Differential survival and feeding rates of three commonly traded gastropods across salinities

Elisabeth Renk1,*, James W. E. Dickey1,2,3,4*, Ross N. Cuthbert5, Elžbieta Kazanavičiūtė1,6, Elizabeta Briski1

1 GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel, Wischhofstraße 1-3, 24148 Kiel, Germany
2 Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 310, 12587 Berlin, Germany
3 Freie Universität Berlin, Institute of Biology, Königin-Luise-Str. 1-3, 14195 Berlin, Germany
4 Berlin-Brandenburg Institute of Advanced Biodiversity Research, Königin-Luise-Str. 2-4, 14195 Berlin, Germany
5 Institute for Global Food Security, School of Biological Sciences, Queen’s University Belfast, Belfast, Northern Ireland, UK
6 Lancaster University, Lancaster, LA1 4YW, UK

Corresponding author: James W. E. Dickey (jamesdickey03@gmail.com)

Abstract

Increasing rates of biological invasions pose major ecological and economic threats globally. The pet trade is one major invasion pathway, and environmental change could mediate the successful establishment and impact of these released or escaped non-native species (NNS). Salinity regime shifts are a pervasive but often overlooked environmental change in aquatic ecosystems. This study investigates the establishment and impact risks posed by three readily available, traded snail species – Melanoides tuberculata, Tarebia granifera and Anentome helena – by assessing their survival and feeding responses across a spectrum of salinity levels (0.2–16 g/kg). Survival differed among the species, with M. tuberculata showing close to 100% survival across the salinity range, T. granifera exhibiting heightened mortality at 16 g/kg, and A. helena displaying no survival at salinities above 12 g/kg. In feeding experiments assessing the more resilient M. tuberculata and T. granifera, the former had greater consumption rates towards both plant- (spinach) and animal-based (daphniid) resources. While salinity and density effects did not affect animal consumption, they both had significant effects on plant consumption, with feeding suppressed for both consumers under a salinity of 8 g/kg relative to freshwater conditions. When combining proportional survival and resource consumption for M. tuberculata and T. granifera, the former had greater impact potential towards both plant and animal resources, highlighting its potential to exert higher ecological impacts. Studies have overlooked the importance of salinity for invasion success and the impact of pet trade species. We therefore propose that these methods provide a screening tool to assess the potential risks of traded species establishing and exerting impacts, and we encourage future studies to account for a broader range of abiotic stressors.

Key words: Anentome helena, feeding rates, Melanoides tuberculata, Relative Impact Potential, risk assessment, salinity, survival, Tarebia granifera
Introduction

Non-native species (NNS) are a major global threat to ecosystems and biodiversity, often causing substantial economic costs (Molnar et al. 2008; Cuthbert et al. 2021a; Diagne et al. 2021; IPBES 2023). An increasingly globalised world, with novel trade and transport routes, has facilitated the spread of NNS via means such as ship ballast water (Smith et al. 1999; Drake and Lodge 2004; Briski et al. 2012), the construction of new corridors like the Suez or Panama Canals (Balzani et al. 2022), and the horticultural and pet trades (Chucholl 2013; Lockwood et al. 2019; Dickey et al. 2023b). Indeed, the aquatic pet trade alone has enabled the establishment of freshwater and marine species in new habitats, with well-known examples including goldfish (*Carassius auratus* Linnaeus, 1758), pond slider terrapins (*Trachemys scripta* Thunberg, 1792) and various crayfish species, which have exerted negative impacts on recipient ecosystems (Vodovsky et al. 2017; Dickey et al. 2018; Britton 2022). In some cases, the species are deliberately released into the wild (Shiu and Stokes 2008; Maceda-Veiga et al. 2019), while in others escapes occur (Patoka et al. 2017). Further, incidental “hitch-hiker” species, like zebra mussels (*Dreissena polymorpha* Pallas, 1771), can be transported via the pet trade (Patoka and Patoková 2021; Dickey et al. 2023c).

Species must be able to withstand broad biotic and abiotic conditions during the invasion process, namely transport, introduction and establishment stages, to become invasive NNS (Blackburn et al. 2011). Indeed, successful invaders often show a heightened tolerance to abiotic stressors, as well as fast somatic growth and high fecundity (Côté and Smith 2018). One important constraining abiotic factor for aquatic invertebrates is salinity, which affects mortality, fecundity, growth and community composition (Zalizniak et al. 2009) and acts in combination with other environmental changes (Cuthbert et al. 2019). Indeed, this stressor poses a threat to biodiversity, and can be a result of agricultural land use, mining discharge, aquaculture effluent, fracking, road salt run-off, as well as rising sea levels (Cunillera-Montcusí et al. 2022; Barrios-Figueroa and Urbina 2023). Broad salinity tolerance facilitates establishment and may lead to greater spread and competitive advantages (Lockwood and Somero 2011). For example, the red lionfish (*Pterois volitans* Linnaeus, 1758), most likely introduced to the Atlantic Ocean through an aquarium spill (Whitfield et al. 2002; Hixon et al. 2016), is expanding its range into lower salinity estuaries of North America (Schofield et al. 2015). As well as helping to predict whether species can establish in freshwater, brackish and marine habitats, understanding the role of salinity can also offer insights into tolerance and performance under freshwater salinisation. Determining the ability of NNS to survive and exert impacts under such abiotic stressors is an important concern for conservationists, and is a crucial consideration for predicting and proactively preventing potentially damaging invasions.

Aquatic snails have frequently established and exerted negative impacts in novel environments after introductions via the pet trade (Preston et al. 2022). One example is the golden apple snail (*Pomacea canaliculata* Lamarck, 1822) in Asia. Native to South America, specimens were originally imported as aquarium pets, as well as food sources and for use in commercial aquaculture (Joshi 2007, de Brito and Joshi 2016). The snail spread rapidly and consequently generated vast economic losses to crops, while also posing a threat to human health as intermediate hosts of zoonotic nematode parasites (Xu et al. 2016; Djeddour et al. 2021). While such high
impact species can be regulated and thus become absent from the pet trade, other species with invasion histories remain readily available, such as *Melanoides tuberculata* (Müller, 1774), *Tarebia granifera* (Lamarck, 1822) and *Anentome helena* (von dem Busch, 1847) (Dickey et al. 2023b). Both the Afro-Asiatic freshwater snail *M. tuberculata* and the South-East Asian *T. granifera* have established non-native populations in tropical and subtropical areas worldwide, partly due to the pet trade (Vaz et al. 1986; Duggan 2010; Work and Mills 2013; Coelho et al. 2018), as well as through deliberate introductions as biocontrol agents (Pointier and Jourdane 2000). Both species have broad generalist diets and abiotic tolerances (Miranda et al. 2010; Weir and Salice 2012; Raw et al. 2016). Indeed, these species have been found in freshwater and estuarine conditions within their non-native ranges (Miranda et al. 2010; Farani et al. 2015). In contrast, the carnivorous “assassin snail” *A. helena*, with a native distribution including Cambodia, Indonesia, Thailand, Vietnam, Laos and Malaysia (Ng et al. 2016), has only a limited non-native range to date, despite its popularity in the pet trade for controlling outbreaks of pest species (Karmakar et al. 2022; Dickey et al. 2023a). Part of one of the few freshwater, stenohaline, potamodromous genera within the almost entirely marine Nassariidae family (Galindo et al. 2016), its only reported non-native occurrence to date has been from a freshwater reservoir in Singapore (Ng et al. 2016).

In recent years, many approaches have been developed to investigate the probability of invasion success and the magnitude of impact (Ruiz et al. 2000; Geller et al. 2010; Dickey et al. 2020; McCard et al. 2021). In this study, we tested the tolerance of three commonly traded snail species (*M. tuberculata*, *T. granifera* and *A. helena*) to salinity changes. We also determined their feeding ability and preferences under those abiotic conditions. First, we examined the survivability of the three study species over a range of salinities, from freshwater to brackish, to mimic pet trade release events (i.e. without any acclimation period). Second, we tested the effect of salinity on the *per capita* feeding rates of two of these species which showed the highest survival, *M. tuberculata* and *T. granifera*, using a method similar to functional response experiments (i.e. the rate of resource consumption by a consumer in relation to resource density), which are regularly used to predict the potential ecological impact a species might exert (Dick et al. 2014; Faria et al. 2023). Finally, we combined survival and feeding rates through the Relative Impact Potential metric to quantify and compare how salinity affects the potential ecological impacts of *M. tuberculata* and *T. granifera* (Dick et al. 2017; Dickey et al. 2020).

**Materials and methods**

**Species acquisition and husbandry**

Our three study species (Suppl. material 2; fig. S2) were ordered from an online vendor (i.e. garnelen-direkt.de): *M. tuberculata* was ordered on May 23rd 2022, *T. granifera* on July 26th 2022, and *A. helena* on October 1st 2022 (280 individuals ordered per species; mean shell lengths of 1.69cm, 1.42cm and 1.77cm, respectively). All three species arrived within 24 hours and were transported in moist, shockproof containers. After arrival, the snails were placed in a climate chamber at GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany, in a 56 L glass aquarium containing constantly oxygenated freshwater per the vendor’s recommendation. All individuals of each species were placed in a separate aquarium.
The temperature of the climate chamber was maintained at 18 °C (± 0.6 °C) with a 16:8h light and dark regime and the water temperature was kept at 19 °C (± 0.8 °C). The two detritivorous species (i.e. *M. tuberculata* and *T. granifera*) were fed *ad libitum* with “Veggi Wafers” (Pleco Tetra, USA), while the carnivorous *A. helena* were fed frozen *Mysis* sp. (Vivantis aqua, Germany).

**Survival experiments**

Animals were acclimated for at least two weeks before experimentation. The survival of snails was determined in eight different salinities: 0.2, 0.6, 1, 2, 5, 8, 12, and 16 g/kg. This range was chosen to represent a spectrum from freshwater - the recommended conditions for all three species in the pet trade - to brackish water representative of estuarine conditions or those of Kiel Fjord in the Baltic Sea. Baltic Sea water was diluted with freshwater or mixed with artificial salt (Aquarium Systems Instant Ocean, France) to reach the desired salinities, as needed. The experiments started by placing ten snails in each 2 L aerated aquarium under the experimental salinity conditions, without prior adaptation, with experiments replicated three times per species and salinity (Suppl. material 2: fig. S2). Salinity was measured and adjusted as needed twice per week, and snails were fed *ad libitum* daily; *M. tuberculata* and *T. granifera* with spinach, and *A. helena* with thawed *Mysis* sp.. Uneaten food was removed once per week to prevent degradation of water quality. Three criteria were applied to determine viability of snails, which can prove difficult due to their ability to retreat into their shells. First, snails were assessed visually for control of movement or attachment to the tank wall. Second, non-moving snails were lightly poked with tweezers, and if they reacted, were deemed living. Third, any snails that did not react to poking were put in freshwater for 60 minutes and if no signs of movement were observed within this time, they were considered dead and removed from the experiment. Live snails were placed back into the same experimental aquarium that they were taken from. The survival status was assessed daily, with the experiments lasting for 30 days. The snails were kept in their respective tanks for an additional 14 days (as per the methods of Casties et al. 2019; Paiva et al. 2020), under the same salinity and feeding regimes, after the survival experiment to determine potential delayed onset mortality.

**Proportional food consumption experiments**

Proportional consumption experiments, designed in the style of functional response trials, tested the feeding rates of the two detritivorous snails, *T. granifera* and *M. tuberculata*, over three salinities. The carnivorous *A. helena* was not tested in these experiments, as it demonstrated high mortalities in higher salinities and was thus deemed low risk under these conditions (see the results section below). One set of trials assessed consumption of a plant-based food resource and the other of an animal-based resource. The experiments were performed in open and aerated plastic bottles (550 ml) in the same climate chamber in which the survival experiments were conducted. All snails had been previously used for the survival experiment and therefore had acclimated to the salinities for at least two months. For *M. tuberculata*, 0.2, 8, and 16 g/kg were chosen as experimental salinities, while for
due to its high mortality at 16 g/kg, the salinities 0.2, 8, and 12 g/kg were chosen. Note that we were primarily interested in how the feeding rates of the two species compared at 0.2 and 8 g/kg, with the 12 and 16 g/kg conditions tested for intraspecific comparisons at their highest respective “survivable” salinity. Five different resource densities were offered to the tested individuals. Each experimental salinity and resource density was replicated five times, resulting in 75 trials for both species under both resource types.

The snails were fed *ad libitum* for at least two weeks and starved for four days before the experiments commenced to standardise hunger levels. The trials ran for five days (120 hours) for both species towards both resources. Spinach was provided as a plant-based food source, and offered in discs, prepared with a hole punch to ensure uniformity (average area 0.210 cm²) (Fig. 3). To ensure that the different experimental salinities did not affect the leaf disc area, controls were run in which spinach discs were kept in the salinities for five days without a consumer. The numbers of spinach discs offered to the gastropods were 0.5, 1, 2, 4, and 8. Spinach was always added to the containers first, followed by one snail each. To determine consumption, all spinach discs were photographed and the area consumed was determined by subtracting the remaining area (measured using ImageJ: Abràmoff et al. 2004) from the average unconsumed leaf disc area (see Suppl. material 2: fig. S3 for image of consumed and unconsumed leaf discs). For the animal resource, frozen *Daphnia* sp. (Vivantis Aqua, Germany) was chosen and offered at densities of 1, 4, 8, 16, and 24 per container. Except for the food source and density, the trial was done identically to the plant-based approach. After the trial, snails were removed and the remaining *Daphnia* sp. counted. The number of *Daphnia* sp. consumed was measured by subtracting the remaining numbers from the initially supplied densities.

**Statistical analysis and visualization**

Sigmoidal mortality curves were constructed for each species for each salinity treatment, described by the following equations (Briski et al. 2008, 2011; Paiva et al. 2018):

\[ y = \frac{100}{1 + e^{-Z(s-Q)}} \]  

(1)

where \( y \) is the proportional mortality, \( Z \) is the mortality rate and \( Q \) is the onset of mortality. In Eqn. 1, \( s \) represents salinity (used for Fig. 1) and in Eqn. 2, \( t \) represents time in days (used for Fig. 3). All curves were constructed using S-Plus 6.1 (S-Plus ® 6.1, 2002; Insightful Corp., Seattle, WA, USA). Raw data are available as Suppl. materials 1, 2.

All further statistical analyses were performed with R v4.0.3. A Cox proportional hazards model was fitted to analyse the survival data by determining the hazard ratio. This ratio is commonly used for survival analysis and compares mortality rates under different conditions (in this case, our salinity levels). Hazard ratios of one indicate no effect of the variable on mortality rate, with those less than one indicative of reduced mortality rate and those greater than one indicating increased mortality rate. The analysis was conducted using the ‘survival’ package (Therneau 2023).
To model the proportional consumption (species, salinity and resource density used as independent variables in the full model) at experimental salinities of 0.2 and 8 g/kg – i.e. the two common salinities for *M. tuberculata* and *T. granifera* – the package ‘glmmTMB’ (Brooks et al. 2017) for fitting generalized linear models (ordered beta family for data with zeros and ones) was applied, with non-significant terms removed step-wise. To determine the shapes of the feeding curves (i.e. whether a decelerating hyperbola Type II-esque curve, or a sigmoidal Type III-esque curve: see Dick et al. 2014), polynomial logistic regression was used by analysing proportional resource consumption across densities for each species, salinity, and resource type (Pritchard et al. 2017). While a positive first-order term indicates a Type III functional response, a negative first-order term characterises Type II functional response. Seven different self-starter models were then fitted using the ‘devtools’ package (Wickham et al. 2002), with asymptotic regression, negative exponential, power curve (Freundlich function; fixed so that 0 < b < 1), logarithmic and Michaelis-Menten used as Type II curves, and 3-parameter Gompertz and logistic sigmoidal used as Type III curves. Akaike Information Criterion (AIC) values were used to determine the best fit.

The potential ecological impact of a NNS under context-dependencies can be quantified using the Impact Potential (IP) metric (Dick et al. 2017; Dickey et al. 2020), typically calculated as the product of consumer maximum feeding rate (FR) - typically the asymptote of the functional response curve or the feeding rate at a fixed, abundant resource density (Dickey et al. 2020) - and some measure of numerical response (NR) to determine population impact.

\[ \text{IP} = \text{FR} \times \text{NR} \quad (3) \]

Here, for each experimental salinity, we used “proportional consumption at maximum resource density” (i.e. consumption per the given area of 8 spinach discs, or per 24 *Daphnia* sp.: FR) derived from our proportional food consumption experiments for FR, and “proportional survival” at the corresponding salinity, employing the results from the preceding survival experiments for NR.

**Results**

**Survival experiments**

The survival experiment indicated clear differences in salinity tolerance among the three species (Tables 1–3, Figs 1–3). Mortality rose rapidly in *A. helena* with increasing salinity, whereas less immediate and severe effects were found in *M. tuberculata* and *T. granifera* (Fig. 1). The multivariate Cox proportional hazards model showed a significant effect of salinity (p < 0.05; HR = 3.37) and species (p < 0.05) and on mortality rate, with *M. tuberculata* and *T. granifera* demonstrating reduced mortality rates relative to *A. helena* (HRs of 0.0047 and 0.0073 respectively, relative to *A. helena*) (Fig. 2).

The highest survival rate was observed for *M. tuberculata*, with four deceased snails out of 240 overall (Figs 1–3). These deaths occurred at various salinity levels of 0.6, 2, 8 and 12 g/kg. No deaths were recorded at the highest salinity (16 g/kg) within the experimental 30-day or the 14-day post-trial observation period (Table 1). *Tarebia granifera* experienced slightly higher mortality than
Elisabeth Renk et al.: Survival and feeding rates of pet trade gastropods

M. tuberculata, but only within the 16 g/kg treatment. There were seven deceased snails recorded at this experimental salinity, with additional deaths recorded during the 14-day post-trial observation period, resulting in twelve snails out of the initial 30 from the 16 g/kg treatment (Table 1). For A. helena, all snails in the 12 and 16 g/kg treatments died within the first 24 hours of the experiment, whereas three snails survived the 30-day trial period at 8 g/kg. However, all of these snails in the 8 g/kg treatment ultimately died within the 14-day post-experiment observation period. For this species, only the 0.2 g/kg treatment had 100% survival during the initial 30-day experiment, with three additional snails dying in freshwater during the 14-day post-experiment observation period (Table 1).

Figure 1. Survival curves in regard to salinity (g/kg) for the three species after (a) 15 and 30 days (b). *Melanoides tuberculata*, *Tarebia granifera* and *Anentome helena*, are displayed in yellow, red and navy respectively.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazard ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. helena (N=240)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>M. tuberculata (N=240)</td>
<td>0.0047 (0.0019 - 0.014)</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>T. granifera (N=240)</td>
<td>0.0073 (0.0029 - 0.014)</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>Salinity (N=720)</td>
<td>3.3737 (2.7655 - 4.129)</td>
<td>&lt;0.001 ***</td>
</tr>
</tbody>
</table>

Figure 2. Forest plot based on the Cox proportional hazards regression model, with species and salinity as covariates. Hazard ratios, the ratios of the mortality rates under our experimental salinities, are shown by black squares with 95% confidence intervals by solid horizontal lines (note also stated numerically in “Hazard ratio” column). Hazard ratios, HRs, greater than one (i.e. to the right of the dashed line) indicate that the covariate is associated with increased risks of mortality, with those less than one (i.e. to the left of the dashed line) associated with decreased risks of mortality. We see significant effects of species and salinity on survival, and using the freshwater *Anentome helena* as our reference, we see that *M. tuberculata* and *Tarebia granifera* have reduced risks of mortality relative to the reference species, with HRs of 0.0047, and 0.0073, or 99.53% and 99.27% less, respectively. The concordance index of 0.94 suggested good predictive accuracy of the model on survival outcomes. Survival is based on results at 30 days under experimental salinity conditions.
Proportional consumption experiments

In the control trials, the surface area of the plant-based food source was unaffected and therefore all consumption was solely attributed to snail consumption. For the two common salinities assessed, proportional consumption was significantly affected by salinity, species and resource density, however, no significant interactions were found (Suppl. material 2: table S1). Consumption was significantly higher for *M. tuberculata* than for *T. granifera* (z = 4.065, p < 0.001), with significantly reduced consumption at 8 versus 0.2 g/kg (z = 7.841, p < 0.001). A significant negative effect of density on proportional consumption was also found (z = 2.557, p = 0.01).

For *M. tuberculata*, those in the 8 g/kg treatment exhibited the lowest consumption, consuming in total 2.27 cm$^2$ out of the 16.31 cm$^2$ offered across each treatment (Suppl. materials 1, 2). This was followed by the 16 g/kg treatment, where snails consumed 7.90 cm$^2$, and the 0.2 g/kg treatment, where they consumed 9.66 cm$^2$. Negative first order terms were found at all three experimental salinities for *M. tuberculata*, with a negative exponential model having the best fit in the 0.2 g/kg treatment, and power curves having the best fit for the 8 and 16 g/kg treatments based on lowest AIC values (Fig. 4a, Suppl. materials 1, 2).

### Table 1. Mortality recorded for a) *Melanoides tuberculata*, b) *Tarebia granifera* and c) *Anentome helena* during the initial 30-day experimental period and then the 14-day observation period after survival trials across salinities.

<table>
<thead>
<tr>
<th>Species</th>
<th>Salinity</th>
<th>Day 30 Mean survival</th>
<th>Day 30 Standard deviation</th>
<th>Day 44 Mean survival</th>
<th>Day 44 Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>a) <em>M. tuberculata</em></td>
<td>0.6ppt</td>
<td>9.667</td>
<td>0.577</td>
<td>9.667</td>
<td>0.577</td>
</tr>
<tr>
<td></td>
<td>1ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2ppt</td>
<td>9.667</td>
<td>0.577</td>
<td>9.667</td>
<td>0.577</td>
</tr>
<tr>
<td></td>
<td>5ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8ppt</td>
<td>9.667</td>
<td>0.577</td>
<td>9.667</td>
<td>0.577</td>
</tr>
<tr>
<td></td>
<td>12ppt</td>
<td>9.667</td>
<td>0.577</td>
<td>9.667</td>
<td>0.577</td>
</tr>
<tr>
<td></td>
<td>16ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>b) <em>T. granifera</em></td>
<td>0.2ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.6ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12ppt</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>16ppt</td>
<td>8.667</td>
<td>2.309</td>
<td>4</td>
<td>5.196</td>
</tr>
<tr>
<td>c) <em>A. helena</em></td>
<td>0.2ppt</td>
<td>10</td>
<td>0</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.6ppt</td>
<td>8.667</td>
<td>1.155</td>
<td>8.667</td>
<td>1.155</td>
</tr>
<tr>
<td></td>
<td>1ppt</td>
<td>9.667</td>
<td>0.577</td>
<td>9</td>
<td>1.732</td>
</tr>
<tr>
<td></td>
<td>2ppt</td>
<td>9.667</td>
<td>0.577</td>
<td>9.333</td>
<td>0.577</td>
</tr>
<tr>
<td></td>
<td>5ppt</td>
<td>9.667</td>
<td>0.577</td>
<td>9.667</td>
<td>0.577</td>
</tr>
<tr>
<td></td>
<td>8ppt</td>
<td>1.333</td>
<td>2.309</td>
<td>0.667</td>
<td>1.154</td>
</tr>
<tr>
<td></td>
<td>12ppt</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>16ppt</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
For *T. granifera*, the 12 g/kg treatment snails exhibited the lowest consumption with 0.47 cm² out of the 16.31 cm² offered (Suppl. materials 1, 2). This was followed by the 8 and 0.2 g/kg treatments, where snails consumed 1.35 cm² and 4.96 cm², respectively. Negative first order terms were found for all proportional consumption trials with spinach. The model with the best curve fit for *T. granifera* spinach consumption at 0.2 g/kg was the negative exponential, at 8 g/kg the logarithmic model and at 12 g/kg the power curve (Fig. 4b, Suppl. materials 1, 2).

Figure 3. Survival curves for *Melanoides tuberculata*, *Tarebia granifera* and *Anentome helena* with proportional deaths for all tested salinities.
For the *Daphnia* sp. trials, after stepwise removal of non-significant terms, there was only a significant effect of species on the proportional consumption, with *M. tuberculata* consuming more than *T. granifera* \( (z = 5.368, p < 0.001; \text{Suppl. material 2: table S1}) \). *Melanoides tuberculata* exhibited the highest *Daphnia* sp. consumption at 8 g/kg, consuming 225 out of 275 *Daphnia* sp. offered (Suppl. materials 1, 2). This was followed by the 0.2 g/kg treatment, with 187 out of 275 *Daphnia* sp. consumed, and the 16 g/kg treatment, with 152 out of 275 consumed (Suppl. materials 1, 2). There was a negative first order term for *M. tuberculata* feeding on *Daphnia* sp. at 0.2 g/kg, while 8 and 16 g/kg treatments displayed positive first order terms. The Michaelis-Menten model best fit the data for 0.2 g/kg treatment and the 3-Parameter Gompertz model for both 8 and for 16 g/kg (Fig. 5a, Suppl. materials 1, 2).

For *T. granifera* feeding on *Daphnia* sp., consumption across all tested salinities displayed negative first order terms. At 0.2 and 8 g/kg power curves had the best fit, with negative exponential model at 12 g/kg treatment (Fig. 5b, Suppl. materials 1, 2). *Tarebia granifera* consumed 97 out of 275 *Daphnia* sp. at 0.2 g/kg, 68 out of 275 at 8 g/kg and 135 out of 275 at 12 g/kg (Suppl. materials 1, 2).

**Relative impact potential**

At the two compared salinities, (i.e. 0.2 and 8 g/kg) the impact potential for spinach consumption of *M. tuberculata* was higher than for *T. granifera*. Within species, spinach consumption of *M. tuberculata* at 0.2 g/kg showed the highest impact potential, closely followed by 16 g/kg, with the lowest impact score at 8 g/kg (Table 2). For *T. granifera* spinach consumption, 0.2 g/kg also had the highest impact potential, with reduced impacts at 8 and 12 g/kg (Table 2).

In the case of *Daphnia* feeding trials, *M. tuberculata* also exerted higher impact potential than *T. granifera* at matched salinities. *Melanoides tuberculata* had the highest impact potential at 8 g/kg, followed by 16 and 0.2 g/kg (Table 3). *Tarebia granifera* showed the highest impact potential at 12, followed by 0.2 and 8 g/kg (Table 3).
Discussion

There is a pressing urgency for invasion scientists to develop methods of effectively predicting, and in turn, proactively preventing damaging NNS introductions into novel ecosystems. Here, focusing on three readily available gastropod species within the pet trade, each with invasion histories to date, we employed methods...
determining survival and feeding rates under sudden exposure to ecologically-relevant experimental salinities. We found clear differences in survival rates across our experimental salinities, with *M. tuberculata* exhibiting close to 100% survival, *T. granifera* showing mortality at 16 g/kg and *A. helena* experiencing 100% mortality at salinities above 12 g/kg. Assessing *per capita* consumption towards plant and animal-based resources, *M. tuberculata* demonstrated higher feeding rates than *T. granifera* for the common experimental salinities of 0.2 and 8 g/kg. This ultimately gave *M. tuberculata* higher impact potential values, and suggests that this species warrants prioritization based on our experimental conditions.

**Survival experiments**

The survival experiments demonstrated distinct salinity tolerance differences between the three study species. While *M. tuberculata* and *T. granifera* have had documented occurrences in estuarine habitats (Miranda et al. 2011; Farani et al. 2015), we sought to determine the ability of individuals sourced from the pet trade to withstand sudden introduction to different salinities, mimicking release events. *Melanoides tuberculata* proved to be the most robust across our experimental salinity spectrum, whereas *T. granifera* survived low and intermediate experimental salinities, but exhibited higher mortality than *M. tuberculata*, notably in the 16 g/kg salinity treatment. Indeed, although two *T. granifera* tanks held at 16 g/kg and comprising 20 individuals survived the initial 30-day trial period, almost all of them ultimately died within 14 days subsequently. This result thus cautions inferences from relatively short-term survival trials in experiments, as results can rapidly change with additional time points. These findings are consistent with shorter term studies featuring wild-caught individuals, with Farani et al. (2015) finding high adult and juvenile *M. tuberculata* survival after 96 hours in 9 and 18 g/kg salinity, and higher adult survival rates at these salinities (94% and 92%, respectively) than in freshwater conditions (68%). Da Silva and Barros (2015) also found survival above 90% after 48 hours in salinities up to 25. Further, Miranda et al. (2010) found *T. granifera* capable of surviving and reproducing in salinities up to 20, and even surviving levels from 30 to 40 for just under a month. Both of these species possess a number of adaptations to survive brief periods of saline stress, such as reducing activity, retreating into their shells and closing their operculum, and through brood pouches which provide protection to developing young prior to birth (Ben-Ami and Hodgson 2005; Miranda et al. 2010). While it is important to acknowledge that tolerances to abiotic stressors can change over time (Spence and Tingley 2020), and that gradual acclimation could enhance survivability, there is a clear need to account for “prior adaptation” (Hufbauer et al. 2012) when assessing the abilities of traded species to survive and exert impacts in novel ecosystems, especially since the pet trade can artificially select for successful invaders (Briski et al. 2018; Gippet and Bertelsmeier 2021).

While *M. tuberculata* and *T. granifera* demonstrated tolerance of most study salinities, *A. helena* experienced mortality at all experimental salinities above 0.2 g/kg, with 100% mortality within the first 24 hours of the trial in 12 and 16 g/kg tanks, supporting the assertion of the genus *Anentome* being stenohaline (Galindo et al. 2016). With salinity stress shown to affect vital functions such as movement, feeding, respiration, excretion, and growth (Pourmozaffar et al. 2020; Barrios-Figueroa and Urbina 2023), there are various avenues through which our mortality end-point
Elisabeth Renk et al.: Survival and feeding rates of pet trade gastropods

...may have occurred. This lack of tolerance to salinity change, as well as predatory performance being reduced at low temperatures (Dickey et al. 2023a) may partly explain the currently limited non-native range of *A. helena*, despite its prevalence in the pet trade. Indeed, with the temperature maintained at a constant level throughout this experiment (approximately 19 °C), representing a relevant summer temperature in temperate regions, it was at the lower end of tolerance for all three study species. Therefore, it may be that the combination of stressors from salinity and temperature affected survival, and under higher temperatures, *A. helena* in particular may have experienced lower mortality (see, for example, Cuthbert et al. 2021b).

**Consumption rates**

All species have a range of salinities at which energy expenditure is optimized, but at elevated levels, gastropods need to invest more energy in osmoregulation via the ATP-fuelled active pumping of ions from the environment (Barrios-Figueroa and Urbina 2023). This energetically costly process likely influences food choice for species with generalist diets, as well as the rate of intake. Here, the proportional consumption experiments provided insights into the feeding behaviours of *M. tuberculata* and *T. granifera* when exposed to two food resources under three experimental salinities. *Melanoides tuberculata* tended to show higher feeding rates than *T. granifera* for both resources, at the two common experimental salinities, and consumed the most spinach in the 0.2 and 16 g/kg treatments, with lower consumption at the intermediate salinity.

For *Daphnia* sp. consumption, *M. tuberculata* again consumed significantly more than *T. granifera* over the two common experimental salinities of 0.2 and 8 g/kg. *Melanoides tuberculata* showed the greatest consumption at 8 followed by 16 and 0.2 g/kg. Interestingly, *M. tuberculata* displayed feeding curves that resembled Type III forms for *Daphnia* sp. consumption at 8 and 16 g/kg, indicating proportionately lower rates of consumption at low resource densities. With the maximum feeding rate also highest at 8 g/kg, this may indicate that salinity has a greater influence on movement, rather than consumption and digestion for *M. tuberculata*. It is worth noting that the intermediate salinity level of 8 g/kg had the highest *Daphnia* sp. consumption for *M. tuberculata* but also the lowest spinach consumption, with a similar pattern shown for *T. granifera* at 12 g/kg. These findings may suggest preferences for animal-based food resources at these salinities, but this requires further testing. Indeed, *Daphnia* sp. may offer greater energy return for investment under conditions of salinity stress for both species. However, it remains unclear why spinach consumption remained high for *M. tuberculata* at the highest experimental salinity. Future research could specifically study this by presenting both resources simultaneously, such as via invader “prey switching” studies (Cuthbert et al. 2018; Joyce et al. 2019). Further, the provided food resources in this study were chosen to determine the broad effects of salinity on the consumption of plant- and animal-based resources for our study species, with spinach and *Daphnia* sp. known to be readily consumed (based on pet ownership websites). However, we encourage future studies to focus on a particular study system and include site-specific resources that the species could encounter if released. For example, while our experimental salinities included those relevant to the Baltic Sea, food resources from this area could be used, such as *Fucus* sp. as an ecologically relevant, brown algae resource. Nevertheless, this study represents one of the few assessing the role of salinity on
pet trade gastropod diets. Salinity and diet effects on fitness have been shown for other taxonomic groups however, and in a study on juvenile Nile tilapia (Oreochromis niloticus Linnaeus, 1758), a two-way salinity and diet treatment interaction (plant-based or containing both animal and plant ingredients) was discovered on growth performance, with individuals on a plant-based diet showing reduced final body weight, average daily gain, total length and standard length at higher salinities (Gonçalves et al. 2022). Another study, on killifish (Fundulus heteroclitus Linnaeus, 1766), demonstrated a preference for higher salinity after consuming high levels of dietary calcium than when unfed (Bucking et al. 2012).

**Impact potential**

Combining survival and feeding rates, *M. tuberculata* had a higher impact potential than *T. granifera* for both food resources at the two common salinities of 0.2 and 8 g/kg, while also possessing a broader salinity tolerance. While our survival study was focused on mimicking release events, questions remain surrounding the effects that longer periods of acclimation, and indeed adaptation over multiple generations, might have for both survival and feeding rates under the combined stressors of temperature and salinity. While *M. tuberculata* has demonstrated a broader salinity tolerance, *T. granifera* has been shown to be tolerant of temperatures between 0 and 47.5 °C, which may give it a greater potential for establishment in temperate zones, with a likely optimum for physiological activities at around 30 °C (Miranda et al. 2010). A more constrained temperature range of 16 °C to 37 °C has been shown for *M. tuberculata* (Okumura and Rocha 2020), and accordingly, the presence of *M. tuberculata* has only been recorded in thermally polluted habitats in Western Europe to date, such as in the Gillbach in Germany (Emde et al. 2016) and the Ebro in Spain (Oscoz et al. 2010). However, much like freshwater seepage zones in South Africa for *T. granifera* (Miranda et al. 2010), these habitats may allow adaptation to cooler conditions over time. Like salinity, temperature may also play a role in the dietary choice. For example, in a study on the diets of opaleye fish (*Girella nigricans* Ayres, 1860), more algal material was consumed at higher temperatures (Behrens and Lafferty 2012), however, this pattern was not shown for the freshwater gastropod, *Lymnaea stagnalis* Linnaeus, 1758 (Zhang et al. 2018).

Another interesting avenue for further research centres on the interactions between these two species with native species, and with each other. Both species have been used as effective biocontrol agents against gastropods which are hosts to harmful parasites, driven by their abilities to rapidly colonize waterbodies and reach high abundances (Pointier and Jourdane 2000). With *T. granifera* shown to exude a chemical cue causing negative taxis responses in other gastropod species, including *M. tuberculata* (Raw et al. 2013), questions remain with regards to the role of abiotic stressors on its competitive ability, and whether European native species could be similarly affected. Further, the shells of *T. granifera* have also been shown to be more resistant to shell-crushing predators in South Africa (Miranda et al. 2016), which may also prove advantageous against, for example, crayfish species in Europe (Renai and Gherardi 2004; Mathers et al. 2022). Understanding the roles of such abiotic stressors on reproduction of these species is also vital and can be incorporated into the impact potential metric, as both *M. tuberculata* and *T. granifera* can reproduce by parthenogenesis (Berry and Kadri
1974). During this study, we observed cases of reproduction for both *M. tuberculata* and *T. granifera*, however, this was not quantified formally. Reproduction was noted at the salinities of 0.2, 8 and 16 g/kg for *M. tuberculata* and at 0.2 and 12 g/kg for *T. granifera*. Further studies should also test the effects of these stressors on “dispersal enhancing” behavioural responses (Dickey et al. 2022; McGlade et al. 2022).

**Conclusions**

The pet trade is a highly dynamic, global industry and every species sold has the potential to be released, or to escape, into the wild. There are still many knowledge gaps concerning species in the trade (Dickey et al. 2023b), especially in terms of their abilities to survive upon release into novel environments and exert impacts following inherent anthropogenic selection. While future studies need to incorporate other abiotic stressors, and also assess how trophically-analogous native species may be affected under similar conditions, we propose that the methods implemented here highlight *M. tuberculata* as a species highly tolerant of salinity, and potentially more capable of establishing and exerting impacts across a spectrum of habitats from freshwater to brackish than other readily available species. Such methods offer potential for prioritising “risky” pet trade species, facilitating intervention to limit their availability, and in turn help to inform the creation of low-risk species lists (Simberloff 2006; Patoka et al. 2018; Dickey et al. 2023b). However, efforts to address the problem of pet abandonment at its source, through the education of potential pet owners, will continue to warrant prioritisation.

**Acknowledgements**

We would like to thank Gregor Steffen for helping with the experiments. Thanks also to the constructive comments from an anonymous reviewer who undoubtedly helped to improve the manuscript.

**Additional information**

**Conflict of interest**

The authors have declared that no competing interests exist.

**Ethical statement**

No ethical statement was reported.

**Funding**

JWED acknowledges financial support from the Alexander von Humboldt Foundation and the Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), and RNC by the Alexander von Humboldt Foundation as well as the Leverhulme Trust (ECF-2021-0001).

**Author contributions**

JWED, ER and EB conceived the study. ER and EK conducted the experiments. ER, JWED, RNC and EB contributed to the statistical analysis and prepared the initial manuscript. All authors provided valuable input to the development of the final manuscript and have given approval for publication.
Data availability

Raw data supporting the findings of this study are available as Supplementary Information. There was no custom code or mathematical algorithm used in the study.

References


Behrens MD, Lafferty KD (2012) Geographic variation in the diet of Opaleye (Girella nigricans) with respect to temperature and habitat. PLOS ONE 7(9): e45901. https://doi.org/10.1371/journal.pone.0045901


Gippet JMW, Bertelsmeier C (2021) Invasiveness is linked to greater commercial success in the global pet trade. Proceedings of the National Academy of Science USA 118. https://doi.org/10.1073/pnas.2016337118


Therneau T (2023) A Package for Survival Analysis in R. https://cran.r-project.org/package=survival


Supplementary material 1

The results from survival and feeding experiments

Authors: Elisabeth Renk, James W. E. Dickey, Ross N. Curthbert, Elžbieta Kazanavičiūtė, Elizabeta Briski
Data type: xlsx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/neobiota.94.125227.suppl1

Supplementary material 2

Supplementary information

Authors: Elisabeth Renk, James W. E. Dickey, Ross N. Curthbert, Elžbieta Kazanavičiūtė, Elizabeta Briski
Data type: pdf
Explanation note: fig. S1. Experiment species Melanoides tuberculata (a), Tarebia granifera (b) and Anentome helena (c). Photographed by Gregor Steffen, Geomar Kiel 2023. fig. S2. Experimental set up for the survival experiments. fig. S3. Examples of uneaten(a) and partially consumed (b) spinach discs.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/neobiota.94.125227.suppl2