 30°C, nor among treatments ranging from 35°C to 45°C. This suggested the threshold of temperature tolerance to be between 30 and 35°C. The second trial, focusing on the 30 to 35°C range of temperatures, also revealed a significant difference among temperature treatments in the mean proportion of dead mussels (Fig. 2B; ANOVA: $F = 96.8$, $df = 7$, $n = 10$, $p < 0.001$). Tukey post-hoc comparisons revealed that mortality differed significantly between the 33 and 34°C treatments (a 58% difference) and between the 35 and 37°C treatments (a 34% difference), indicating a temperature tolerance threshold of 33°C. There was no significant difference in mortality among treatments ranging from 28 to 33°C, nor between the 34 and 35°C treatments. Fitting a sigmoidal curve to the data, substituting x for $[30.0 - 0.88 \times T]$, revealed an LT_{50} of 34.1°C after 6 h exposure for juvenile *Mytilus trossulus*.

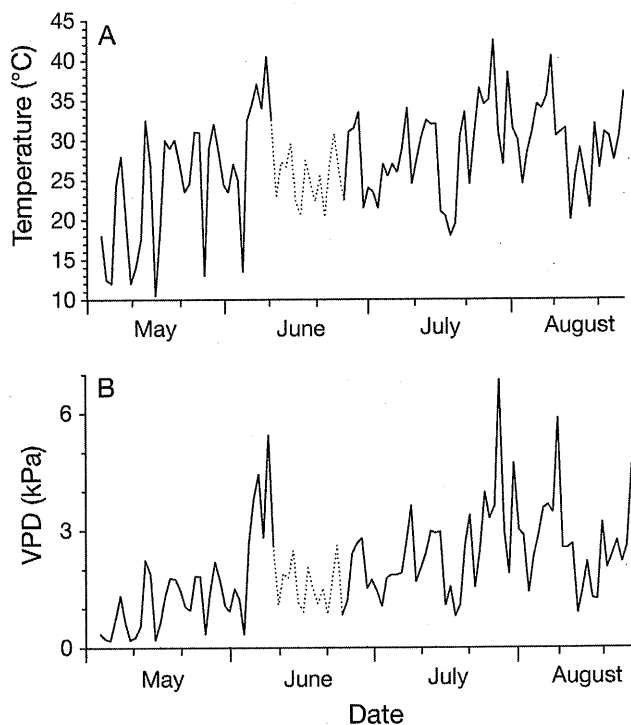


Fig. 1. (A) Maximal daily mid-intertidal temperatures and (B) predicted maximal daily mid-intertidal vapour pressure deficit (VPD) at the rock surface for May to August, 2011. Dotted: predicted data for 9–21 June, as measured data were lost due to damaged data loggers

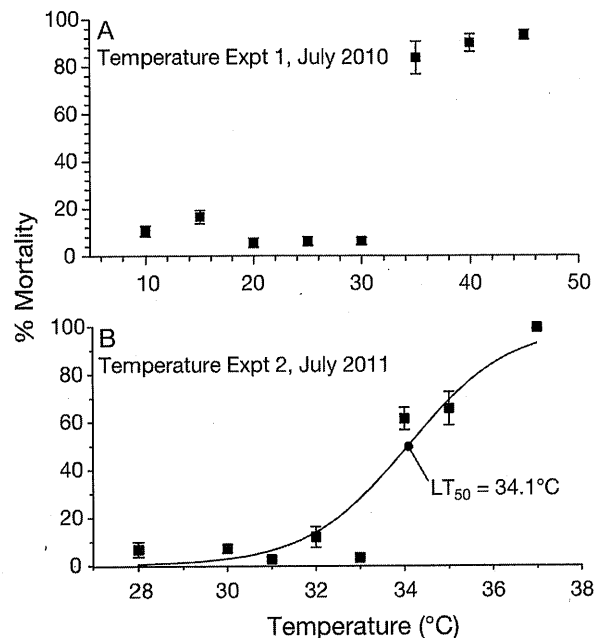


Fig. 2. *Mytilus trossulus*. Effect of prolonged exposure to various temperature treatments on mortality of newly settled mussels (1–2 mm shell length). (A) Expt 1: exposing mussels to temperatures ranging from 10 to 45°C in 5°C intervals. (B) Expt 2: exposing mussels to the narrower temperature range of 28 to 37°C in 1°C intervals to determine the threshold of temperature tolerance. Means \pm SE. Solid line in (B) is the sigmoidal curve used to determine the median lethal temperature (LT₅₀)

The temperature loggers recorded a total of 12 d in 2010 and 13 d in 2011 that exhibited rock surface temperatures $>33^{\circ}\text{C}$ (i.e. above the temperature tolerance threshold for mussels <2 mm SL) and persisting for >1 h (Fig. 3). The majority of these days had temperatures that persisted $>33^{\circ}\text{C}$ for only 1 to 2 h, but twice in July 2010 and once in July 2011 the temperatures persisted $>33^{\circ}\text{C}$ 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 d that exceeded 33°C and never for more than 3 h. Temperatures in May 2011 never exceeded 33°C . Mussels 1 to 2 mm SL settling on bare surfaces could, therefore, be exposed to potentially lethal temperatures in the field, but this occurs relatively rarely throughout the recruitment season, and temperatures above the threshold do not often persist for periods >2 h.

Sensitivity to desiccation

Juvenile mussels had low tolerance to high VPD levels (Fig. 4). There was a significant difference in

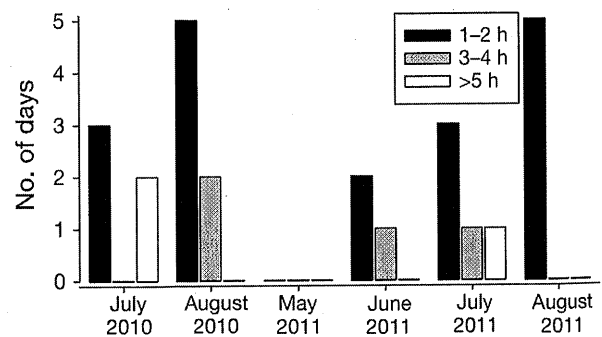


Fig. 3. Number of days during the 2010 and 2011 settlement seasons when the intertidal rock surface near the upper limit of adult mussel distribution exceeded 33°C for 1–2, 3–4, and >5 at low tide

mean mortality among VPD treatments (ANOVA: $F = 68.42$, $df = 3$, $n = 10$, $p < 0.001$), and Tukey post-hoc comparisons indicated that mortality differed significantly between the 0.58 and 1.17 kPa treatments, mortality being 72% higher in the latter. There was no significant difference between the 0.12 and 0.58 kPa treatments, nor between the 1.17 and 1.75 kPa treatments. This suggests the threshold VPD lies between 0.58 and 1.17 kPa. Fitting a sigmoidal curve to the data, substituting x for $[2.69 \times \text{VPD} - 2.71]$, revealed an LD₅₀ of 1.01 kPa after 6 h exposure for 1 to 2 mm SL *Mytilus trossulus*.

Most days of summer 2011 had low tide VPD levels that met or exceeded the LD₅₀ for juvenile mussels and were often sustained for many consecutive hours (Fig. 5). May 2011 had few days with potentially harmful VPD levels, with 67% of the month having VPD levels below the threshold level. In contrast, VPD levels exceeded the threshold level every day in July for >1 h, and on most days harmful VPD levels

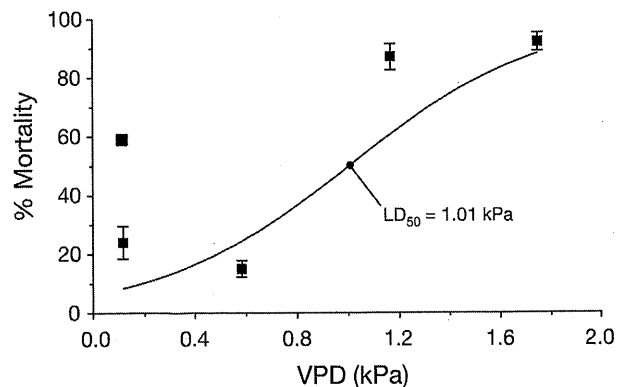


Fig. 4. *Mytilus trossulus*. Effect of prolonged exposure to various vapour pressure deficits (VPD) on mortality of juvenile mussels (1–2 mm shell length). Means \pm SE

were sustained for >5 h. Harmful VPD levels sustained for >9 h, spanning almost the full duration of low tide, occurred in June, July, and August.

Our analysis of water content by the algae *Cladophora columbiana* revealed that it 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 weight. The amount of water that was contained within tufts of algae after low tide aerial exposure was proportional to the blotted weight of the algae (Linear regression: $F_{1,8} = 26.5$, $R^2 = 0.77$, $p < 0.001$).

Ontogeny of desiccation tolerance

Mussel mortality differed among VPD treatments and among size classes. A significant interaction between size class and VPD (Table 1) revealed that the trend in mean mortality among VPD levels differed among size classes. Fig. 6 reveals that higher treatment VPD levels led to increased mortality in mussels 1 to 2 mm SL, but not in mussels ≥ 3 mm SL.

In this experiment, the 1 to 2 mm SL mussels experienced lower mortality at 1.17 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 causing higher mortality for 1 to 2 mm SL mussels is consistent between both experiments.

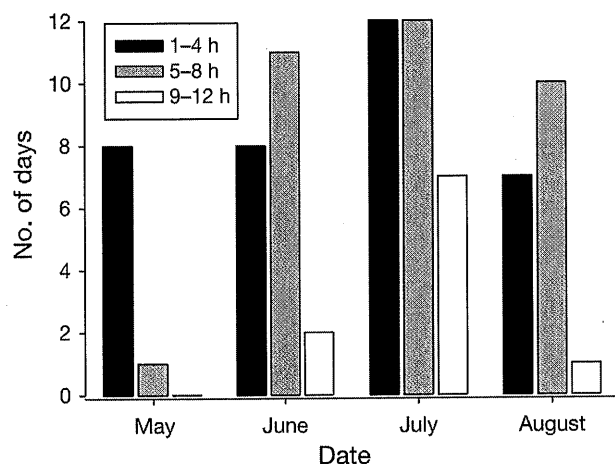


Fig. 5. Number of days during the 2011 settlement season that vapour pressure deficit (VPD) was ≥ 1.01 kPa (mussel desiccation sensitivity threshold) for 1–4, 5–8, and 9–12 h during low tide

Table 1. *Mytilus trossulus*. Two-factor ANOVA of the mean mortality of mussels in each of 4 size classes exposed to various vapour pressure deficit (VPD) levels for 6 h ($n = 5$)

Source	df	MS	F	p
Size class	3	0.363	43.532	<0.001
VPD	2	0.082	9.840	<0.001
Size class \times VPD	6	0.026	3.088	0.012
Residual	48	0.008		

DISCUSSION

Sensitivity to heat

Extreme temperatures may not be as important a mortality factor as previously presumed for recently settled *Mytilus trossulus* on Vancouver Island, British Columbia. Mortality of juvenile *M. trossulus* (1–2 mm SL) did increase at high temperatures, but only at temperatures $>33^\circ\text{C}$, indicating that these recent settlers are able to tolerate the majority of temperature conditions experienced in their natural environment. 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. $>33^\circ\text{C}$), but they were most often sustained for only 1 to 2 h. This is not likely long enough to kill a newly settled mussel 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 >1 h. These high temperature events occurred with similar frequency in August of 2010 and 2011, suggesting that the frequency of such temperature events is consistent from

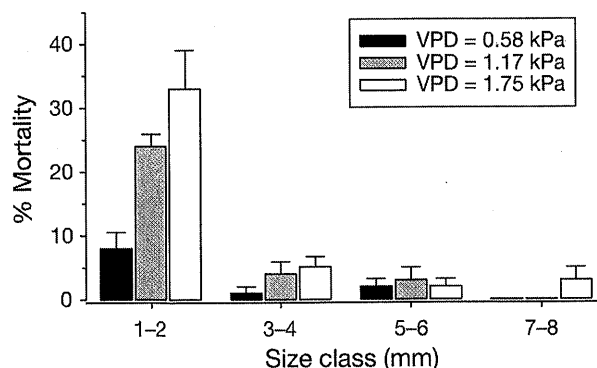


Fig. 6. *Mytilus trossulus*. Effect of vapour pressure deficit (VPD) on mortality of mussels within 4 size classes. Means \pm SE

year to year. However, these high temperature events occurred on <20% of the total number of days monitored during the settlement season, and <1% had a temperature event that persisted above the threshold level for >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 heat being an important mortality factor for newly settled *M. trossulus*. Our 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 to 2 mm SL mussels might nevertheless reduce their ability to survive. In our study, there were several days during which temperatures at or above the threshold level were sustained for 1 to 2 h, and several days during which temperatures were sustained 1 to 3°C below the threshold level for 3 to 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 *Mytilus 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 heat 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 LT₅₀ of *Lottia gigantea* to decrease by ~5°C. An interaction such as this, coupled with repeated exposure to sublethal temperatures, might, therefore, reduce the survivorship of 1 to 2 mm SL mussels during the first few days and weeks of life in the intertidal zone.

Sensitivity to desiccation

Unlike extreme heat, exposure to high desiccation levels appears to be an important threat to the survival of 1 to 2 mm SL *Mytilus trossulus* on the west

coast of Vancouver Island. These recently settled juvenile *M. trossulus* were highly sensitive to VPD levels >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 in the field. Substantial mortality was observed after exposing mussels to 6 h of high desiccation levels in laboratory experiments. In addition, lethal levels of desiccation 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. Although not examined in this study, the duration of exposure to desiccation 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 *Mytilus 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 level of 1.75 kPa caused <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. *Mytilus trossulus* settle primarily in filamentous algae (Suchanek 1978, Martel et al. 1999), a structurally complex microhabitat. We determined that tufts of filamentous algae are able to retain large amounts of water when exposed at low tide, thus eliminating desiccation within the tufts. We 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 (authors' 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 fila-

mentous algae may, therefore, be to obtain protection from frequent exposure to desiccation. This would be consistent with Gosselin and Chia (1995), who demonstrated that similar microhabitats provide protection from desiccation to newly hatched *Nucella emarginata*.

Ontogeny of desiccation tolerance

Tolerance to desiccation changed rapidly with increasing body size in juvenile *Mytilus 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 levels, yet mussels in the next size class (3–4 mm SL) were significantly more tolerant. The tolerance threshold to desiccation by juvenile *M. trossulus*, therefore, increases substantially when the individual reaches ~2 mm SL. Our results revealed that bare rock surfaces within the vertical range of *M. trossulus* in the intertidal zone (~2.0 to 2.75 m) are frequently exposed to desiccation levels at or above the tolerance threshold for newly settled mussels. In addition, these high levels of desiccation were often sustained for the full duration of low tide, which typically lasted 5 to 8 h but on some days spanned 13 h. 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 to 2 mm SL *M. trossulus* may be repeatedly exposed to potentially lethal levels of desiccation for several weeks before growing to desiccation-resistant size.

The difference in desiccation tolerance observed between mussels in the 2 desiccation experiments only slightly changes the likelihood of exposure to potentially harmful VPD levels during the settlement and early growth season. The 2 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. We fitted a second sigmoidal curve to mortality data for 1 to 2 mm SL mussels from the ontogeny of desiccation tolerance experiment, which revealed an LD₅₀ 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 >1 h, and on most days harmful VPD levels were sustained for >4 h. Although the tolerance of 1 to 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 is likely to be a potentially important mortality factor for 1 to 2 mm SL *Mytilus trossulus*.

The size threshold at which mussels become resistant to desiccation 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 to 2.5 mm SL (Sigurdsson et al. 1976, de Blok & Tan Maas 1977). In addition, Hunt & 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 that the size of juvenile mussels at settlement in adult habitat (2.0 to 2.5 mm SL) is the minimum size where mussels would be expected to be 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 sensitivity to desiccation, 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 *Mytilus 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

furoid algae as climate changes. A recent report examining intertidal community changes over 10 yr 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.

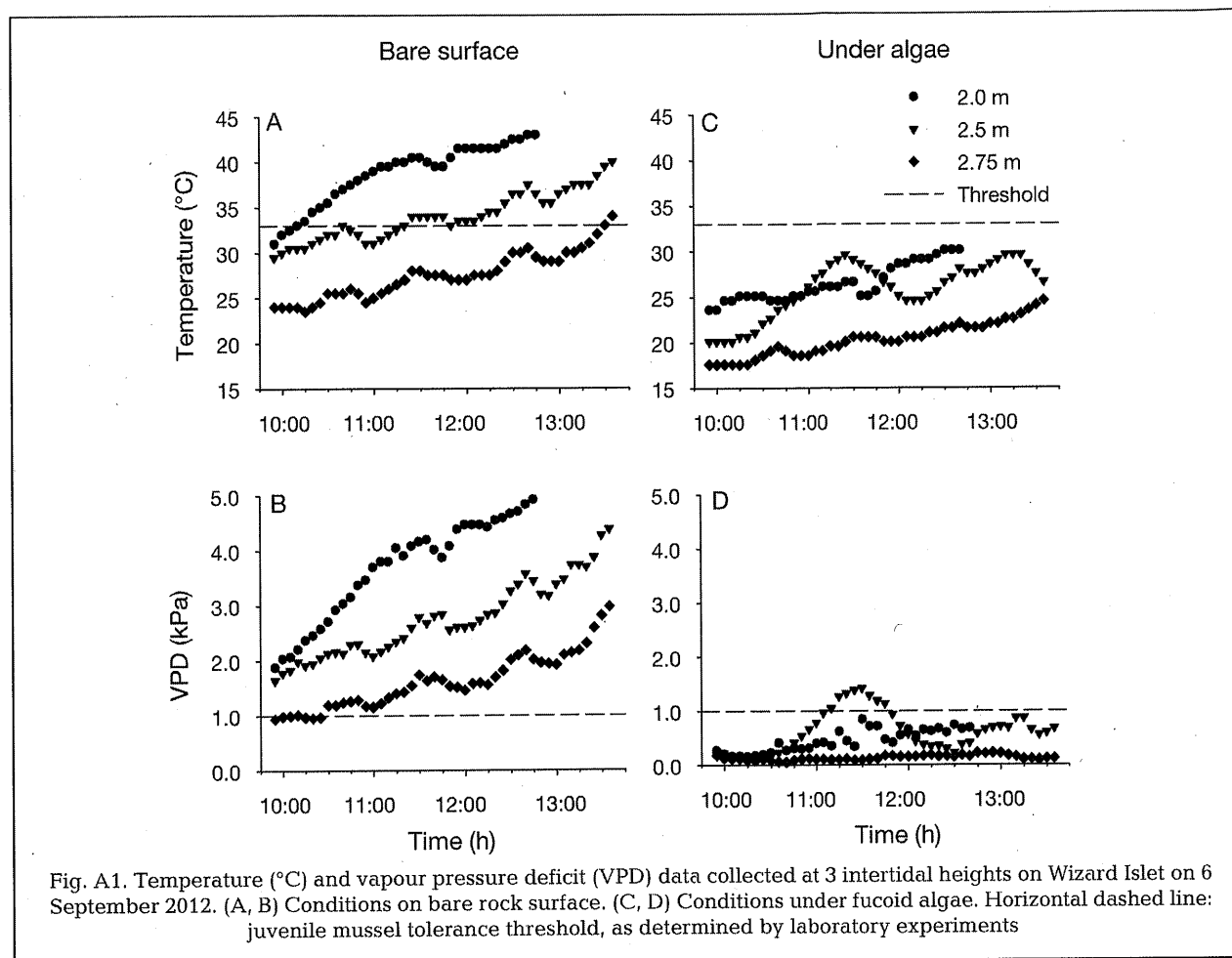
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LITERATURE CITED

- Addington RN, Mitchell RJ, Oren R, Donovan LA (2004) Stomatal sensitivity to vapor pressure deficit and its relationship to hydraulic conductance in *Pinus palustris*. *Tree Physiol* 24:561–569
- Anderson DB (1936) Relative humidity or vapor pressure deficit. *Ecology* 17:277–282
- Anestis A, Pörtner HO, Karagiannis D, Angelidis P, Staikou A, Michaelidis B (2010) Response of *Mytilus galloprovincialis* (L.) to increasing seawater temperature and to martellosis: metabolic and physiological parameters. *Comp Biochem Physiol A* 156:57–66
- Baudinette RV (1972) Energy metabolism and evaporative water loss in the California ground squirrel. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 81: 57–72
- Bayne BL (1964) Primary and secondary settlement in *Mytilus edulis* L. (Mollusca). *J Anim Ecol* 33:513–523
- Bayne LB, Bayne JC, Carefoot CT, Thompson JR (1976) The physiological ecology of *Mytilus californianus* Conrad 1. Metabolism and energy balance. *Oecologia* 22:211–228
- Buckley BA, Owen ME, Hofmann GE (2001) Adjusting the thermostat: the threshold induction temperature for the heat-shock response in intertidal mussels (genus *Mytilus*) changes as a function of thermal history. *J Exp Biol* 204:3571–3579
- de Blok JW, Tan-Maas M (1977) Function of byssus threads in young postlarval *Mytilus*. *Nature* 267:558
- Denny MW, Miller LP, Harley CDG (2006) Thermal stress on intertidal limpets: long-term hindcasts and lethal limits. *J Exp Biol* 209:2420–2431
- Freeman MF, Tukey JW (1950) Transformations related to the angular and the square root. *Ann Math Stat* 21: 607–611
- Gosselin LA (1997) An ecological transition during juvenile life in a marine snail. *Mar Ecol Prog Ser* 157:185–194
- Gosselin LA, Chia FS (1995) Characterizing temperate rocky shores from the perspective of an early juvenile snail: the main threats to survival of newly hatched *Nucella emarginata*. *Mar Biol* 122:625–635
- Gosselin LA, Jones LA (2010) Effects of solar radiation on barnacle settlement, early post-settlement mortality and community development in the intertidal zone. *Mar Ecol Prog Ser* 407:149–158
- Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. *Mar Ecol Prog Ser* 146:265–282
- Harley CDG (2008) Tidal dynamics, topographic orientation, and temperature-mediated mass mortalities on rocky shores. *Mar Ecol Prog Ser* 371:37–46
- Helmuth B (1998) Intertidal mussel microclimates: predicting the body temperature of a sessile invertebrate. *Ecol Monogr* 68:51–74
- Helmuth B (1999) Thermal biology of rocky intertidal mussels: quantifying body temperatures using climatological data. *Ecology* 80:15–34
- Hofmann GE, Somero GN (1995) Evidence for protein damage at environmental temperature: seasonal changes in levels of ubiquitin conjugates and Hsp70 in the intertidal mussel *Mytilus trossulus*. *J Exp Biol* 198:1509–1518
- Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Mar Ecol Prog Ser* 155:269–301
- Hunt HL, Scheibling RE (1998) Spatial and temporal variability of patterns of colonization by mussels (*Mytilus trossulus*, *M. edulis*) on a wave-exposed rocky shore. *Mar Ecol Prog Ser* 167:155–169
- Johnson SB, Geller JB (2006) Larval settlement can explain the adult distribution of *Mytilus californianus* Conrad but not of *M. galloprovincialis* Lamarck or *M. trossulus* Gould in Moss Landing, central California: evidence from genetic identification of spat. *J Exp Mar Biol Ecol* 328: 136–145
- Jones SJ, Mieszkowska N, Wetthey DS (2009) Linking thermal tolerances and biogeography: *Mytilus edulis* (L.) at its southern limit on the east coast of the United States. *Biol Bull (Woods Hole)* 217:73–85
- Katul GG, Palmroth S, Oren R (2009) Leaf stomatal responses to vapour pressure deficit under current and CO₂-enriched atmosphere explained by the economics of gas exchange. *Plant Cell Environ* 32:968–979
- Kennedy VS (1976) Desiccation, higher temperatures and upper intertidal limits of 3 species of sea mussels (Mollusca: Bivalvia) in New Zealand. *Mar Biol* 35:127–137
- Koch H (1989) Desiccation resistance of the supralittoral amphipod *Traskorchestia traskiana* (Stimpson, 1857). *Crustaceana* 56:162–175
- Lane DJW, Beaumont AR, Hunter JR (1985) Byssus drifting and the drifting threads of the young post-larval mussel *Mytilus edulis*. *Mar Biol* 84:301–308
- LeBlanc N, Landry T, Stryhn H, Tremblay R, McNiven M, Davidson J (2005) The effect of high air and water temperature on juvenile *Mytilus edulis* in Prince Edward Island, Canada. *Aquaculture* 243:185–194
- Martel AL, Robles C, Beckenback K, Smith MJ (1999) Distinguishing early juveniles of Eastern Pacific mussels (*Mytilus* spp.) using morphology and genomic DNA. *Invertebr Biol* 118:149–164
- McDonald JH, Seed R, Koehn RK (1991) Allozymes and metamorphic characters of 3 species of *Mytilus* in the Northern and Southern hemispheres. *Mar Biol* 111: 323–333
- Miller LP, Harley CDG, Denny MW (2009) The role of temperature and desiccation stress in limiting the local-scale

- distribution of the owl limpet, *Lottia gigantea*. *Funct Ecol* 23:756–767
- Montalto L, Ezcurra de Drago I (2003) Tolerance to desiccation of an invasive mussel, *Limnoperna fortunei* (Dunker, 1857) (Bivalvia, Mytilidae), under experimental conditions. *Hydrobiologia* 498:161–167
 - Newell CR, Hidu H, McAlice BJ, Podniesinski G, Short F, Kindblom L (1991) Recruitment and commercial seed procurement of the blue mussel *Mytilus edulis* in Maine. *J World Aquacult Soc* 22:134–152
 - Pedersen TM, Hansen JLS, Josefson AB, Hansen BW (2008) Mortality through ontogeny of soft-bottom marine invertebrates with planktonic larvae. *J Mar Syst* 73: 185–207
 - Petes LE, Menge BA, Murphy GD (2007) Environmental stress decreases survival, growth, and reproduction in New Zealand mussels. *J Exp Mar Biol Ecol* 351:83–91
 - Porri F, Zardi GI, McQuaid CD, Radloff S (2007) Tidal height, rather than habitat selection for conspecifics, controls settlement in mussels. *Mar Biol* 152:631–637
 - Ricciardi A, Serrouya R, Whoriskey FG (1995) Aerial exposure tolerance of zebra and quagga mussels (Bivalvia: Dreissenidae): implications for overland dispersal. *Can J Fish Aquat Sci* 52:470–477
 - Roberts DA, Hofmann GE, Somero GN (1997) Heat-shock protein expression in *Mytilus californianus*: Acclimatization (Seasonal and tidal-height comparisons) and acclimation effects. *Biol Bull (Woods Hole)* 192:309–320
 - Rodenhuis D, Bennett K, Werner A, Murdock TQ, Bronaugh D (2007) Hydro-climatology and future climate impacts in British Columbia. *Pacific Climate Impacts Consortium*, University of Victoria, Victoria, BC
 - Schiel DR, Steinbeck JR, Foster MS (2004) Ten years of induced ocean warming causes comprehensive changes in marine benthic communities. *Ecology* 85:1833–1839
 - Schneider KR, Van Thiel LE, Helmuth B (2010) Interactive effects of food availability and aerial body temperature on the survival of 2 intertidal *Mytilus* species. *J Therm Biol* 35:161–166
 - Shanks AL (2009) Barnacle settlement versus recruitment as indicators of larval delivery. I. Effects of post-settlement mortality and recruit density. *Mar Ecol Prog Ser* 385: 205–216
 - Sigurdsson JB, Titman CW, Davies PA (1976) The dispersal of young post-larval bivalve molluscs by byssus threads. *Nature* 262:386–387
 - Siqueira MB, Katul GG, Tanny J (2012) The effect of the screen on the mass, momentum, and energy exchange rates of a uniform crop situated in an extensive screen-house. *Boundary-Layer Meteorol* 142:339–363
 - Somero GN (2002) Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. *Integr Comp Biol* 42:780–789
 - Strathmann MF (ed) (1987) Phylum Mollusca, Class Bivalvia, Chapter 14. In: *Reproduction and development of marine invertebrates of the northern Pacific coast*. University of Washington Press, Seattle, WA, p 309–353
 - Suchanek TH (1978) The ecology of *Mytilus edulis* L. in exposed rocky intertidal communities. *J Exp Mar Biol Ecol* 31:105–120
 - Sukhotin AA, Lajus DL, Lesin PA (2003) Influence of age and size on pumping activity and stress resistance in the marine bivalve *Mytilus edulis* L. *J Exp Mar Biol Ecol* 284: 129–144
 - Tomanek L, Zuzow MJ (2010) The proteomic response of the mussel congeners *Mytilus galloprovincialis* and *M. trossulus* to acute heat stress: implications for thermal tolerance limits and metabolic costs of thermal stress. *J Exp Biol* 213:3559–3574
 - Tsuchiya M (1983) Mass mortality in a population of the mussel *Mytilus edulis* L. caused by high temperature on rocky shores. *J Exp Mar Biol Ecol* 66:101–111
 - van Houten YM, van Rijn PCJ, Tanigoshi LK, van Stratum P, Bruin J (1995) Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. *Entomol Exp Appl* 74:225–234
 - Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size-structured populations. *Annu Rev Ecol Syst* 15:393–425
 - Wolcott TG (1973) Physiological ecology and intertidal zonation in limpets (Acrnaea): a critical look at 'limiting factors'. *Biol Bull (Woods Hole)* 145:389–422
 - World Meteorological Organization (WMO) (2008) Guide to meteorological instruments and methods of observation. Appendix 4B, WMO-No. 8 (CIMO Guide). WMO, Geneva

Appendix 1



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