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# Effects of osmotic and thermal shock on the invasive aquatic mudsnail *Potamopyrgus antipodarum*: mortality and physiology under stressful conditions

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## Abstract

Invasive freshwater species, such as the exotic mollusc *Potamopyrgus antipodarum* (New Zealand mudsnail), can frequently survive under harsh conditions, including brackish and hypoxic environments. We experimentally assessed the effects of osmotic (0, 10, 20, 25 and 30 psu) and thermal (20 °C) shock on mortality, activity and physiology of *P. antipodarum* collected at Capitol Lake, Olympia, Washington, USA, during winter and spring seasons when environmental temperature was 5 and 10 °C respectively. We measured standard metabolic rate and enzymatic activities (malate dehydrogenase, lactate dehydrogenase, alanopine dehydrogenase) in snails after a 10-day acclimation period at high salinity. Significantly higher mortalities were observed at higher salinities; the strongest effects occurred on snails collected at the end of winter, and exposed to 30 psu and 20 °C (100% mortality in 3 days). When snails were collected during the spring, 100% mortality was observed after 40 days at 30 psu and 20 °C. Standard metabolic rates were significantly lower when snails were exposed to salinities of 25 and 30 psu, even after 10 days of acclimation. Enzymatic activities showed small but significant declines after 10 days at 30 psu reflecting the declines observed in overall metabolism. The physiological tolerances to temperature and salinity displayed by this population of *P. antipodarum* make its eradication from Capital Lake difficult to achieve.

## Keywords

Ecophysiology, enzymatic activity, invasive species, mortality, New Zealand mudsnail, salinity

## Introduction

After their initial introduction and establishment, exotic species often become invasive, spread to new territories, and cause major ecosystem changes with negative socioeconomic impacts (Pimentel et al. 2000; Sala et al. 2000). Due to their fast spread and wide range of impacts, exotic freshwater molluscs are a major concern in ecosystems around the planet (Sousa et al. 2014; Boltovskoy 2015). Development of control methods is an important step to stop the spread and mitigate the impacts produced by invasive molluscs.

The effects of environmental conditions on physiological performance are relevant to understanding changes in the behaviors and distributions of species (Pörtner and Farrell 2008; Somero 2012). Moreover, the study of ecologically relevant physiological variables has direct application in models predicting the spread and control of invasive species (e.g., Fly and Hilbish 2013; Xiao et al. 2014). Particularly, variables that can be used to indicate stress under extreme conditions, such as metabolic rate or enzymatic activity, can be of major importance to understand distribution limits and the efficiency of control methods. For example, the effect of extreme salinity conditions on mortality and physiological variables of several invasive molluscs were studied in order to control and predict their distribution (Duncan and Klekowski 1967; Alexander Jr and McMahon 2004; Sylvester et al. 2013; Boltovskoy 2015; Yang et al. 2018; Underwood et al. 2019).

The New Zealand mudsnail (*Potamopyrgus antipodarum*; Tateidae, Mollusca) is an aquatic freshwater species native to New Zealand that has been frequently introduced, becoming invasive, in Oceania, Asia, Europe, and North America (Ponder 1988; Kerans et al. 2005). It has recently been found in Chile, establishing a foothold in South America, too (Collado 2014; Collado et al. 2019). This species can be spread passively by other organisms (on the feet, pelts, or plumage of birds and mammals) and by human vectors such as in the mud adhered to boats or motor vehicles (Alonso and Castro-Díez 2008). *Potamopyrgus antipodarum* has biological and physiological characteristics that allow it to survive in dry environments, freezing temperatures, and a wide range of salinity conditions (Hylleberg and Siegismund 1987; Siegismund and Hylleberg 1987; Costil et al. 2001; Alonso and Castro-Díez 2008, 2012; Leclair and Cheng 2011). In a review of the salinity tolerance of *P. antipodarum*, Leclair and Cheng (2011) described this species surviving under salinities up to 20–27 psu (practical salinity units). Hoy et al. (2012) found populations living and reproducing at higher salinities in the Columbia River estuary, USA.

This species was first detected in our study site, Capitol Lake, Olympia, Washington (WA), USA, in 2009 (Leclair and Cheng 2011). This artificial lake is isolated from the southern Puget Sound estuary by a dam that when opened can allow seawater into the freshwater, changing the salinity to values close to 30 psu (Leclair and Cheng 2011). Flushing with seawater was practiced as control method in March 2010 when authorities attempted to eliminate this species (Leclair and Cheng 2011). Although more snails died than predicted by Leclair and Cheng's model, survival rate was usu-

ally over 70–80%. Consequently, this species still remains in the lake, reproducing and spreading successfully. In the current study, we examined the effects of salinity and temperature on mortality and activity of *P. antipodarum*. Additionally, we measured the effects of temperature and salinity on Standard Metabolic Rate (SMR) and enzymatic activities of *P. antipodarum* in order to study its metabolic response to stressful environments. Our working hypothesis is that mortality will increase at higher salinity concentrations regardless of temperature and season. However, based on previous field studies, we predict that season (winter or spring) or/and temperature will affect results by increasing mortality at higher temperatures for each season. It is expected that SMR will increase with higher temperatures. However, oxygen consumption rate, and consequently SMR will decrease at higher salinities, due to the operculum closing behavior of this species affecting survival capacity under extreme conditions. Additionally, we measured the effects of decreasing oxygen concentration on respiration rates.

## Methods

### Specimen collection

Snails were collected from the nearshore benthos in the vicinity of Marathon Park, Capitol Lake, Olympia WA, USA (47°02'14"N, 122°54'39"W) in two seasons: winter (March 1, water temperature 5 °C) and spring (April 5, water temperature 10 °C). The undersides of submerged stones and rocks were examined for snails. Specimens were manually removed and placed in Ziploc bags. These bags were transported in coolers to the laboratory and placed into small holding aquaria (40 L) kept in a temperature-controlled room set at 5 and 10 °C, for the first and second rounds of experiments, respectively. Sampling was carried out under a permit of the Washington State Department of Fish and Wildlife, and followed Level 2 decontamination procedures of their Invasive Species Management Protocols (Tweit et al. 2012). Additionally, all materials used to collect and handle snails were maintained at –30 °C for at least one week after use to ensure total disinfection. Final identification was carried out in the laboratory under a stereoscopic microscope using field guides and descriptions of *P. antipodarum* and native snails (Frest and Johannes 1999; Crosier and Molloy 2017). We note that more than 99% of the snails we collected in Capitol Lake were *P. antipodarum*.

### Tolerance to osmotic and thermal shock

In order to test the effects of osmotic and thermal shock on the mortality of *P. antipodarum*, two experiments were carried out under controlled conditions. The two exposure experiments, one per collection date, were conducted at five salinity concentrations (0, 10, 20, 25, and 30 psu) and three temperatures (5, 10, and 20 °C). Each experiment was performed in a complete factorial design, including

all combinations of salinity concentrations with 5 and 20 °C, or 10 and 20 °C for the first and second experiment, respectively. After 4 days of maintenance in the lab at the collection temperature, snails were randomly transferred to glass chambers (300 ml) filled with 250 ml of water at one of the salinity concentrations. Experimental chambers were capped with plastic mesh, and placed in 40-L aquaria with freshwater filled to same level as the water in the experimental chamber; the whole aquarium was covered using a plastic film to further guard against escape. The free space in the experimental chambers between the water surface and the top mesh allowed snails to remain outside of the water, simulating real conditions at the shore of the lake. Five chambers at each salinity/temperature were used totaling 50 experimental chambers per experiment. The number of snails per experimental chamber was increased from five to ten in order to improve the results in spring experiments when more snails were available.

The five different salinities (0, 10, 20, 25, and 30 psu) were obtained using Instant Ocean sea salt (0, 10, 20, 25, and 30 g/L) and water from the City of Olympia's Artesian Well (water quality data available at http://olympiawa.gov/~/media/Files/PublicWorks/Utility-Inserts/WQR/Artesian-Test-Results.pdf). Target salinity levels were verified using a Red Sea seawater refractometer (Model R12018) after initial preparation and during experiments. Water at the different salinity concentrations and temperatures were stored in large (40 L) carboys in the same experimental room and used to exchange the water in the experimental chambers each week. Although minimal, when evaporation occurred in the experimental chambers, water level was raised to the original level by adding distilled water.

For each experiment, two water temperatures were used, one at the same acclimation value (the same temperature of the lake when snails were collected, either 5 or 10 °C) and one at 20 °C without any acclimation time (thermal shock). While, the acclimation temperature was set for the room in general, two of the aquaria were kept at 20 °C using aquarium heaters, and the temperature in each aquarium was monitored using four data loggers, one per 40-L aquaria (thermal bottom sensors, iButton).

Each experimental chamber and the aquaria themselves were gently aerated, and snails were fed with food grade *Spirulina* three times per week. Mortality was checked every day during the first week and every few days afterwards. Snails were regarded as dead when no reaction was detected under a stereomicroscope after stimulation with a dissection needle in the operculum area. Dead snails were kept for 1–2 h in water at 0 psu to verify that there was no recovery after they were removed from the experimental chambers. Dead snails were kept at −30 °C for at least a week before being discarded. In addition to mortality, activity (active/inactive snails) as the number of open or closed snails (Pascual and Drake 2008) and reproductive activity (presence/absence of neonates released into the chambers and observed under a stereomicroscope) were recorded for each experimental chamber. Experiments were finished when most of the treatments reached more than 90% mortality, resulting in different time durations for each experiment, 7 and 105 days for winter and spring, respectively.

**Oxygen consumption**

Standard metabolic rates of *P. antipodarum* were measured at 10 and 20 °C on 231 specimens collected in the spring and kept in experimental chambers as described for mortality experiments. These additional experimental chambers were kept in the same 40 L tanks used for the spring mortality experiments. Due to the change in the activity level observed during the mortality experiments at the two temperatures, respiration rates were measured at several salinities, acclimation times and oxygen levels to test metabolic changes associated with these variables. First, a respiration consumption baseline was determined measuring oxygen consumption at 0 psu for 10 and 20 °C. Then, for the remaining salinities (10, 20, 25, and 30 psu), specimens were acclimated for 0, 2, 4, 6, 8, and 10 days before oxygen consumption measurements. Consequently, at least 8 additional chambers at four salinities and two temperatures were used for respiration experiments. All these snails were fed with *Spirulina* at 48 h, just after three snails were removed and used for respiration measurements.

Rates of oxygen consumption were measured on individual specimens using PreSens type B2-NTH fiber optic oxygen optodes connected to a PreSens Microx TX3 temperature-compensated oxygen meter (Precision Sensing, Regensburg, Germany). Sensors were calibrated at two points using an aqueous 5% sodium sulfite solution for oxygen-free water and gently stirred filtered water (at 10 and 20 °C) for oxygen-saturated water. Data were recorded on a personal computer through a serial connector. Three specimens were chosen at random from one experimental chamber, transferred into three different glass syringes with oxygen saturated (100%) filtered water (0.22 μm) containing antibiotics (100 mg $l^{-1}$ each of erythromycin and ampicillin) at each salinity, and incubated until oxygen saturation reached 0%. Antibiotics were added to decrease bacterial effects (Rutherford and Thuesen 2005). The volume of the syringe was set at 0.1 or 0.2 ml for measurements carried out at 10 and 20 °C respectively, which lasted around 1–2 h. Syringes were sealed with Luer-type fittings with Teflon septa for insertion of the oxygen sensor similar to methods described by Rutherford and Thuesen (2005) and Paolucci et al. (2010).

Total lengths of snails were measured before respiration experiments using an electronic caliper, and weight was calculated according to the length-ash free dry weight (AFDW) relationships of Eklöf et al. (2017). All snails were fixed in liquid $N_2$ after respiration and kept for further enzymatic analyses. Control experiments were performed in an identical fashion to respiration experiments for ~4 h using empty snail shells. Controls showed no significant bacterial respiration over the time in which oxygen consumption experiments were conducted. Linear regressions of oxygen concentrations against time were performed using Graphical Analysis Vernier Software (Sarasota, FL, USA). The rate of oxygen consumption was estimated from the 50–75% oxygen concentration interval and used in statistical comparisons. In order to examine the effect of oxygen concentration on metabolism, additional oxygen consumption rates were estimated at 75, 45, 25, and 5% of saturation for snails measured at 0 psu.

**Enzymatic activity**

The following enzymes were screened to select appropriate indicators of aerobic and anaerobic metabolic potential: malate dehydrogenase (MDH, E.C. 1.1.1.37), lactate dehydrogenase (LDH, E.C. 1.1.1.27), octopine dehydrogenase (E.C. 1.5.1.11), alanopine dehydrogenase (ADH, E.C. 1.5.1.17), tauropine dehydrogenase (E.C. 1.4.99.2) and strombine dehydrogenase (E.C. 1.5.1.22). Malate dehydrogenase, an important metabolic enzyme that provides oxalacetate to citrate synthase for the first step of the citric acid cycle, was selected as an indicator of aerobic metabolic potential. Lactate dehydrogenase, the terminal enzyme in glycolysis that contributes to both aerobic and anaerobic metabolic pathways, was selected as an indicator of glycolytic potential. Molluscs can use several different –opine dehydrogenases for anaerobic respiration, and in our survey of enzymatic activities, alanopine dehydrogenase displayed activities an order of magnitude higher than the others, and ADH was chosen for analyses. Enzymatic activities of MDH, ADH, and LDH were measured on freshly collected snails in spring (0 psu-0 acclimation time), and after 10 days in the lab at two different temperatures (10 and 20 °C) at 30 psu, since these are the most extreme conditions in which we expected to see differences.

Whole animals were weighed on a Mettler analytical balance while still frozen and homogenized using Duall hand held glass homogenizers kept on ice. Specimens were diluted at 1:99 parts weight/volume with 0.01 M tris homogenization buffer, pH 7.5 at 10 °C. Aliquots of homogenate were transferred to microfuge tubes and centrifuged at 6600 g for 10 minutes at 5 °C. All assays were performed within 1 h of homogenization using a Hewlitt-Packard diode array spectrophotometer equipped with a water-jacketed cuvette holder. Measurements of enzyme activity were made in 2-ml quartz cuvettes at 20 °C under non-limiting conditions in order to estimate maximum metabolic potential and followed procedures essentially as those described previously (Childress and Somero 1979; Seibel et al. 2000). Enzyme activities are expressed as units (μmoles of substrate converted to product per minute) per gram ash-free dry weight of animal (AFDW).

MDH activity measurements were carried out in a cocktail solution containing 50 mM Imidizole/HCl buffer (pH 7.0 at 20 °C), 20 mM $MgCl_2$, 0.4 mM oxaloacetate, and 150 μM NADH. LDH activity measurements were performed in a cocktail solution containing 80 mM tris/HCl buffer (pH 7.2 at 20 °C), 2 mM sodium pyruvate, 150μM NADH, and 100 mM KCl. LDH assay reactions were started by addition of the sample supernatant, and the decrease in absorbance at 340 nm due to NADH oxidation was recorded. For ADH, LDH activity after the addition of homogenate supernatant was recorded as background activity, and this background rate was then subtracted from the overall rate after the assay reaction was initiated by addition of alanine (2 mM) to arrive at the ADH activity of the sample.

**Statistics**

The effects of salinity and temperature (independent variables) treatments on mortality (dependent variable) were analyzed in two different two-way ANOVA, one for the

winter and another for the spring experiments. The effects of the same two independent variables on activity (dependent variable) were assessed again using two-way ANOVA (one for winter and another for spring). The average number of active snails in each of the five chambers per salinity treatment across the full experimental time was used as a variable, rather that the accumulative mortality. Relationships between metabolism (SMR, response variable) and two categorical independent variables were performed using two General Linear Models with Analysis of Covariance (GLM-ANCOVA) and Tukey HSD post hoc test, controlling for the effects of AFDW as covariate. While one GLM-ANCOVA used salinity and temperature as categorical independent variables, the second GLM-ANCOVA used acclimation time and temperature. Differences between oxygen consumption rates of *P. antipodarum*, at different oxygen levels (75, 45, 25, and 5%) at 10 and 20 °C were assessed using a two-way ANOVA and Tukey post hoc comparisons. All analyses were performed in Statistica 7.0. Data were checked for normality (Shapiro-Wilk test) and homoscedasticity (Cochran's test and Levene's test).

## Results

### Tolerance to osmotic and thermal shock

Snail mortality was significantly higher at higher salinities, but it was also affected by water temperature, showing significant interaction between these two variables in both seasons (Table 1). In winter, experiments only lasted 7 days, because thermal shock at higher salinity resulted in 100% mortality after several days (Fig. 1). In spring, experiments were carried out for 105 days as snails were much more resistant to higher temperature and salinities (Fig. 1). In general, salinity had a strong and significant effect on mortality in both seasons and temperature conditions (two-way ANOVA, $F = 33.17$, $p < 0.001$ and $F = 63.69$ and $p < 0.001$ for the winter and spring, respectively; Fig. 2, Table 1). The strongest effect was found in snails collected during the winter and exposed to 20 psu or more, reaching between 60 and 100% of mortality in only 3 days (Tukey HSD test, $p < 0.001$; Fig. 2 upper panel; Suppl. material 1: Table S1). Conversely, at higher salinities during spring, significantly higher mortalities were observed only at 25 and 30 psu as compared to lower salinities (Tukey HSD test, $p < 0.01$; Fig. 2 lower panel; Suppl. material 1: Table S1). Mortality reached 100% after 40 days in the highest salinity concentration. In general, there was not a strong effect on mortality when snails were kept at low salinities, 0 or 10 psu.

Thermal shock had a lower impact on mortality (~55% at 0 psu after 7 days at 20 °C in winter) compared to the effect of higher salinity (100% at 30 psu after 5 days at 5 °C in winter). A similar trend was seen in the spring (Fig. 2). In general, snail mortality due to higher salinity was slightly higher when a thermal shock treatment (20 °C) was applied (Fig. 2), but this effect was only significantly higher than acclimation temperature on two occasions (0 and 20 psu for winter and spring, respectively; Tukey HSD test, $p < 0.01$; Suppl. material 1: Table S1). No other significant differences in mortality between temperature treatments at a determined salinity concentration were observed (Table 1).

**Table 1.** Results of two-way ANOVA test assessing effects of salinity (0, 10, 20, 25, and 30 psu) and temperature (5, 10, and 20 °C) on mortality and mean activity of *Potamopyrgus antipodarum* during winter and spring. DF = degrees of freedom.

| **Mortality** | | | | |
|---|---|---|---|---|
| **Winter** | **DF** | **MS** | ***F*** | ***P*** |
| Salinity | 4 | 15100.0 | 38.92 | <0.001 |
| Temperature | 1 | 3200.0 | 8.25 | <0.007 |
| Interaction | 4 | 1420.0 | 3.66 | <0.05 |
| Residuals | 39 | 388.0 | – | – |
| **Spring** | **DF** | **MS** | ***F*** | ***P*** |
| Salinity | 4 | 12890.6 | 75.31 | <0.001 |
| Temperature | 1 | 12409.9 | 72.51 | <0.001 |
| Interaction | 4 | 3048.1 | 17.81 | <0.001 |
| Residuals | 39 | 171.2 | – | – |
| **Activity** | | | | |
| **Winter** | **DF** | **MS** | ***F*** | ***P*** |
| Salinity | 4 | 4187.8 | 16.92 | <0.001 |
| Temperature | 1 | 70.7 | 0.29 | 0.595925 |
| Interaction | 4 | 438.1 | 1.77 | 0.153958 |
| Residuals | 40 | 247.5 | – | – |
| **Spring** | **DF** | **MS** | ***F*** | ***P*** |
| Salinity | 4 | 2227.4 | 83.63 | <0.001 |
| Temperature | 1 | 628.4 | 23.59 | <0.001 |
| Interaction | 4 | 12.5 | 0.47 | 0.758753 |
| Residuals | 40 | 26.6 | – | – |

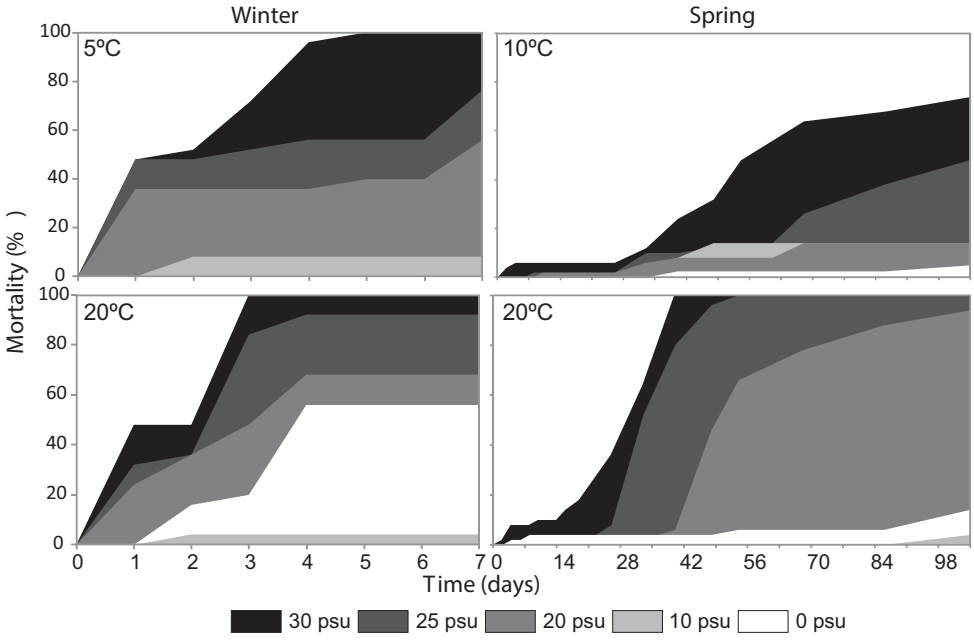


**Figure 1.** Mortality of the New Zealand mudsnail, *Potamopyrgus antipodarum*, during two exposure experiments (winter and spring season) at different salinities (0, 10, 20, 25, and 30 psu) and temperatures (lake temperature and 20 °C). $n$ = 5 for winter experiments and $n$ = 10 for spring experiments.

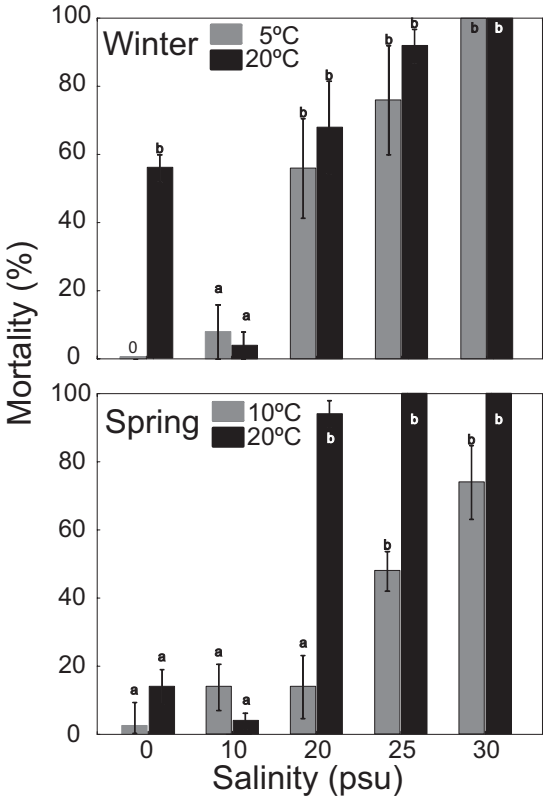


**Figure 2.** Mortality of the New Zealand mudsnail, *Potamopyrgus antipodarum*, collected during the winter and spring at five salinities. Mortality values (mean percentage ± SE) are given at the end of 5 and 50 days for the winter and spring experiments, respectively. Grey and black bars show water temperature (5 and 10 °C) of Capitol Lake at the time of capture and thermal shock treatments (20 °C), respectively. Different letters indicate significant difference in mortality between salinity treatments within each experimental temperature ($p < 0.05$ ANOVA, Tukey post hoc comparisons).

## Snail activity

The mean activity significantly decreased at higher salinities in both seasons, but thermal shock significantly reduced activity only during spring (Table 1). In both experiments, snails remained mostly open and active at salinities of 0 and 10 psu in all temperature treatments, and the percentage of active snails only occasionally fell below 80% at low salinities (Fig. 3). At 20 psu, snails remained active at the start of all the experiments and treatments, however the percentage of active snails decreased to values around 50 or even 0% after 4 days of exposure in winter, or after 21 days in spring at 20 °C (Fig. 3). Snails that survived longer than this remained active to the end of the experiment. In the 10 °C experiments at 25 and 30 psu, most snails died, but the remaining snails also remained active.

The New Zealand mudsnail is parthenogenetic and ovoviviparous, and snails reproduced during some experiments. Neonate snails were mostly observed in 0 and 10 psu experiments, but occasionally at 20 psu. Neonates were only present during the spring experiment after day 40 and 54 in the experimental chambers at 20 and 10 °C, respectively. Neonates were not quantified, and no neonates were observed in the much shorter experiments in winter.

## Oxygen consumption

Oxygen consumption rates of 231 snails were measured individually in respiration chambers in the spring (Figure 4; Suppl. material 1: Table S2). The mean length (± SD) of specimens used for respiration measurements was 3.93 ± 0.36 mm, and the calculated mean ash free dry weight (± SD) was 0.61 ± 0.11 mg. At least 3 specimens were measured at each combination of temperature (10 and 20 °C), salinity (0, 10, 20, 25, and 30 psu), and acclimation time (0, 2, 4, 6, 8, and 10 days). Standard metabolic rate of *P. antipodarum* was significantly affected by temperature, salinity, and acclimation time (Figs 4, 5). For comparisons, oxygen consumption rates measured between 50–75% air saturation were use. The oxygen consumption rates of *P. antipodarum* at 0 psu were significantly higher at higher temperatures with 35.9 ± 9.4 μmol $O_2\, g_{AFDW}^{-1}\, h^{-1}$ at 10 °C and 67.7 ± 15.2 μmol $O_2 g_{AFDW}^{-1} h^{-1}$at 20 °C, respectively (Tukey post hoc, $p < 0.001$; Fig. 4, Table 2; Suppl. material 1: Table S3). The resulting effect of temperature is equivalent to a $Q_{10}$ of 1.9.

Osmotic shock from 0 to 10 psu (no acclimation period) produced a decrease in the average SMR to 23.9 ± 2.1 and 55.2 ± 11.9 μmol $O_2\, g_{AFDW}^{-1}\, h^{-1}$ for 10 and 20 °C, respectively ($Q_{10}$ = 2.3), showing significant differences between these temperature conditions (Tukey post hoc, $p < 0.01$; Fig. 4, Table 2; Suppl. material 1: Table S3), but not between salinities. SMR decreased significantly when snails were exposed to higher salinities of 20 psu or more at the same temperature (Fig. 4, GLM-ANCOVA, Tukey-HSD post hoc test $p < 0.01$), reaching the lowest respiration rates at 30 psu and 10 °C (1.5 ± 1.8 μmol $O_2\, g_{AFDW}^{-1}\, h^{-1}$).

After 2 days of acclimation at 20 °C, the overall average SMR of snails showed a significant increase (Fig. 5). Conversely, at 10 °C SMR remained low without significant differences across acclimation days. At 30 psu, oxygen consumption was always lower than oxygen consumption at lower salinities regardless of acclimation time at 20 °C (Fig. 5; lower panel). This effect of high salinity on respiration rate was not as clear at 10 °C (Fig. 5).

## Standard metabolic rate at low oxygen concentration

At 10 °C, SMR was not significantly affected by oxygen concentration above 25% saturation (Fig. 6; Suppl. material 1: Table S5). At 20 °C, SMR was significantly higher at 75% saturation compared with intermediate oxygen levels (Fig. 6; Suppl. material 1: Table S5). A significant drop in SMR was observed when oxygen levels reached ~5% of saturation (two-way ANOVA, Tukey post hoc test, $p < 0.05$; Fig. 6).

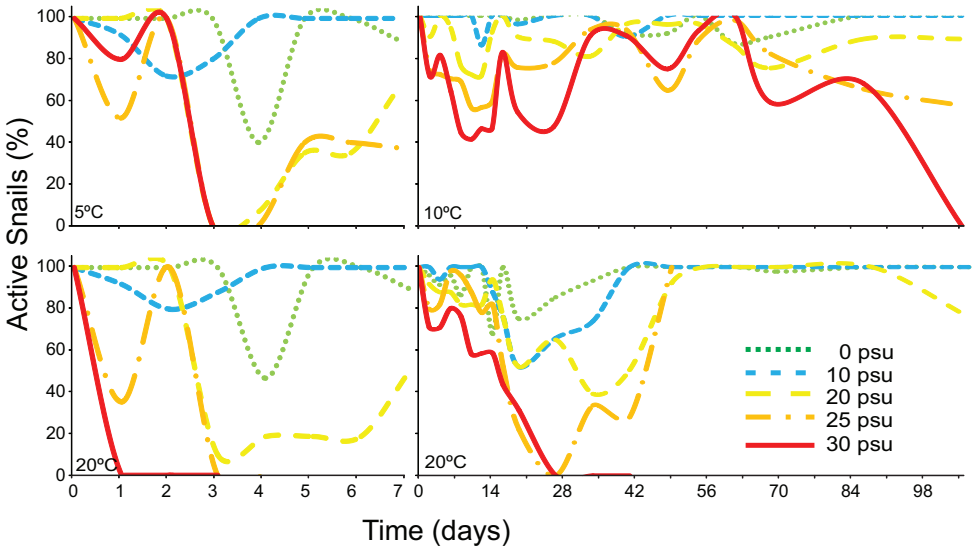


**Figure 3.** Activity of the New Zealand mudsnail, *Potamopyrgus antipodarum*, under different salinity conditions. Active snails were snails with an open operculum. Experiments were carried out during the winter (left panels) and spring (right panels). Values are based on the number of snails remaining after mortality.

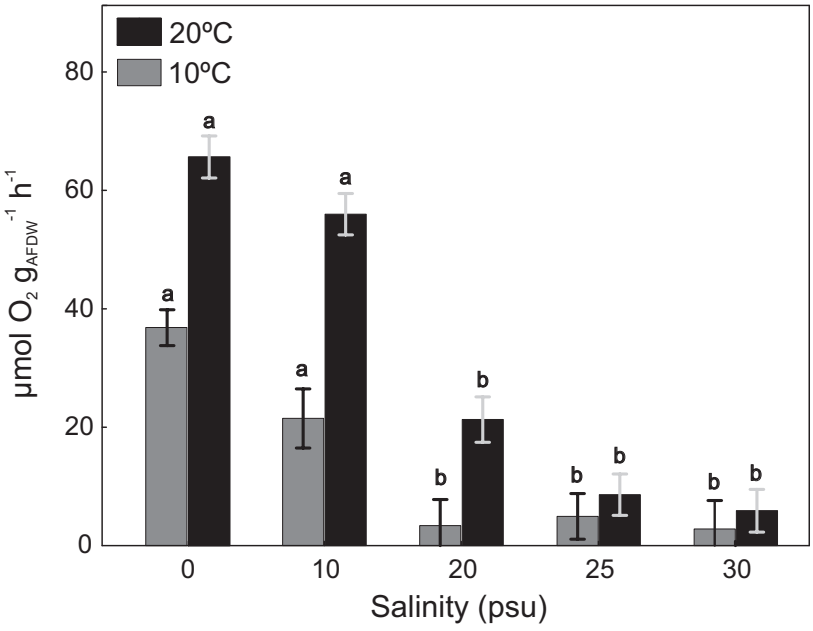


**Figure 4.** Oxygen consumption rates of *Potamopyrgus antipodarum* at two temperatures and five salinities. Different letters indicate significant difference ($p < 0.05$ GLM-ANCOVA, Tukey post hoc comparisons) between standard metabolic rate (mean ± SE) between salinity treatments within the same temperature conditions. Rates were measured between 50–75% air saturation.

**Table 2.** Results of GLM-ANCOVA analysis assessing effects of salinity (0, 10, 20, 25, and 30 psu), temperature (5, 10, and 20 °C), and acclimation time (0, 2, 4, 6, 8, and 10 days) on the standard metabolic rate of *Potamopyrgus antipodarum*. DF = degrees of freedom. AFDW = ash free dry weight.

| | DF | MS | *F* | *P* |
|---|---|---|---|---|
| AFDW | 1 | 637.8 | 8.76 | 0.0051 |
| Temperature | 1 | 3477.8 | 47.74 | <0.001 |
| Salinity | 4 | 4774.8 | 65.54 | <0.001 |
| Temp*Salinity | 4 | 470.9 | 6.46 | 0.0004 |
| Residuals | 41 | 72.8 | | |
| | **DF** | **MS** | ***F*** | ***P*** |
| AFDW | 1 | 15167.3 | 33.01 | <0.001 |
| Temperature | 1 | 75814.3 | 165.02 | <0.001 |
| Acclimation time | 5 | 3831.8 | 8.34 | <0.001 |
| Temp*Acclimation | 5 | 2175.1 | 4.73 | 0.0004 |
| Residuals | 220 | 459.4 | | |

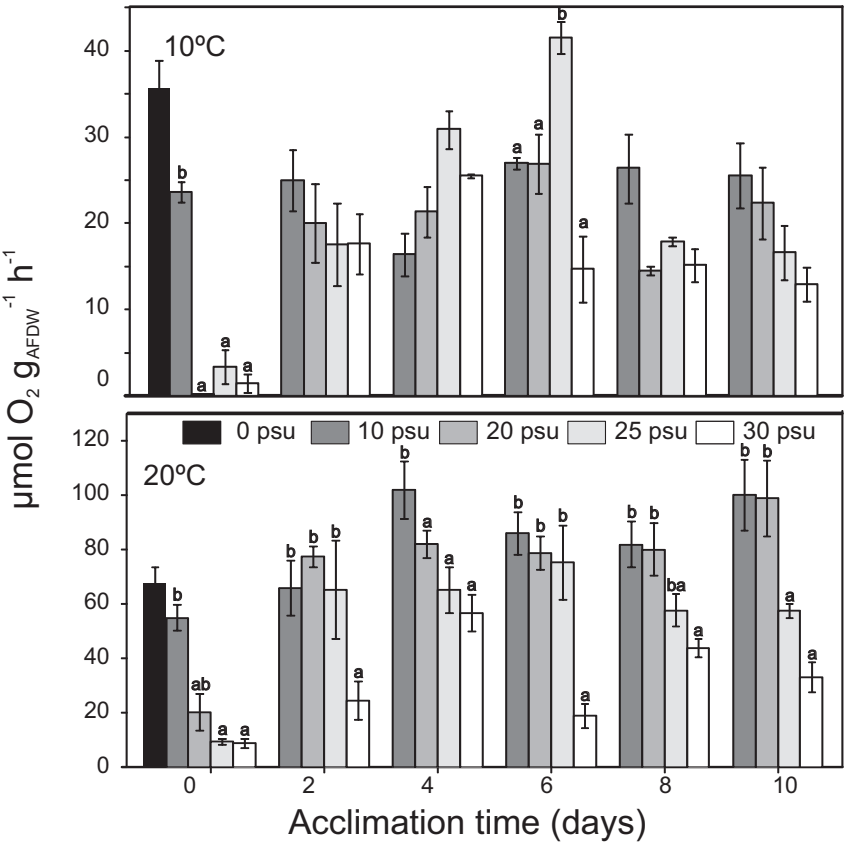


**Figure 5.** Average oxygen consumption rates for *Potamopyrgus antipodarum* at different acclimation times (0–10 days) when the snail was exposed at different water temperatures (10 and 20 °C) and at different salinities. Different letters indicate significantly different oxygen consumption rate ($p < 0.05$ GLM-ANCOVA, Tukey post hoc comparisons) between salinity treatments within each acclimation time. Snails were collected during spring. Rates were measured between 50–75% air saturation.

**Table 3.** Enzymatic activities of the New Zealand mudsnail, *Potamopyrgus antipodarum*, under three experimental conditions: Temperature, 10 °C, salinity 0 and 30 psu, and acclimation time 0 and 10 days. AFDW: ash free dry weight. n.d.: no data.

| | **Enzymatic Activity (units $g_{AFDW}^{-1}$, mean ± SE, *n*)** | | |
|---|---|---|---|
| | **Treatment Conditions (T, S, Incubation period)** | | |
| Enzyme | 10 °C, 0 psu, 0 days | 10 °C, 30psu, 10 days | 20 °C, 30 psu, 10 days |
| Malate dehydrogenase | 19.143 ± 1.675, 9 | 14.298 ± 0.711, 3 | 10.023 ± 1.303, 6 |
| Lactate dehydrogenase | 1.140 ± 0.496, 9 | 0.410 ± 0.043, 3 | 0.312 ± 0.032, 6 |
| Alanopine dehydrogenase | 3.653 ± 0.241, 9 | 2.808 ± 0.387, 3 | 2.572 ± 0.244, 6 |
| Octopine dehydrogenase | 0.320 ± 0.128, 5 | n.d. | n.d. |
| Tauropine dehydrogenase | 0.710 ± 0.206, 6 | n.d. | n.d. |
| Strombine dehydrogenase | 0.953 ±0.169, 7 | n.d. | n.d. |

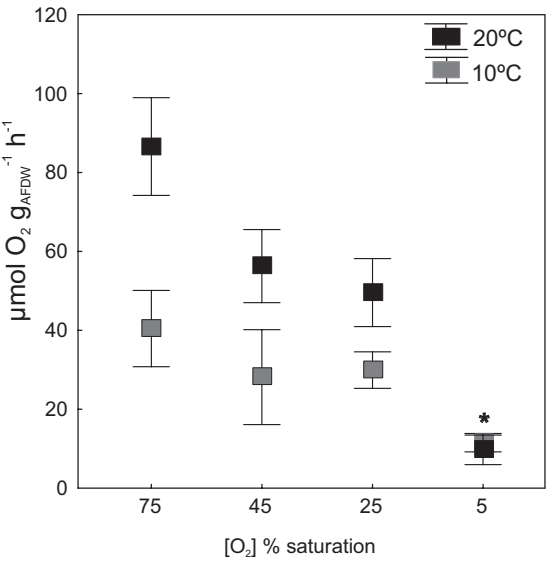


**Figure 6.** Average oxygen consumption rates of the New Zealand mudsnail, *Potamopyrgus antipodarum*, at different oxygen levels when exposed to 0 psu salinity conditions at 10 and 20 °C. Values are mean ± SE. Rates at 5% saturation are significantly lower than the other rates at the same temperature (*, $p < 0.05$, ANOVA, Tukey post hoc comparisons).

## Enzymatic activities

Activities of MDH, LDH, ADH and other –opine dehydrogenases are given in Table 3. Alanopine dehydrogenase was found to be the main anaerobic enzyme in *P. antipodarum*, and ADH activities are ~10× higher than those of LDH. Activities of all enzymes were significantly lower after 10 days at higher salinities (Fig. 7, Table 3). Lactate dehydrogenase showed the largest average difference (73% lower); MDH activity was on average 39% lower, and ADH activity had the smallest average difference (–26%).

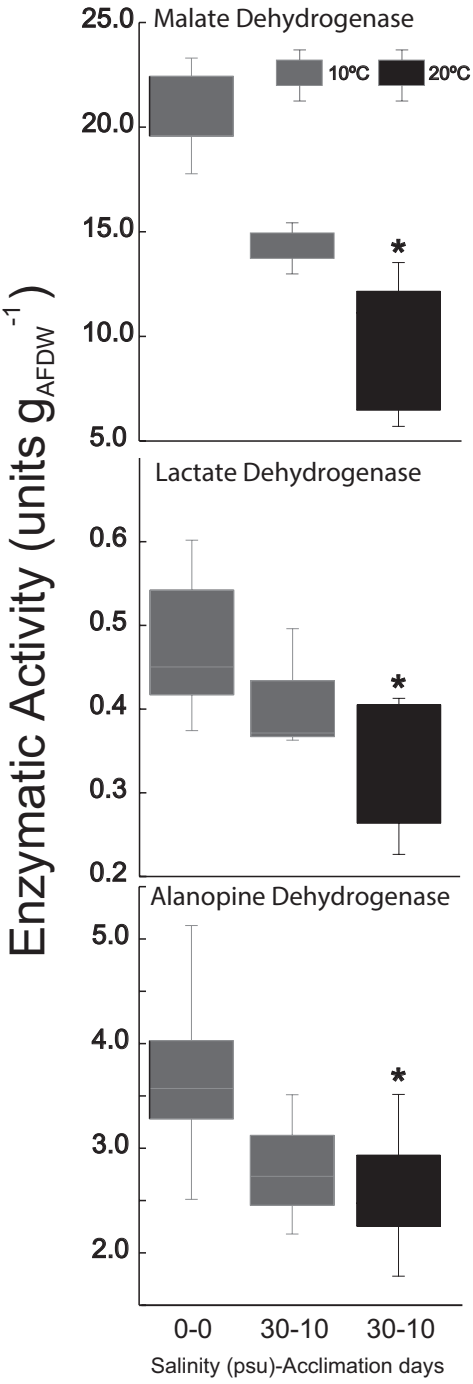


**Figure 7.** Enzymatic activities of the New Zealand mudsnail, *Potamopyrgus antipodarum*, in freshly collected specimens and under two experimental conditions. Boxes represent 50% of enzyme activities, and whiskers are the total range of data. Enzymatic activities in specimens incubated for 10 days at 20 °C and 30 psu are significantly different from those at 0 psu for all three enzymes (*, $p < 0.05$, ANOVA, Tukey post hoc comparisons). All activities were measured at 20 °C.

## Discussion

### Mortality

The increase in the mortality rate of *P. antipodarum* with an increase in salinity observed during this study agrees with results obtained in previous studies (Duncan and Klekowski 1967; Costil et al. 2001; Leclair and Cheng 2011; Hoy et al. 2012). The time of exposure and the high salinity needed to reach mortality rates close to 100% render high temperature and high salinity control methods difficult to apply successfully at an environmental scale such as Capitol Lake, Washington. The mortality results demonstrate that controlling this species by osmotic shock will probably fail due to its high tolerance, behavior and plasticity to become acclimated to high salinity concentrations in agreement with previous studies (Duncan and Klekowski 1967; Hoy et al. 2012). In the Columbia River estuary, populations of *P. antipodarum* displayed high salinity tolerance with 80% survival after three weeks at 34 psu (Hoy et al. 2012).

The previous attempt to control *P. antipodarum* in Capitol Lake through backflushing the lake with seawater (Leclair and Cheng 2011) probably failed due to the short period of time (4 days), low salinity (7.5–24.9 psu) and moderate water temperature ~9 °C. Our results demonstrated that a salinity of 30 psu needs to be applied for at least 7–40 days, depending mostly on season and temperature, to reach mortalities close to 100%. A higher tolerance to salinity in our population sample, as compared to that predicted by Leclair and Cheng (2011), may be partially explained by a possible selection process of the surviving snails after backflushing the lake with seawater in 2010. Increased salinity tolerances were also reported for this species in its invasive range as compared to native range populations (Drown et al. 2011). Our mortality results (Figs 1, 2) support the hypothesis of Drown et al. (2011) that the 'performance optimum' of invasive lineages of *P. antipodarum* is shifted to higher salinities.

Seasonal acclimation temperature affected both the salinity concentration at which the highest mortality was observed as well as the speed of mortality. These experimental results were consistent with those reported in previous studies (Hylleberg and Siegismund 1987; Cheng and LeClair 2011; Leclair and Cheng 2011; Moffitt and James 2012). Our results demonstrate that mortality at different salinities can be greatly affected by the season. At temperatures approaching freezing, populations of *P. antipodarum* may be highly stressed (Moffitt and James 2012), and winter may be the best season to carry out control measures. Although logistically intriguing, backflushing the lake with high temperature (20 °C) seawater (>30 psu) during three days in winter could potentially be used as an eradication strategy. Clearly, these snails prefer slightly brackish water over freshwater, and using low salinity seawater in eradication methods will not be effective.

### Activity

Snails were significantly most active at lower salinities, and the effect of temperature reducing activity was clear only during the spring. The capacity to avoid stressful con-

ditions by retracting into shells and closing the operculum as observed during our experiment can provide temporary tolerance to high salinity, but this is only a short-term solution to osmotic stress. This species can survive more-or-less normally at 20 psu or less. Although Duncan and Klekowski (1967) found that *P. antipodarum* (as *P. jenkinsi*) could not reproduce at over 12–18 psu, we observed the release of living neonates even at 20 psu. Although we did not quantify number of neonates, these were first observed at 20 °C after 40 days at 0 and 10 psu. Two weeks later the first snails at 10 °C were also observed at these same temperatures. In our study, neonates displayed active locomotor activity even at 20 psu. The presence of neonates during experiments has also been reported by other investigators as a response to stressful conditions (Bruce et al. 2009; Moffitt and James 2012; Romero-Blanco and Alonso 2019). Similar to survival rate, differences in reproduction probability have been observed between populations of this species (Drown et al. 2011), with higher reproduction in invasive or clonal populations compared to native sexually reproducing populations.

**Standard metabolic rate**

The oxygen consumption rates we measured for *P. antipodarum* are similar to those measured by Duncan and Klekowski (1967) and Hudson (1975). Our results showed that *P. antipodarum* can acclimate to higher salinities and that this acclimation is affected by temperature. Following an initial shock response to higher salinities where aerobic metabolism was suppressed, SMR become depressed at 25 and 30 psu after 8–10 days. This decline in metabolism is more noticeable at 20 °C than at 10 °C. Although, we did not measure SMR at different acclimation times at 0 psu, it is reasonable to suppose that these values will remain stable across time as was observed at 10 and 20 psu for both temperatures. Additionally, our results demonstrated a significant decrease in oxygen consumption rates of *P. antipodarum* at lower oxygen concentrations. Below 70–50% oxygen saturation, *P. antipodarum* becomes an oxy-conformer. This is similar to the oxyconformation response observed for *P. antipodarum* in its native range (Hudson 1975) and for other snail species (McMahon and Russell-Hunter 1978).

**Enzymatic activities**

It is well known that many molluscs can survive under prolonged periods of hypoxia or even anoxia by exploiting various anaerobic pathways (Grieshaber et al. 1994). Malate dehydrogenase, lactate dehydrogenase, and alanopine dehydrogenase activities in *P. antipodarum* showed significant declines after 10 days at 30 psu. These declines in both anaerobic and aerobic metabolic potentials track the overall decline in whole animal metabolism as measured by oxygen consumption.

Similar to some other gastropods (Baldwin and England 1982; Grieshaber et al. 1994), alanopine dehydrogenase was found to be the main anaerobic enzyme in

*P. antipodarum*. The –opine dehydrogenases have generally low substrate specificity and can utilize a variety of substrates (Gäde and Grieshaber 1986). The lower activities of octopine dehydrogenase, strombine dehydrogenase and tauropine dehydrogenase could all be due to ADH using those different substrates. Genetic identifications of those other enzymes are needed to confirm their presence in the New Zealand mudsnail. Regardless, MDH, LDH, and ADH were all successfully used herein as indicators of environmental stress (cf. Dahlhoff 2004) for *P. antipodarum*.

### Impacts

The future of Capitol Lake remains uncertain. If the dam is permanently breached and the lake restored to estuarine conditions, it is unlikely that wintertime temperatures and salinities would often exceed the thermal and osmotic tolerances of this population of *P. antipodarum*. However, it seems possible that summertime temperatures at low tide and summertime salinities at high tide could pass the upper thermal and osmotic tolerances of this population. This could result in the eradication of the New Zealand mudsnail from this ecosystem. However, if this species makes its way upstream into areas of permanent freshwater, it will likely continue to successfully seed the estuary for many years to come.

Additionally, it seems possible that tolerance to high salinity conditions, probably based on phenotypic plasticity (Drown et al. 2011), will allow this species, not only to survive if Capitol Lake is restored to an estuarine ecosystem, but also to spread to other coastal environments of mid-range salinities. Potentially, its high tolerance to environmental stress due to its physiological plasticity and anaerobic potential may give this species a seemingly greater competitive capacity over other aquatic species as has been previously suggested (Sardiña et al. 2015; Rakauskas et al. 2018). However, this advantage may change under future contexts of increasing temperatures (Sardiña et al. 2015).

## Data resources

The data underpinning the analysis reported in this paper are deposited in the Zenodo Data Repository (https://doi.org/10.5281/zenodo.3567136).

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## Supplementary material 1

**Tables S1–S5**

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Data type: measurements.

Explanation note: **Table S1.** Complementary statistic result (Tukey post hoc comparisons) for figure 2, which is showing results of two-way ANOVA test assessing effects of salinity (0, 10, 20, 25, and 30 psu) and temperature (5, 10, and 20°C) on mortality of *Potamopyrgus antipodarum* during winter and spring. DF = degrees of freedom. **Table S2.** Standard Metabolic Rate of the New Zealand mudsnail, *Potamopyrgus antipodarum*, in freshly collected snails and 2–10 days of acclimation at five salinity levels (0, 10, 20, 25, and 30 psu) and two temperature conditions (10 and 20°C). **Table S3.** Statistic results (GLM-ANCOVA, Tukey post hoc comparisons) for the oxygen consumption rates of *Potamopyrgus antipodarum* at two temperatures and five salinities (Figure 4). Rates were measured between 50–75% air saturation. **Table S4.** Lower panels. Statistical results for the average oxygen consumption rates for *Potamopyrgus antipodarum* at different acclimation times (0–10 days) when the snail was exposed to higher salinity conditions at different salinities (Fig. 5 lower panels). Contrast show significantly different oxygen consumption rate ($p < 0.05$ GLM-ANCOVA, Tukey post hoc comparisons) across the same temperature (upper panel) or between salinity treatments within each acclimation time (lower panels). Snails were collected during spring. Rates were measured between 50–75% air saturation. **Table S5.** Statistical results (ANOVA, Tukey post hoc comparisons) for the Figure 6. Average oxygen consumption rates of the New Zealand mudsnail, *Potamopyrgus antipodarum*, at different oxygen levels when exposed to 0 psu salinity conditions at 10 and 20 °C. Values are mean ± SE. Rates at 5% saturation are significantly lower than the other rates at the same temperature (*, $p < 0.05$, ANOVA, Tukey post hoc comparisons).



Link: https://doi.org/10.3897/neobiota.54.39465.suppl1